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(56) **References Cited**

U.S. PATENT DOCUMENTS

6,183,965	B1	2/2001	Verdine et al.
6,372,712	B1	4/2002	Briesewitz et al.
6,686,454	B1	2/2004	Yatscoff et al.
6,713,607	B2	3/2004	Caggiano et al.
7,220,552	B1	5/2007	Crabtree et al.
7,396,660	B2	7/2008	Huang et al.
7,851,183	B2	12/2010	Zotchev et al.
8,664,186	B2	3/2014	Aigle et al.
9,119,853	B2	9/2015	Moss et al.
9,211,291	B2	12/2015	Berkenblit et al.
9,250,237	B2	2/2016	Liu et al.
9,260,484	B2	2/2016	Briesewitz et al.
9,428,845	B1	8/2016	Verdine et al.
9,989,535	B2	6/2018	Verdine et al.
10,039,839	B2	8/2018	Verdine et al.
10,203,323	B2	2/2019	Verdine et al.
10,466,249	B2	11/2019	Verdine et al.
10,533,016	B2	1/2020	Verdine et al.
10,800,787	B2	10/2020	Bodhuri et al.
10,948,495	B2	3/2021	Verdine et al.
10,989,710	B2	4/2021	Verdine et al.
11,008,334	B2	5/2021	Ostrem et al.
11,059,830	B2	7/2021	Verdine et al.
11,566,007	B2	1/2023	Koltun et al.
11,608,346	B2	3/2023	Koltun et al.
11,644,460	B2	5/2023	Verdine et al.
11,690,915	B2	7/2023	Aay et al.
11,739,074	B2	8/2023	Aggen et al.
11,952,352	B2	4/2024	Gill et al.
11,987,590	B2	5/2024	Verdine et al.
2002/0110874	A1	8/2002	Khosla et al.
2002/0147133	A1	10/2002	Briesewitz et al.
2003/0153053	A1	8/2003	Reid
2003/0175901	A1	9/2003	Reeves et al.
2004/0087496	A1	5/2004	Kim et al.
2004/0157768	A1	8/2004	Or et al.
2005/0233431	A1	10/2005	Ashley et al.
2007/0203168	A1	8/2007	Zhao
2007/0218502	A1	9/2007	Hahn et al.
2007/0265333	A1	11/2007	Fu et al.
2011/0117606	A1	5/2011	Jorgensen et al.
2012/0208720	A1	8/2012	Kashiwagi et al.
2012/0270800	A1	10/2012	Verdine et al.
2013/0072439	A1	3/2013	Nash et al.
2013/0344030	A1	12/2013	Steadman et al.
2014/0073581	A1	3/2014	Liu et al.
2014/0256717	A1	9/2014	Fernandez et al.
2014/0316104	A1	10/2014	Fischer et al.
2015/0250896	A1	9/2015	Zhao

(Continued)

FOREIGN PATENT DOCUMENTS

CN	107001387	A	8/2017
EP	0194972	A2	9/1986

(Continued)

OTHER PUBLICATIONS

U.S. Appl. No. 61/418,038, Johns Hopkins University.
"Registration No. 333-235968: Amendment No. 2 to Form S-1 Registration Statement Under The Securities Act of 1933 for Revolution Medicines, Inc.," United States Securities and Exchange Commission, Washington, D.C., 20549, dated Feb. 11, 2020 (354 pages).

(Continued)

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(57) **ABSTRACT**

The disclosure features macrocyclic compounds, and pharmaceutical compositions and protein complexes thereof, capable of inhibiting Ras proteins, and their uses in the treatment of cancers.

8 Claims, 6 Drawing Sheets

(56) **References Cited**

U.S. PATENT DOCUMENTS

2015/0307855	A1	10/2015	Yuzawa et al.
2016/0083373	A1	3/2016	Xu
2016/0199506	A1	7/2016	Verdine et al.
2016/0296528	A1	10/2016	Fernandez et al.
2016/0341719	A1	11/2016	Verdine et al.
2017/0190734	A1	7/2017	Aciro et al.
2018/0318434	A1	11/2018	Verdine et al.
2020/0132597	A1	4/2020	Miao et al.
2020/0197391	A1	6/2020	Jin et al.
2020/0199102	A1	6/2020	Mulvihill et al.
2021/0130303	A1	5/2021	Koltun et al.
2021/0130326	A1	5/2021	Aggen et al.
2021/0130369	A1	5/2021	Koltun et al.
2021/0285955	A1	9/2021	Mulvihill et al.
2021/0405060	A1	12/2021	Verdine et al.
2022/0082556	A1	3/2022	Verdine et al.
2022/0105185	A1	4/2022	Aay et al.
2022/0143202	A1	5/2022	Verdine et al.
2022/0144849	A1	5/2022	Verdine et al.
2022/0396589	A1	12/2022	Buckl et al.
2023/0002412	A1	1/2023	Canales et al.
2023/0100838	A1	3/2023	Pitzen et al.
2023/0106174	A1	4/2023	Koltun et al.
2023/0212152	A1	7/2023	Arnold et al.
2023/0226186	A1	7/2023	Aay et al.
2023/0233569	A1	7/2023	Singh et al.
2023/0234929	A1	7/2023	Koltun et al.
2023/0303591	A1	9/2023	Buckl et al.
2023/0374035	A1	11/2023	Koltun et al.
2024/0084305	A1	3/2024	Liu et al.
2024/0108630	A1	4/2024	Corcoran et al.
2024/0139185	A1	5/2024	Jin et al.
2024/0262847	A1	8/2024	Burnett et al.
2024/0277796	A1	8/2024	Seamon
2024/0352036	A1	10/2024	Li et al.
2024/0352038	A1	10/2024	Li et al.
2024/0368190	A1	11/2024	Li et al.
2024/0383920	A1	11/2024	Li et al.
2024/0426833	A1	12/2024	Mulvihill et al.

FOREIGN PATENT DOCUMENTS

EP	0393934	A1	10/1990
EP	0562853	A1	9/1993
EP	1079859	B1	7/2010
KR	10-2009-0041971	A	4/2009
WO	WO-86/02080	A1	4/1986
WO	WO-95/32294	A1	11/1995
WO	WO-96/20216	A1	7/1996
WO	WO-96/41807	A1	12/1996
WO	WO-98/01546	A2	1/1998
WO	WO-98/07743	A1	2/1998
WO	WO-98/12217	A1	3/1998
WO	WO-99/61055	A1	12/1999
WO	WO-00/47724	A2	8/2000
WO	WO-01/36460	A2	5/2001
WO	WO-01/36612	A1	5/2001
WO	WO-01/90070	A2	11/2001
WO	WO-03/033010	A1	4/2003
WO	WO-2007/133352	A2	11/2007
WO	WO-2008/069824	A2	6/2008
WO	WO-2010/031185	A1	3/2010
WO	WO-2010/034243	A1	4/2010
WO	WO-2010/088573	A1	8/2010
WO	WO-2012/075048	A2	6/2012
WO	WO-2012/078915	A1	6/2012
WO	WO-2012/131371	A1	10/2012
WO	WO-2012/131377	A1	10/2012
WO	WO-2012/174489	A2	12/2012
WO	WO-2013/001310	A1	1/2013
WO	WO-2013/022818	A1	2/2013
WO	WO-2013/185090	A1	12/2013
WO	WO-2013/185093	A1	12/2013
WO	WO-2013/185103	A1	12/2013
WO	WO-2014/009774	A1	1/2014
WO	WO-2014/121942	A1	8/2014
WO	WO-2014/169167	A1	10/2014
WO	WO-2014/176488	A1	10/2014
WO	WO-2014/187959	A2	11/2014
WO	WO-2015/132784	A1	9/2015
WO	WO-2016008011	A1	1/2016
WO	WO-2016/112279	A1	7/2016
WO	WO-2016/112295	A1	7/2016
WO	WO-2016/160362	A1	10/2016
WO	WO-2017/058768	A1	4/2017
WO	WO-2017/059207	A1	4/2017
WO	WO-2017085198	A1	5/2017
WO	WO-2018/081592	A2	5/2018
WO	WO-2018/091634	A1	5/2018
WO	WO-2018/187401	A1	10/2018
WO	WO-2018/187423	A1	10/2018
WO	WO-2018/217651	A1	11/2018
WO	WO-2020/101736	A1	5/2020
WO	WO-2020/106640	A1	5/2020
WO	WO-2020/132597	A1	6/2020
WO	WO-2021052499	A1	3/2021
WO	WO-2021/086833	A1	5/2021
WO	WO-2021/091956	A1	5/2021
WO	WO-2021/091967	A1	5/2021
WO	WO-2021/091982	A1	5/2021
WO	WO-2021/108683	A1	6/2021
WO	WO-2021/257736	A1	12/2021
WO	WO-2022/060583	A1	3/2022
WO	WO-2022/060836	A1	3/2022
WO	WO-2022/137085	A1	6/2022
WO	WO-2022/137174	A1	6/2022
WO	WO-2022/139304	A1	6/2022
WO	WO-2022/235945	A1	11/2022
WO	WO-2022235725	A1	11/2022
WO	WO-2023/060253	A1	4/2023

OTHER PUBLICATIONS

"SMART™ Drugs: Engineering Nature's Solution to the Undruggable Target Challenge," WarpDrive Bio, 2016, available <http://www.warpdrivebio.com/docs/Warp%20Drive%20Bio_SMART%20Drugs%20Platform_2016.pdf> (31 pages).

"Substructure Search Report on Specifically Substituted Macrocycles—Substances Only," prepared by Science IP, dated Dec. 17, 2014 (6177 pages).

"Translating Frontier Oncology Targets to Outsmart Cancer™," Corporate Overview Q3-2020, Revolution Medicines, Aug. 20, 2020 (35 pages).

Aebi et al., "Synthesis, Conformation, and Immunosuppressive Activities of Three Analogues of Cyclosporin A Modified in the 1-Position," J Med Chem. 33(3):999-1009 (1990).

Allain et al., "Cyclophilin B mediates cyclosporin A incorporation in human blood T-lymphocytes through the specific binding of complexed drug to the cell surface," Biochem J. 317 (Pt 2):565-70 (1996).

Andrei et al., "Stabilization of protein-protein interactions in drug discovery," Expert Opin Drug Discov. 12(9):925-40 (2017) (17 pages).

Antunes et al., "A mutational analysis defines Vibrio fischeri LuxR binding sites," J Bacteriol. 190(13):4392-7 (2008).

Araujo et al. "Mechanisms of response and tolerance to active Ras inhibition in KRAS-mutant NSCLC," Cancer Discov. (Jul. 2024) (87 pages).

Archibald et al., "Discovery and Evaluation of Potent, Cysteine-based alpha4beta1 Integrin Antagonists," Bioorg Med Chem Lett. 10(9):993-995 (2000).

Baillie, "Targeted Covalent Inhibitors for Drug Design," Covalent Inhibitor Drug Discovery & Development Symposium PBSS, Feb. 7, Foster City, California. (2019) (16 pages).

Banaszynski et al., "Characterization of the FKBP.rapamycin.FRB ternary complex," J Am Chem Soc. 127(13):4715-21 (2005).

Banteli et al., "A synthesis of the C1-N12 tripeptide fragment of sanglifehrin A," Tetrahedron Letters. 40(11):2109-2112 (Mar. 12, 1999).

(56) **References Cited**

OTHER PUBLICATIONS

Baranasic et al., "Draft Genome Sequence of *Streptomyces rapamycinicus* Strain NRRL 5491, the Producer of the Immunosuppressant Rapamycin," Genome Announc. 1(4):e00581-13 (2013) (2 pages).
Bastin et al., "Salt selection and optimisation procedures for pharmaceutical new chemical entities," Org Process Res Dev 4(5):427-35 (2000).
Bayle et al., "Rapamycin analogs with differential binding specificity permit orthogonal control of protein activity," Chem Biol. 13(1):99-107 (2006).
Bender et al., "Periodate Oxidation of alpha-Keto gamma-Lactams. Enol Oxidation and beta-Lactam Formation. Mechanism of Periodate Hydroxylation Reactions," J Org Chem. 43(17):3354-3362 (1978).
Benjamin et al., "Rapamycin passes the torch: a new generation of mTOR inhibitors," Nat Rev Drug Discov. 10(11):868-80 (2011).
Bhuyan et al., "Antioxidant activity of peptide-based angiotensin converting enzyme inhibitors," Org Biomol Chem. 10(11):2237-47 (2012).
Blodgett et al., "Unusual transformations in the biosynthesis of the antibiotic phosphinothricin tripeptide," Nat Chem Biol. 3(8):480-5 (2007).
Braun et al., "A bifunctional molecule that displays context-dependent cellular activity," J Am Chem Soc. 125(25):7575-80 (Jun. 2003).
Briesewitz et al., "Affinity modulation of small-molecule ligands by borrowing endogenous protein surfaces," Proc Natl Acad Sci U.S.A. 96(5):1953-8 (1999).
Bruce, "In vivo protein complex topologies: sights through a cross-linking lens," Proteomics. 12(10):1565-75 (2012).
Burgess et al., "Controlled translocation of palladium(II) within a 22 ring atom macrocyclic ligand," Dalton Trans. 43(45):17006-16 (2014) (12 pages).
Chaurasia et al., "Molecular insights into the stabilization of protein-protein interactions with small molecule: The FKBP12-rapamycin-FRB case study," Chem Phys Lett. 587:68-74 (2013).
Che et al., "Inducing protein-protein interactions with molecular glues," Bioorg Med Chem Lett. 28(15):2585-92 (Aug. 2018) (18 pages).
Chevalier et al., "Straightforward synthesis of bioconjugatable azo dyes. Part 1: Black Hole Quencher-1 (BHQ-1) scaffold," Tetrahedron Lett. 55(50):6759-63 (2014).
Ding et al., "Insights into Bacterial 6-Methylsalicylic Acid Synthase and Its Engineering to Orsellinic Acid Synthase for Spirotetronate Generation," Chem Biol. 17(5):495-503 (2010).
Doak et al., "Cyclophilin Succumbs to a Macrocyclic Chameleon," J Med Chem. 61(21):9469-9472 (Nov. 2018).
Dyson et al., Chemistry of synthetic medicines. *May's Chemistry of Synthetic Drugs, Fifth Edition*. Moscow: Mir. pp. 12-19 (1964) (9 Pages).
Eberle et al., "Preparation of Functionalized Ethers of Cyclosporin A," Tetrahedron Lett. 35(35):6477-6480 (1994).
English Translation of Office Action for Russian Patent Application No. 2022111759, dated Mar. 6, 2024 (11 pages).
Findlay et al., "The structure of demethoxyrapamycin," Can J Chem. 60:2046-7 (1982).
Garg et al., "Elucidation of the Cryptic Epimerase Activity of Redox-Inactive Ketoreductase Domains from Modular Polyketide Synthases by Tandem Equilibrium Isotope Exchange," J Am Chem Soc. 136(29):10190-10193 (2014).
Gill et al., "Discovery of Small Molecule Inhibitors of the Oncogenic, GTP-Bound (ON) Form of $KRAS^{G12C}$ and $KRAS^{G13C}$," Revolution Medicines (Sep. 2019) (1 page).
Gill, "Discovery of Small Molecule Inhibitors of Oncogenic Mutants of Ras," Revolution Medicines, ACS, Apr. 2, Orlando (2019) (23 pages).
Gordon et al., "A SARS-CoV-2 Protein Interaction Map Reveals Targets for Drug Repurposing," Nature. 583(7816):459-68 (Apr. 2020).
Guerra et al., "LAL regulators SCO0877 and SCO7173 as pleiotropic modulators of phosphate starvation response and actinorhodin biosynthesis in *Streptomyces coelicolor*," PLoS One. 7(2):e31475 (2012) (11 pages).
Guo et al., "Rapamycin-inspired macrocycles with new target specificity," Nat Chem. 11(3):254-263 (Mar. 2019) (13 pages).
Halford, "Covalent drugs go from fringe field to fashionable endeavor: Designing molecules that make bonds with their biological targets is in vogue," Chemical and Engineering News, 98(43) (Nov. 2020) (6 pages).
Hansson et al., "Bioengineering and Semisynthesis of an Optimized Cyclophilin Inhibitor for Treatment of Chronic Viral Infection," Chem Biol. 22(2):285-92 (2015) (24 pages).
He et al., "The LuxR family members GdmRI and GdmRII are positive regulators of geldanamycin biosynthesis in *Streptomyces hygroscopicus* 17997," Arch Microbiol. 189(5):501-10 (2008).
Hoffman, "The origins and evolution of "controlled" drug delivery systems," J Control Release. 132(3):153-63 (Dec. 2008) (Epub Aug. 2008).
Holderfield et al. "Concurrent inhibition of oncogenic and wild-type Ras-GTP for cancer therapy," Nature. 629(8013):919-926 (May 2024) (33 pages) (supplementary information included).
Hong et al., "Evidence for an iterative module in chain elongation on the azalomycin polyketide synthase," Beilstein J Org Chem. 12:2164-2172 (2016).
Horn et al., "Draft Genome Sequence of *Streptomyces iranensis*," Genome Announc. 2(4):e00616-14 (2014) (2 Pages).
Horton et al., "Engineering hybrid genes without the use of restriction enzymes: gene splicing by overlap extension," Gene. 77(1):61-8 (1989).
Hosted et al., "Use of rpsL for dominance selection and gene replacement in *Streptomyces roseosporus*," J Bacteriol. 179(1): 180-6 (1997).
Huang et al., "Conjugation to Albumin-Binding Molecule Tags as a Strategy to Improve Both Efficacy and Pharmacokinetic Properties of the Complement Inhibitor Compstatin," ChemMedChem. 9(10):2223-6 (2014).
Huang et al., "Enhanced rapamycin production in *Streptomyces hygroscopicus* by integrative expression of aveR, a LAL family transcriptional regulator," World J Microbiol Biotechnol. 27:2103-9 (2011).
Hubler et al., "Synthetic routes to $NEtXaa^4$-cyclosporin A derivatives as potential anti-HIV I drugs," Tetrahedron Lett. 41:7193-6 (2000).
International Preliminary Report on Patentability for International Application No. PCT/US2016/012631, mailed Jul. 20, 2017 (7 pages).
International Preliminary Report on Patentability for International Application No. PCT/US2017/027215, mailed Oct. 25, 2018 (7 pages).
International Search Report and Written Opinion for International Application No. PCT/US17/58805, mailed Aug. 27, 2018 (16 pages).
International Search Report and Written Opinion for International Application No. PCT/US2016/012631, mailed Mar. 16, 2016 (13 pages).
International Search Report and Written Opinion for International Application No. PCT/US2016/012656, mailed Mar. 21, 2016 (14 pages).
International Search Report and Written Opinion for International Application No. PCT/US2016/054691, mailed Feb. 15, 2017 (28 pages).
International Search Report and Written Opinion for International Application No. PCT/US2017/027215, mailed Jul. 10, 2017 (13 pages).
International Search Report and Written Opinion for International Application No. PCT/US2017/058800, mailed Apr. 3, 2018 (21 pages).
International Search Report and Written Opinion for International Application No. PCT/US2018/025991, mailed Jun. 26, 2018 (13 pages).

(56) **References Cited**

OTHER PUBLICATIONS

International Search Report and Written Opinion for International Application No. PCT/US2018/026014, mailed Aug. 7, 2018 (31 pages).
International Search Report and Written Opinion for International Application No. PCT/US2019/068100, mailed Feb. 24, 2020 (16 pages).
Ishizawa et al., "TRAP display: a high-speed selection method for the generation of functional polypeptides," J Am Chem. 135(14):5433-40 (2013).
Janes et al., "Targeting KRAS Mutant Cancers with a Covalent G12C-Specific Inhibitor," Cell., 172(3):578-589.e17 (Jan. 2018) (30 pages).
Jarvis, "Have drug hunters finally cracked KRas?—After decades of failures, researchers see promise in fresh approaches to developing drugs that block cancer's toughest target," Chemical & Engineering News. 94(23):28-33. <https://cen.acs.org/articles/94/123/drug-hunters-finally-cracked-KRas.html>, retrieved on Oct. 14, 2018 (2016) (9 pages).
Jiang et al. "Translational and Therapeutic Evaluation of Ras-GTP Inhibition by RMC-6236 in Ras-Driven Cancers," Cancer Discov. 14(6):994-1017 (Jun. 2024) (39 pages) (supplemental information included).
Kawakami et al., "In vitro selection of multiple libraries created by genetic code reprogramming to discover macrocyclic peptides that antagonize VEGFR2 activity in living cells," ACS Chem Biol. 8(6):1205-14 (2013).
Kelsey, "Approaches to Inhibiting Ras-Driven Tumors Beyond $KRAS^{G12C}$," Ras Targeted Drug Development, Revolution Medicines, Sep. 16, 2020 (24 pages).
Kendrew et al., "Recombinant strains for the enhanced production of bioengineered rapalogs," Metab Eng. 15:167-73 (2013).
Kuhn et al., "Synthesis of Functional Ras Lipoproteins and Fluorescent Derivatives," J Am Chem Soc. 123(6):1023-35 (2001).
Kuramochi et al., "Identification of Small Molecule Binding Molecules by Affinity Purification Using a Specific Ligand Immobilized on PEGA Resin," Bioconjug Chem. 19(12):2417-26 (2008).
Kümmerer, "Pharmaceuticals in the Environment," Annual Review of Environment and Resources. 35:57-75 (Nov. 2010) (Epub Aug. 18, 2010) (22 pages).
Laureti et al., "Identification of a bioactive 51-membered macrolide complex by activation of a silent polyketide synthase in *Streptomyces ambofaciens*," Proc Natl Acad Sci USA. 108(15):6258-63 (2011).
Laureti et al., Supporting Material for "Identification of a bioactive 51-membered macrolide complex by activation of a silent polyketide synthase in *Streptomyces ambofaciens*," Proc Natl Acad Sci U.S.A. 108(15):6258-63 (2011), accessed via <https://www.pnas.org/content/suppl/2011/03/24/1019077108.DCSupplemental> (41 pages).
Lee et al., "Current implications of cyclophilins in human cancers," J Exp Clin Cancer Res. 29(1):97 (2010) (6 pages).
Leskiw et al., "TTA codons in some genes prevent their expression in a class of developmental, antibiotic-negative, *Streptomyces* mutants," Proc Natl Acad Sci USA. 88(6):2461-5 (1991).
Li et al., "A simple and efficient route to the FKBP-binding domain from rapamycin," available in PMC Sep. 28, 2012, published in final edited form as: Tetrahedron Lett. 52(39):5070-2 (2011) (7 pages).
Luengo et al., "Structure-activity studies of rapamycin analogs: evidence that the C-7 methoxy group is part of the effector domain and positioned at the FKBP12-FRAP interface," Chem Biol. 2(7):471-81 (1995).
Mackman et al., "Discovery of a Potent and Orally Bioavailable Cyclophilin Inhibitor Derived from the Sanglifehrin Macrocycle," J Med Chem. 61(21):9473-9499 (Aug. 2018).
Majumder et al. "Interaction of aryl hydrocarbon receptor-interacting protein-like 1 with the farnesyl moiety," J Biol Chem. 288(29):21320-21328 (2013).
McGregor et al., "Expanding the Scope of Electrophiles Capable of Targeting K-Ras Oncogenes," Biochemistry. 56(25):3178-3183 (2017).
Meyer et al., "Selective palladation of a large (32 ring atom) macrocyclic ligand at a bis(N-heterocyclic carbene) coordination pocket through transmetallation of the corresponding mercury(II) derivative," Dalton Trans. 41(46):14059-67 (2012) (10 pages).
Mo et al., "Interspecies Complementation of the LuxR Family Pathway-Specific Regulator Involved in Macrolide Biosynthesis," J Microbiol Biotechnol. 26(1):66-71 (2016).
Moore et al., "Ras-targeted therapies: is the undruggable drugged?" available in PMC Feb. 1, 2021, published in final edited form as: Nat Rev Drug Discov. 19(8):533-52 (Aug. 2020) (43 pages).
Mullard, "Cracking KRAS," Nature Publishing Group 18(12) (Nov. 2019) (14 pages).
Murphy et al., "Isolation and characterisation of amphotericin B analogues and truncated polyketide intermediates produced by genetic engineering of *Streptomyces nodosus*," Org Biomol Chem. 8(16):3758-70 (2010).
Non-Final Office Action for U.S. Appl. No. 15/974,923, dated Apr. 15, 2019 (6 pages).
Notification of Reasons for Rejection for Japanese Application No. 2017-555427, mailed Aug. 28, 2018 (12 pages).
Ochi et al., "New strategies for drug discovery: activation of silent or weakly expressed microbial gene clusters," Appl Microbiol Biotechnol. 97(1):87-98 (2013).
Ostrem et al., "Direct small-molecule inhibitors of KRAS: from structural insights to mechanism-based design," Nat Rev Drug Discov. 15(11):771-785 (2016).
Ostrem et al., "K-Ras(G12C) inhibitors allosterically control GTP affinity and effector interactions," Nature. 503(7477):548-51 (2013) (14 pages).
Papageorgiou et al., "Improved binding affinity for cyclophilin A by a cyclosporin derivative singly modified at its effector domain," J Med Chem. 37(22):3674-6 (1994).
Pei et al., "Targeting Ras with Macromolecules," Cold Spring Harb Perspect Med. 8(3):a031476 (Mar. 2018) (14 pages).
Pfeifer et al., "Biosynthesis of complex polyketides in a metabolically engineered strain of *E. coli*," Science. 291(5509):1790-2 (2001) (4 pages).
Pollock et al., "Development of Inhibitors of the Activated Form of $KRAS^{G12C}$," AACR Targeting Ras-Driven Cancers, Dec. 9-12, San Diego, California (Dec. 2018) (Abstract Included) (2 pages).
Power et al., "Engineered Synthesis of 7-Oxo- and 15-Deoxy-15-Oxo-Amphotericins: Insights into Structure-Activity Relationships in Polyene Antibiotics," Chem Biol. 15(1):78-86 (2008).
PubChem CID 130196149, <https://pubchem.ncbi.nlm.nih.gov/compound/130196149>, retrieved on Apr. 1, 2020 (10 pages).
Quesniaux et al., "Cyclophilin binds to the region of cyclosporine involved in its immunosuppressive activity," Eur J Immunol. 17(9):1359-65 (1987).
Quesniaux et al., "Study of the conformation of cyclosporine in aqueous medium by means of monoclonal antibodies," Int J Pept Protein Res. 31(2):173-85 (1988).
Ranganathan et al., "Knowledge-based design of bimodular and trimodular polyketide synthases based on domain and module swaps: a route to simple statin analogues," Chem Biol. 6(10):731-41 (1999).
Ray et al., "New Electrophiles and Strategies for Mechanism-Based and Targeted Covalent Inhibitor Design," Biochemistry. 58: 5234-5244 (Dec. 2019).
Reid et al. "A model of structure and catalysis for ketoreductase domains in modular polyketide synthases," Biochemistry. 42(1):72-79 (2003).
Revill et al., "Genetically engineered analogs of ascomycin for nerve regeneration," J Pharmacol Exp Ther. 302(3):1278-85 (2002).
Revolution Medicines, "Translating Frontier Oncology Targets to Outsmart Cancer™: Corporate Overview Q4-2020." dated Nov. 12, 2020 (30 pages).
Ruan et al., "Binding of rapamycin analogs to calcium channels and FKBP52 contributes to their neuroprotective activities," Proc Natl Acad Sci U S A. 105(1):33-8 (2008).
Rudolph, "Covalent Modification In Drug Discovery—A Chemist's Perspective," Pharmaceutical & BioScience Society. Dated Feb. 7, 2019 (39 pages).

(56) **References Cited**

OTHER PUBLICATIONS

Saeidian et al. "Effect of aromaticity and ring strain on proton affinity of aziridine and amidine skeletons: a DFT study," Journal of the Iranian Chemical Society. 17:1731-1741 (Mar. 2020) (11 pages).
Schulze et al. "Chemical remodeling of a cellular chaperone to target the active state of mutant KRAS," Science. 381(6659):794-799 (Aug. 2023) (79 pages) (supplemental information included).
Schulze et al., "Tri-Complex Inhibitors of the Oncogenic, GTP-Bound Form of $KRAS^{G12C}$ Overcome RTK-Mediated Escape Mechanisms and Drive Tumor Regressions in Vivo," Revolution Medicines (Oct. 2019) (1 page).
Schutt, "Safety Considerations for Covalent Inhibitors," Pharmaceutical & BioScience Society. Dated Feb. 7, 2019 (36 pages).
Schwecke et al., "The biosynthetic gene cluster for the polyketide immunosuppressant rapamycin," Proc Natl Acad Sci U S A. 92(17):7839-43 (1995).
Sebastiano et al., "Impact of Dynamically Exposed Polarity on Permeability and Solubility of Chameleonic Drugs Beyond the Rule of 5," J Med Chem. 61:4189-4202 (Apr. 2018).
Shigdel et al., "Genomic discovery of an evolutionarily programmed modality for small-molecule targeting of an intractable protein surface," Proc Natl Acad Sci U S A. 117(29):17195-203 (Jul. 2020).
Sieber et al., "Novel inhibitors of the calcineurin/NFATc hub—alternatives to CsA and FK506?" Cell Commun Signal. 7:25 (2009) (19 pages).
Sieburth et al., "SUR-8, a conserved Ras-binding protein with leucine-rich repeats, positively regulates Ras-mediated signaling in C. elegans," Cell. 94(1):119-30 (Jul. 1998).
Simanshu et al., "Ras Proteins and Their Regulators in Human Disease," available in PMC Jun. 29, 2018, published in final edited form as: Cell. 170(1):17-33 (2017) (34 pages).
Smith, "Translating Frontier Oncology Targets to Outsmart Cancer," Ras-Targeted Drug Discovery Summit, Revolution Medicines, Sep. 19, 2019 (29 pages).
Smulik et al., "Synthesis of cyclosporin A-derived affinity reagents by olefin metathesis," Org Lett. 4(12):2051-4 (2002).
Steadman et al., "Discovery of Potent Cyclophilin Inhibitors Based on the Structural Simplification of Sanglifehrin A," J Med Chem. 60:1000-1017 (2017).
Stewart et al., "Development of Inhibitors of the Activated Form of $KRAS^{G12C}$," AACR Targeting Ras-Driven Cancers, Dec. 9-12, San Diego, California. Poster B37 (2018) (1 page).
Sun et al. "Design and structure-based study of new potential FKBP12 inhibitors," Biophys J. 85(5):3194-3201 (2003).
Supplementary European Search Report for European Application No. 16852685.3, dated Feb. 4, 2019 (8 pages).
Supplementary Partial European Search Report for European Application No. 17863519.9, dated Jun. 15, 2020 (16 pages).
Supplementary Partial European Search Report for European Patent Application No. 17865512.2, dated May 7, 2020 (20 pages).
Sweeney et al., "From chemical tools to clinical medicines: non-immunosuppressive cyclophilin inhibitors derived from the cyclosporin and sanglifehrin scaffolds," J Med Chem. 57(17):7145-59 (2014) (63 pages).
Sànchez-Tilló et al., "Cyclophilin A is required for M-CSF-dependent macrophage proliferation," Eur J Immunol. 36(9):2515-24 (2006).
Takakusagi et al., "Efficient one-cycle affinity selection of binding proteins or peptides specific for a small-molecule using a T7 phage display pool," Bioorg Med Chem. 16(22):9837-46 (2008).
Tanaka et al., "Clinical Acquired Resistance to $KRAS^{G12C}$ Inhibition through a Novel KRAS Switch-II Pocket Mutation and Polyclonal Alterations Converging on Ras-MAPK Reactivation," Cancer Discov. 11(8):1913-1922 (Apr. 6, 2021).
Tang et al., "Generation of New Epothilones by Genetic Engineering of a Polyketide Synthase in Myxococcus xanthus," J Antibiot (Tokyo). 58(3):178-184 (2005).
UniProtKB Accession No. A0A061A618, Sep. 3, 2014, available <http://www.uniprot.org/uniprot/A0A061A6I8>, (12 pages).
UniProtKB Accession No. Q54296, "Polyketide synthase," <https://www.uniprot.org/uniprot/A0A61A6I8.txt?version=14>, retrieved May 29, 2020 (12 pages).
UniProtKB Accession No. Q54296, Nov. 1, 1996, available <http://www.uniprot.org/uniprot/Q54296>, (12 pages).
UniProtKB Accession No. Q54297, Nov. 1, 1996, available <https://www.uniprot.org/uniprot/Q54297.txt>, (3 pages).
Upadhyaya et al., "Direct Ras Inhibitors Identified From a Structurally Rigidified Bicyclic Peptide Library," available in PMC Oct. 21, 2015, published in final edited form as: Tetrahedron. 70(42):7714-7720 (2014) (15 pages).
Vakiti et al., "Stereoselective synthesis of C17-C34 fragment of antascomicin A," Tetrahedron Lett. 55(47):6438-40 (2014).
Vignot et al., "mTOR-targeted therapy of cancer with rapamycin derivatives," Ann Oncol. 16(4):525-37 (2005).
Vocadlo et al. "Mechanistic insights into glycosidase chemistry," Curr Opin Chem Biol. 12:539-555 (2008) (17 pages).
Wagner et al., "New naturally occurring amino acids," Angew Chem Int Ed Engl. 22(11):816-28 (1983).
Wang et al., "Thermodynamic analysis of cyclosporin a binding to cyclophilin a in a lung tumor tissue lysate," Anal Chem. 76(15):4343-8 (2004).
Wasko et al. "Tumour-selective activity of Ras-GTP inhibition in pancreatic cancer," Nature. 629(8013):927-936 (May 2024) (31 pages).
Weissman et al., "Combinatorial biosynthesis of reduced polyketides," Nat Rev Microbiol. 3(12):925-36 (2005).
Weissman, "Genetic engineering of modular PKSs: from combinatorial biosynthesis to synthetic biology," Nat Prod Rep. 33(2):203-230 (2016).
Wildes et al., "Inhibition of the Oncogenic, GTP-Bound Form of $KRAS^{G12C}$ by Second Generation, Tri-Complex Inhibitors Overcomes RTK-Mediated Escape Mechanisms," The FASEB Regulation and Function of Small GTPases Conference, Jun. 23-28, Olean, New York (Abstract Included) (Jun. 2019) (2 pages).
Wilson et al., "Comparative X-ray structures of the major binding protein for the immunosuppressant FK506 (tacrolimus) in unliganded form and in complex with FK506 and rapamycin," Acta Cryst. D51:511-21 (1995).
Wright et al., "Multivalent binding in the design of bioactive compounds," Curr Org Chem. 5(11):1107-31 (2001).
Wu et al., "Creating diverse target-binding surfaces on FKBP12: synthesis and evaluation of a rapamycin analogue library," available in PMC Sep. 12, 2012, published in final edited form as: ACS Comb Sci. 13(5):486-95 (2011) (22 pages).
Wu et al., "Inhibition of ras-effector interactions by cyclic peptides," Med Chem Commun. 4(2):378-82 (2013).
Wu et al., "Synthesis of Ketone Analogues of Prolyl and Pipecolyl Ester FKBP12 Ligands," J Med Chem. 45(16):3558-3568 (2002).
Zhang et al., "Bifunctional Small-Molecule Ligands of K-Ras Induce Its Association with Immunophilin Proteins," Angew Chem Int Ed Engl. 131:16460-5 (Nov. 2019).
Zhou et al., "Biophysical and biochemical characterization of $KRAS^{G12C}$ inhibition through a novel modality," AACR Targeting Ras-Driven Cancers, Dec. 9-12, San Diego, California. Poster A06 (2018) (1 page).
Chen et al., "Adaptive and Reversible Resistance to Kras Inhibition in Pancreatic Cancer Cells," Cancer Res. 78(4): 985-1002 (19 pages) (Feb. 2018).
Choi et al., "Challenges in Ras therapeutics in pancreatic cancer," Semin Cancer Biol. 54: 101-108 (8 pages) (Feb. 2019).

FIG. 1A

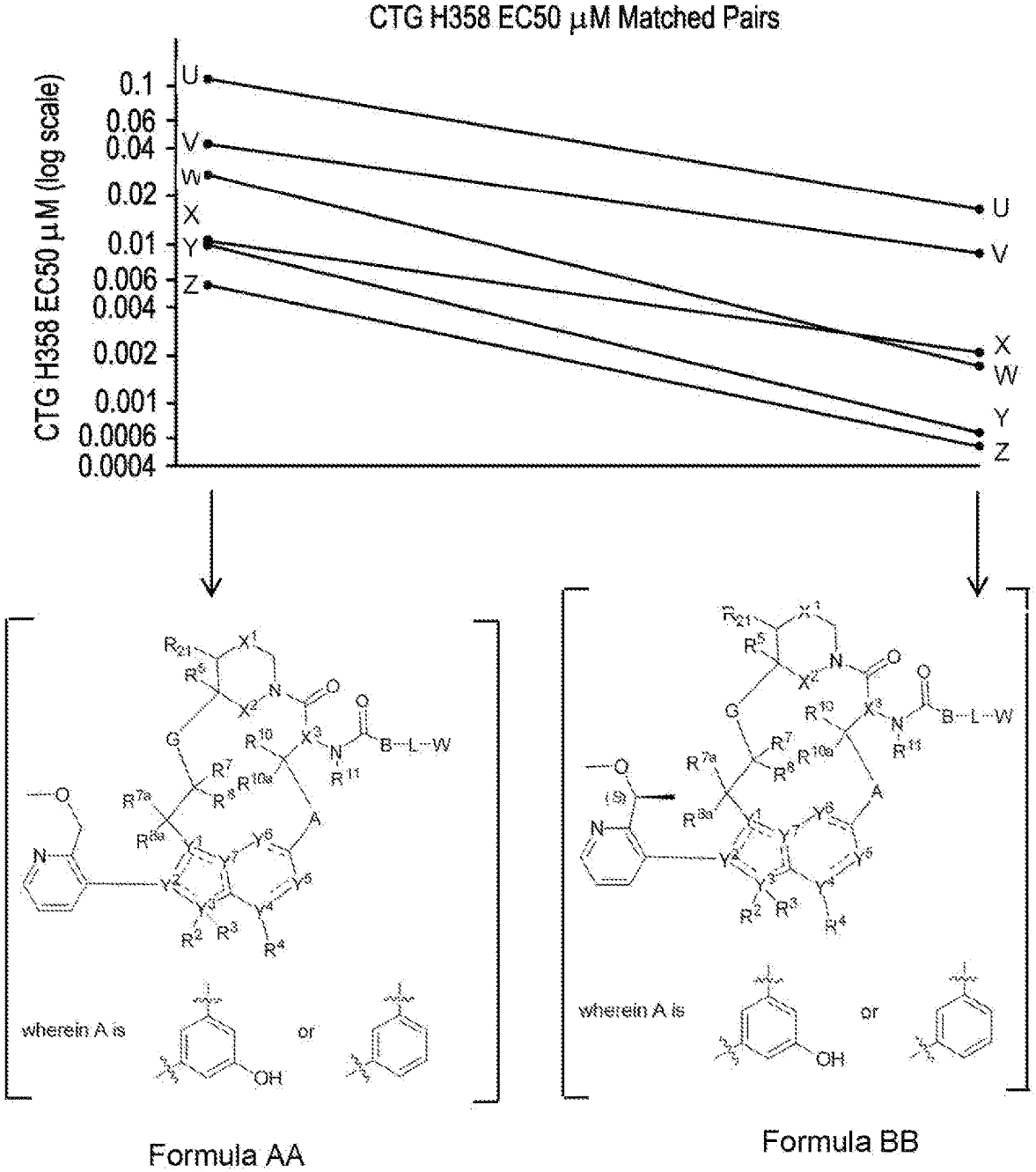

CTG H358 EC50 μM Matched Pairs
CTG H358 EC50 μM (log scale)
0.1
0.06
0.04
0.02
0.01
0.006
0.004
0.002
0.001
0.0006
0.0004
U
V
W
X
Y
Z
wherein A is
or
OH
Formula AA
Formula BB

FIG. 1B

pERK H358 EC50 μM Matched Pairs

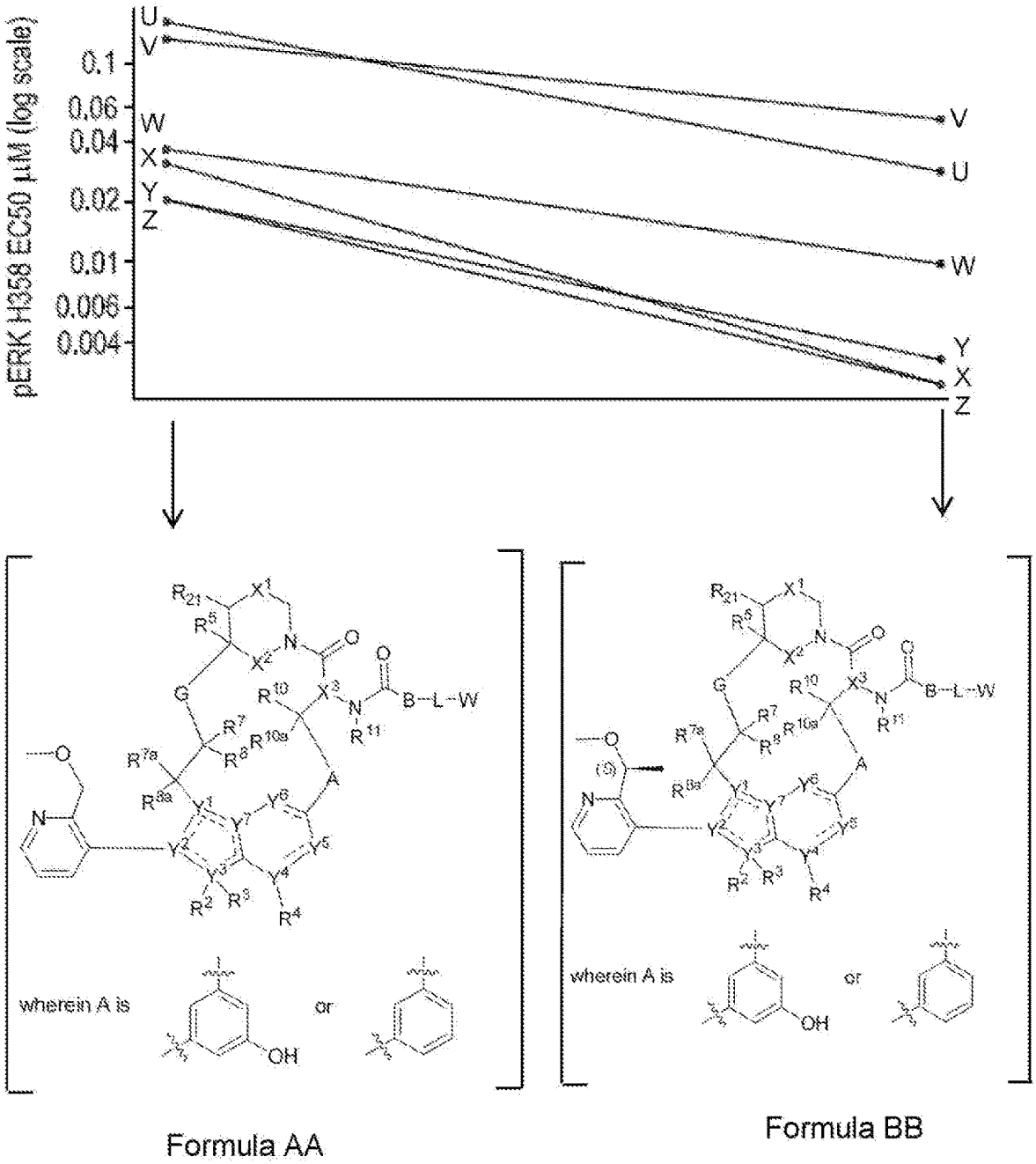


FIG. 2A

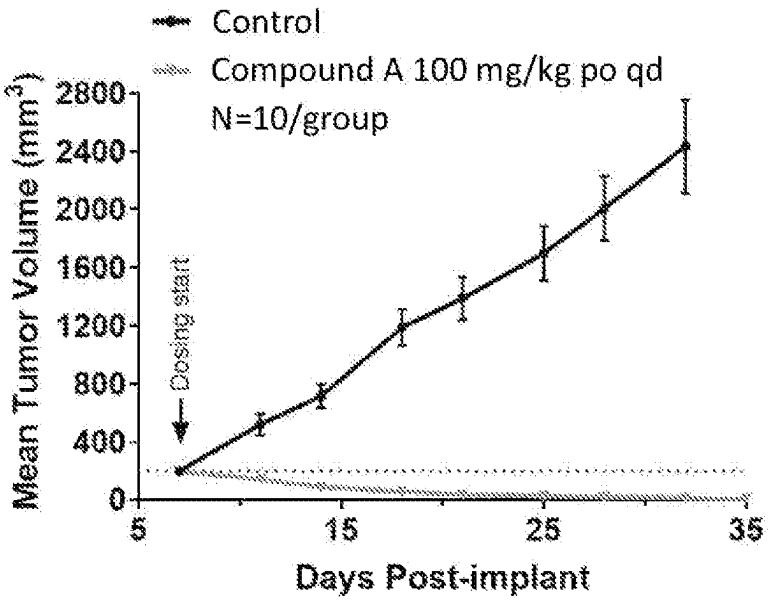

Control
Compound A 100 mg/kg po qd
N=10/group
Mean Tumor Volume (mm3)
2800
2400
2000
1600
1200
800
400
0
Dosing start
5
15
25
35
Days Post-implant

FIG. 2B

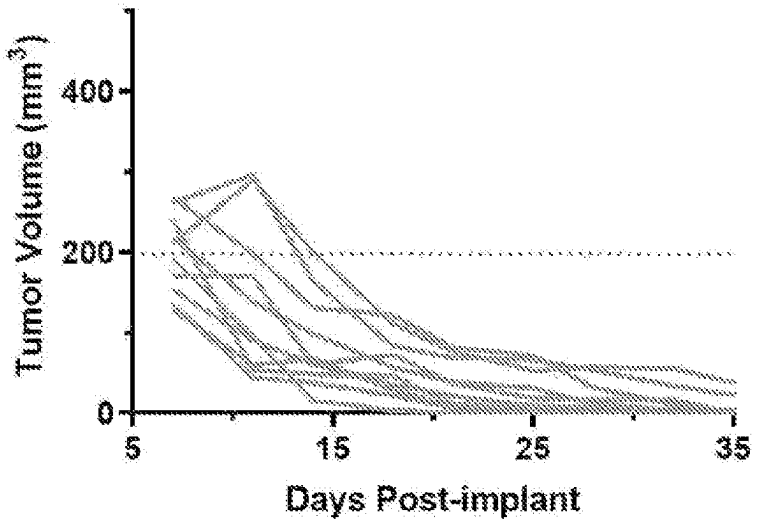

Tumor Volume (mm3)
400
200
0
5
15
25
35
Days Post-implant

FIG. 3A

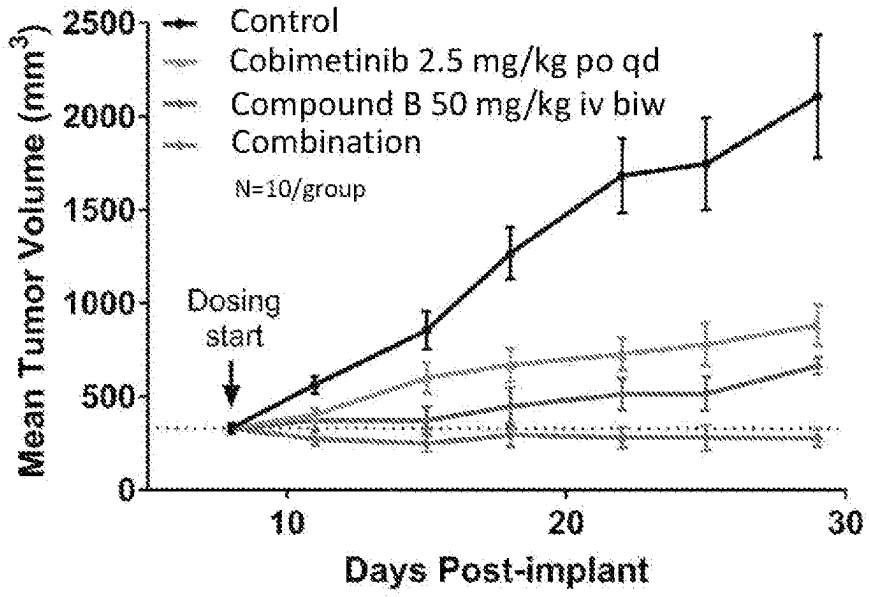

Control
Cobimetinib 2.5 mg/kg po qd
Compound B 50 mg/kg iv biw
Combination
N=10/group
Dosing start
Mean Tumor Volume (mm3)
2500
2000
1500
1000
500
0
10
20
30
Days Post-implant

FIG. 3B

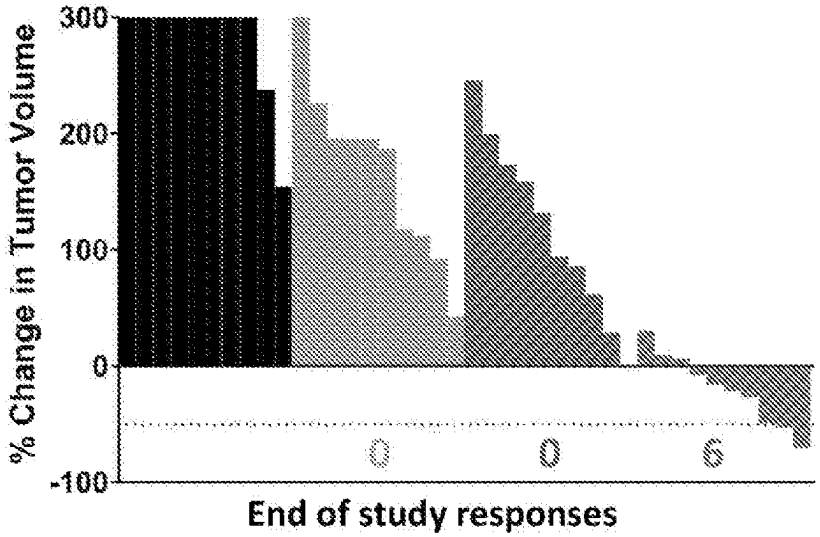

% Change in Tumor Volume
300
200
100
0
-100
0
0
6
End of study responses

FIG. 4A

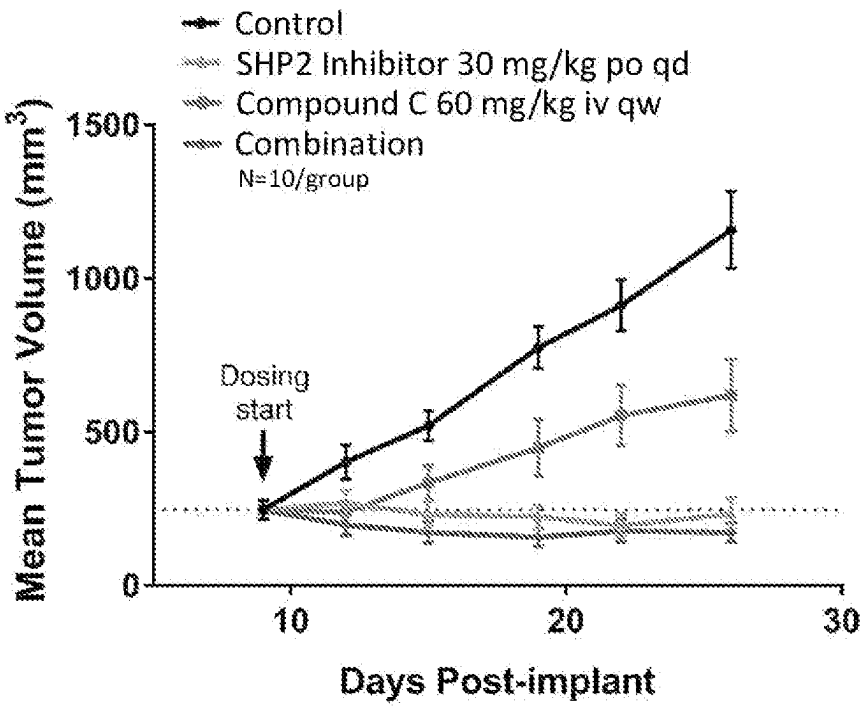

Control
SHP2 Inhibitor 30 mg/kg po qd
Compound C 60 mg/kg iv qw
Combination
N=10/group
Mean Tumor Volume (mm3)
1500
1000
500
0
Dosing start
10
20
30
Days Post-implant

FIG. 4B

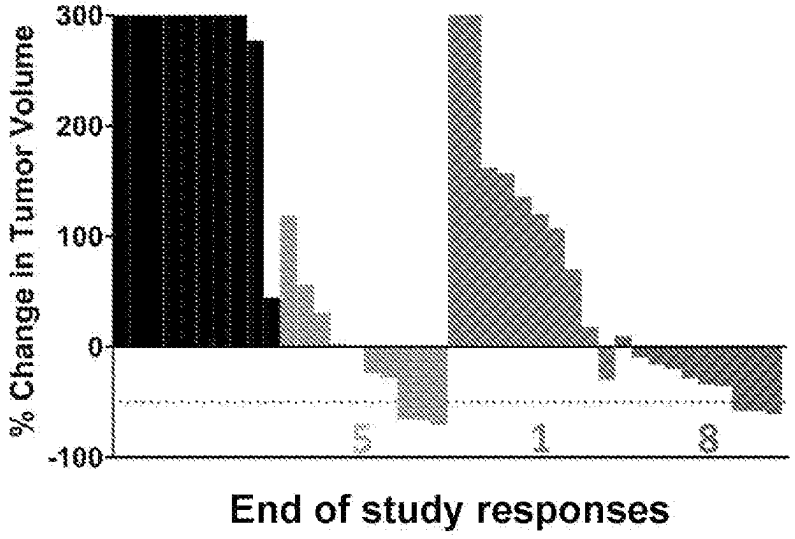
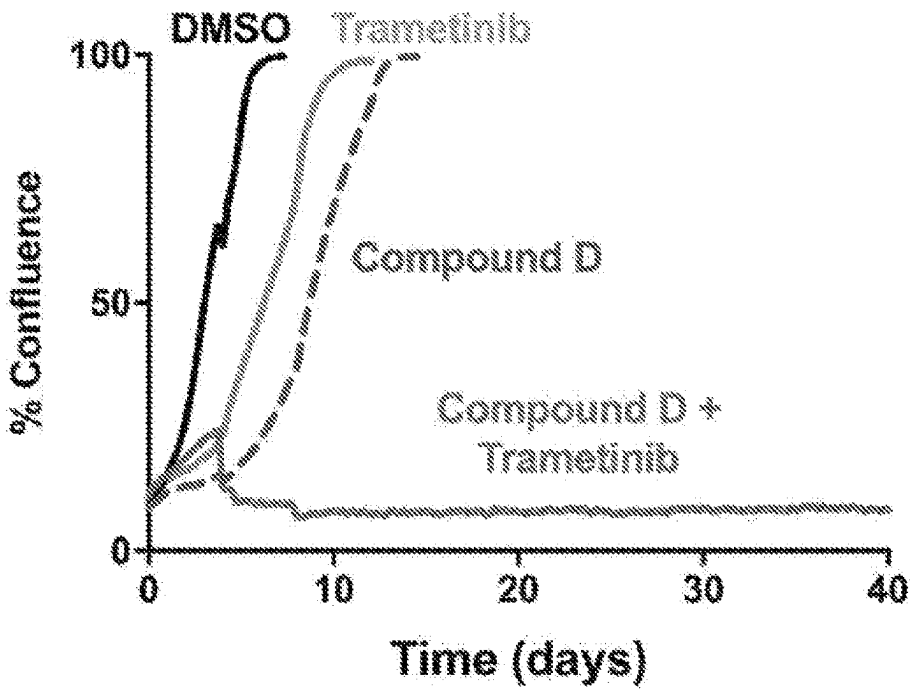

% Change in Tumor Volume
300
200
100
0
-100
5
1
8
End of study responses

FIG. 5


DMSO
Trametinib
Compound D
Compound D +
Trametinib
% Confluence
100
50
0
0
10
20
30
40
Time (days)

RAS INHIBITORS

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims the benefit of priority to U.S. Application No. 62/930,355, filed on Nov. 4, 2019; U.S. Application No. 62/951,652, filed on Dec. 20, 2019; U.S. Application No. 63/000,357, filed on Mar. 26, 2020; U.S. Application No. 63/011,636, filed on Apr. 17, 2020; and U.S. Application No. 63/043,588, filed on Jun. 24, 2020, all of which are hereby incorporated by reference in their entirety.

BACKGROUND

The vast majority of small molecule drugs act by binding a functionally important pocket on a target protein, thereby modulating the activity of that protein. For example, cholesterol-lowering drugs known as statins bind the enzyme active site of HMG-CoA reductase, thus preventing the enzyme from engaging with its substrates. The fact that many such drug/target interacting pairs are known may have misled some into believing that a small molecule modulator could be discovered for most, if not all, proteins provided a reasonable amount of time, effort, and resources. This is far from the case. Current estimates are that only about 10% of all human proteins are targetable by small molecules. Bojadzic and Buchwald, Curr Top Med Chem 18: 674-699 (2019). The other 90% are currently considered refractory or intractable toward above-mentioned small molecule drug discovery. Such targets are commonly referred to as "undruggable." These undruggable targets include a vast and largely untapped reservoir of medically important human proteins. Thus, there exists a great deal of interest in discovering new molecular modalities capable of modulating the function of such undruggable targets.

It has been well established in literature that Ras proteins (K-Ras, H-Ras and N-Ras) play an essential role in various human cancers and are therefore appropriate targets for anticancer therapy. Indeed, mutations in Ras proteins account for approximately 30% of all human cancers in the United States, many of which are fatal. Dysregulation of Ras proteins by activating mutations, overexpression or upstream activation is common in human tumors, and activating mutations in Ras are frequently found in human cancer. For example, activating mutations at codon 12 in Ras proteins function by inhibiting both GTPase-activating protein (GAP)—dependent and intrinsic hydrolysis rates of GTP, significantly skewing the population of Ras mutant proteins to the "on" (GTP-bound) state (Ras(ON)), leading to oncogenic MAPK signaling. Notably, Ras exhibits a picomolar affinity for GTP, enabling Ras to be activated even in the presence of low concentrations of this nucleotide. Mutations at codons 13 (e.g., G13D) and 61 (e.g., Q61K) of Ras are also responsible for oncogenic activity in some cancers.

Despite extensive drug discovery efforts against Ras during the last several decades, a drug directly targeting Ras is still not approved. Additional efforts are needed to uncover additional medicines for cancers driven by the various Ras mutations.

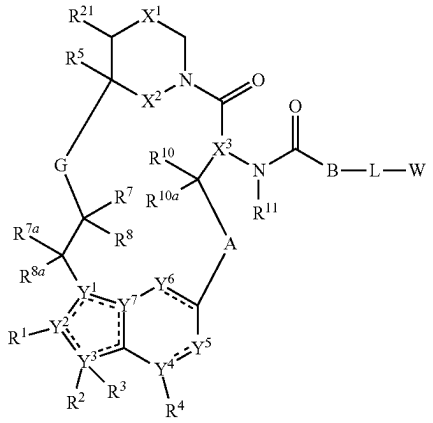
SUMMARY

Provided herein are Ras inhibitors. The approach described herein entails formation of a high affinity three-component complex, or conjugate, between a synthetic

ligand and two intracellular proteins which do not interact under normal physiological conditions: the target protein of interest (e.g., Ras), and a widely expressed cytosolic chaperone (presenter protein) in the cell (e.g., cyclophilin A). More specifically, in some embodiments, the inhibitors of Ras described herein induce a new binding pocket in Ras by driving formation of a high affinity tri-complex, or conjugate, between the Ras protein and the widely expressed cytosolic chaperone, cyclophilin A (CYPA). Without being bound by theory, the inventors believe that one way the inhibitory effect on Ras is effected by compounds of the invention and the complexes, or conjugates, they form is by steric occlusion of the interaction site between Ras and downstream effector molecules, such as RAF and PI3K, which are required for propagating the oncogenic signal.

As such, in some embodiments, the disclosure features a compound, or pharmaceutically acceptable salt thereof, of structural Formula I:

Formula I



wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is —N(H or CH_3)C(O)—(CH_2)— where the amino nitrogen is bound to the carbon atom of —CH(R^{10})—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 10-membered heteroarylene;

B is absent, —CH(R^9)—, >C=C$R^9R^{9'}$, or >C$R^9R^{9'}$ where the carbon is bound to the carbonyl carbon of —N(R^{11})C(O)—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, —C(O)O—CH(R^6)— where C is bound to —C(R^7R^8)—, —C(O)NH—CH(R^6)— where C is bound to —C(R^7R^8)—, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, a haloacetal, or an alkynyl sulfone;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or $S(O)_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R^1 is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 is CH, CH_2, or N;

Y^6 is C(O), CH, CH_2, or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl, or

R^1 and R^2 combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

R^2 is absent, hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form C═CR$^{7'}$R$^{8'}$; C═N(OH), C═N(O—C_1-C_3 alkyl), C═O, C═S, C═NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{7a} and R^{8a} are, independently, hydrogen, halo, optionally substituted C_1-C_3 alkyl, or combine with the carbon to which they are attached to form a carbonyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is H, F, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl; or

R^9 and L combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

$R^{9'}$ is hydrogen or optionally substituted C_1-C_6 alkyl; or

R^9 and $R^{9'}$, combined with the atoms to which they are attached, form a 3 to 6-membered cycloalkyl or a 3 to 6-membered heterocycloalkyl;

R^{10} is hydrogen, halo, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl;

R^{10a} is hydrogen or halo;

R^{11} is hydrogen or C_1-C_3 alkyl; and

R^{21} is hydrogen or C_1-C_3 alkyl (e.g., methyl).

Also provided are pharmaceutical compositions comprising a compound of Formula I, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient.

Further provided is a conjugate, or salt thereof, comprising the structure of Formula IV:

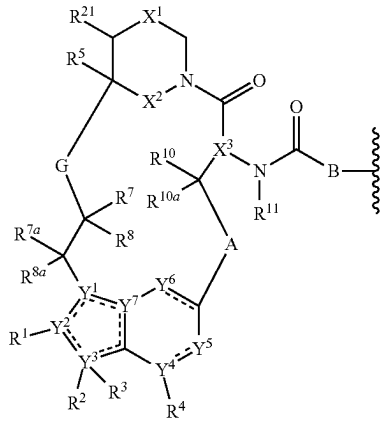
M-L-P Formula IV

wherein L is a linker;

P is a monovalent organic moiety; and

M has the structure of Formula V:

Formula V



wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is —N(H or CH_3)C(O)—(CH_2)— where the amino nitrogen is bound to the carbon atom of —CH(R^{10})—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is absent, —CH(R^9)—, >C═C$R^9R^{9'}$, or >C$R^9R^{9'}$ where the carbon is bound to the carbonyl carbon of —N(R^{11})C(O)—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to

6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, —C(O)O—CH(R^6)— where C is bound to —C(R^7R^8)—, —C(O)NH—CH(R^6)— where C is bound to —C(R^7R^8)—, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or $S(O)_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 is CH, CH_2, or N;

Y^6 is C(O), CH, CH_2, or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl, or

R^1 and R^2 combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

R^2 is absent, hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form C═C$R^{7'}R^{8'}$; C═N(OH), C═N(O—C_1-C_3 alkyl), C═O, C═S, C═NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{7a} and R^{8a} are, independently, hydrogen, halo, optionally substituted C_1-C_3 alkyl, or combine with the carbon to which they are attached to form a carbonyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is H, F, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl, or

R^9 and L combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

$R^{9'}$ is hydrogen or optionally substituted C_1-C_6 alkyl; or

R^9 and $R^{9'}$, combined with the atoms to which they are attached, form a 3 to 6-membered cycloalkyl or a 3 to 6-membered heterocycloalkyl;

R^{10} is hydrogen, halo, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl;

R^{10a} is hydrogen or halo;

R^{11} is hydrogen or C_1-C_3 alkyl; and

R^{21} is H or C_1-C_3 alkyl.

Also provided is a method of treating cancer in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a compound of the present invention, or a pharmaceutically acceptable salt thereof.

In some embodiments, a method is provided of treating a Ras protein-related disorder in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a compound of the present invention, or a pharmaceutically acceptable salt thereof.

Further provided is a method of inhibiting a Ras protein in a cell, the method comprising contacting the cell with an effective amount of a compound of the present invention, or a pharmaceutically acceptable salt thereof.

It is specifically contemplated that any limitation discussed with respect to one embodiment of the invention may apply to any other embodiment of the invention. Furthermore, any compound or composition of the invention may be used in any method of the invention, and any method of the invention may be used to produce or to utilize any compound or composition of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1**A and FIG. **1**B: These figures illustrate a matched pair analysis of potencies of certain compounds of the present invention (Formula BB) (points on the right) and corresponding compounds of Formula AA (points on the left) wherein a H is replaced with (S)Me in the context of two different cell-based assays. The y axes represent pERK EC50 (FIG. **1**A) or CTG IC50 (FIG. **1**B) as measured in an H358 cell line.

FIG. 2A and FIG. 2B: A compound of the present invention, Compound A, drove deep regressions in vivo in a NSCLC (KRAS G12C) xenograft model. Some animals exhibited complete responses (CR)=3 consecutive tumor measurements ≤30 mm3. FIG. 2A shows Compound A dosed at 100 mg/kg by daily oral gavage led to tumor regression in NCI-H358 KRASG12C xenograft model, which is a sensitive model to KRASG12C inhibition alone. The spaghetti titer plot (FIG. 2B) displaying individual tumor growth is shown next to the tumor volume plot (FIG. 2A).

FIG. 3A and FIG. 3B: A compound of the present invention, Compound B, drove tumor xenograft regressions in combination with a MEK inhibitor, cobimetinib, in a NSCLC (KRAS G12C) model. FIG. 3A shows the combination of intermittent intravenous administration of Compound B at 50 mg/kg plus daily oral administration of cobimetinib at 2.5 mg/kg drove tumor regression, whereas each single agent led to tumor growth inhibition. End of study responses were shown as waterfall plots (FIG. 3B), which indicate 6 out 10 mice had tumor regression in the combination group, whereas no tumor regressions recorded in each single agent group.

FIG. 4A and FIG. 4B: A compound of the present invention, Compound C, dosed weekly with daily SHP2 inhibitor, RMC-4550, drove xenograft regressions in a NSCLC (KRAS G12C) model. In FIG. 4A, the combinatorial activity of once weekly intravenous administration of Compound C at 60 mg/kg plus daily oral administration of SHP2 inhibitor at 30 mg/kg is shown. End of study responses in individual tumors were plotted as a waterfall plot (FIG. 4B).

FIG. 5: A compound of the present invention, Compound D, combined with a MEK inhibitor, trametinib, suppressed in vitro growth durably in a long-term cell growth NSCLC (KRAS G12C) model.

DEFINITIONS AND CHEMICAL TERMS

In this application, unless otherwise clear from context, (i) the term "a" means "one or more"; (ii) the term "or" is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternative are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or"; (iii) the terms "comprising" and "including" are understood to encompass itemized components or steps whether presented by themselves or together with one or more additional components or steps; and (iv) where ranges are provided, endpoints are included.

As used herein, the term "about" is used to indicate that a value includes the standard deviation of error for the device or method being employed to determine the value. In certain embodiments, the term "about" refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of a stated value, unless otherwise stated or otherwise evident from the context (e.g., where such number would exceed 100% of a possible value).

As used herein, the term "adjacent" in the context of describing adjacent atoms refers to bivalent atoms that are directly connected by a covalent bond.

A "compound of the present invention" and similar terms as used herein, whether explicitly noted or not, refers to Ras inhibitors described herein, including compounds of Formula I and subformula thereof, and compounds of Table 1 and Table 2, as well as salts (e.g., pharmaceutically acceptable salts), solvates, hydrates, stereoisomers (including atropisomers), and tautomers thereof.

The term "wild-type" refers to an entity having a structure or activity as found in nature in a "normal" (as contrasted with mutant, diseased, altered, etc) state or context. Those of ordinary skill in the art will appreciate that wild-type genes and polypeptides often exist in multiple different forms (e.g., alleles).

Those skilled in the art will appreciate that certain compounds described herein can exist in one or more different isomeric (e.g., stereoisomers, geometric isomers, atropisomers, tautomers) or isotopic (e.g., in which one or more atoms has been substituted with a different isotope of the atom, such as hydrogen substituted for deuterium) forms. Unless otherwise indicated or clear from context, a depicted structure can be understood to represent any such isomeric or isotopic form, individually or in combination.

Compounds described herein can be asymmetric (e.g., having one or more stereocenters). All stereoisomers, such as enantiomers and diastereomers, are intended unless otherwise indicated. Compounds of the present disclosure that contain asymmetrically substituted carbon atoms can be isolated in optically active or racemic forms. Methods on how to prepare optically active forms from optically active starting materials are known in the art, such as by resolution of racemic mixtures or by stereoselective synthesis. Many geometric isomers of olefins, C═N double bonds, and the like can also be present in the compounds described herein, and all such stable isomers are contemplated in the present disclosure. Cis and trans geometric isomers of the compounds of the present disclosure are described and may be isolated as a mixture of isomers or as separated isomeric forms.

In some embodiments, one or more compounds depicted herein may exist in different tautomeric forms. As will be clear from context, unless explicitly excluded, references to such compounds encompass all such tautomeric forms. In some embodiments, tautomeric forms result from the swapping of a single bond with an adjacent double bond and the concomitant migration of a proton. In certain embodiments, a tautomeric form may be a prototropic tautomer, which is an isomeric protonation states having the same empirical formula and total charge as a reference form. Examples of moieties with prototropic tautomeric forms are ketone-enol pairs, amide-imidic acid pairs, lactam-lactim pairs, amide-imidic acid pairs, enamine-imine pairs, and annular forms where a proton can occupy two or more positions of a heterocyclic system, such as, 1H- and 3H-imidazole, 1H-, 2H- and 4H-1,2,4-triazole, 1H- and 2H-isoindole, and 1H- and 2H-pyrazole. In some embodiments, tautomeric forms can be in equilibrium or sterically locked into one form by appropriate substitution. In certain embodiments, tautomeric forms result from acetal interconversion.

Unless otherwise stated, structures depicted herein are also meant to include compounds that differ only in the presence of one or more isotopically enriched atoms. Exemplary isotopes that can be incorporated into compounds of the present invention include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorus, sulfur, fluorine, chlorine, and iodine, such as ^{2}H, ^{3}H, ^{11}C, ^{13}C, ^{14}C, ^{13}N, ^{15}N, ^{15}O, ^{17}O, ^{18}O, ^{32}P, ^{33}P, ^{35}S, ^{18}F, ^{36}Cl, ^{123}I and ^{125}I. Isotopically-labeled compounds (e.g., those labeled with ^{3}H and ^{14}C) can be useful in compound or substrate tissue distribution assays. Tritiated (i.e., ^{3}H) and carbon-14 (i.e., ^{14}C) isotopes can be useful for their ease of preparation and detectability. Further, substitution with heavier isotopes such as deuterium (i.e., ^{2}H) may afford certain therapeutic advantages resulting

from greater metabolic stability (e.g., increased in vivo half-life or reduced dosage requirements). In some embodiments, one or more hydrogen atoms are replaced by ^{2}H or ^{3}H, or one or more carbon atoms are replaced by ^{13}C- or ^{14}C-enriched carbon. Positron emitting isotopes such as ^{15}O, ^{13}N, ^{11}C, and ^{18}F are useful for positron emission tomography (PET) studies to examine substrate receptor occupancy. Preparations of isotopically labelled compounds are known to those of skill in the art. For example, isotopically labeled compounds can generally be prepared by following procedures analogous to those disclosed for compounds of the present invention described herein, by substituting an isotopically labeled reagent for a non-isotopically labeled reagent.

As is known in the art, many chemical entities can adopt a variety of different solid forms such as, for example, amorphous forms or crystalline forms (e.g., polymorphs, hydrates, solvate). In some embodiments, compounds of the present invention may be utilized in any such form, including in any solid form. In some embodiments, compounds described or depicted herein may be provided or utilized in hydrate or solvate form.

At various places in the present specification, substituents of compounds of the present disclosure are disclosed in groups or in ranges. It is specifically intended that the present disclosure include each and every individual subcombination of the members of such groups and ranges. For example, the term "C_1-C_6 alkyl" is specifically intended to individually disclose methyl, ethyl, C_3 alkyl, C_4 alkyl, C_5 alkyl, and C alkyl. Furthermore, where a compound includes a plurality of positions at which substituents are disclosed in groups or in ranges, unless otherwise indicated, the present disclosure is intended to cover individual compounds and groups of compounds (e.g., genera and subgenera) containing each and every individual subcombination of members at each position.

The term "optionally substituted X" (e.g., "optionally substituted alkyl") is intended to be equivalent to "X, wherein X is optionally substituted" (e.g., "alkyl, wherein said alkyl is optionally substituted"). It is not intended to mean that the feature "X" (e.g., alkyl) per se is optional. As described herein, certain compounds of interest may contain one or more "optionally substituted" moieties. In general, the term "substituted", whether preceded by the term "optionally" or not, means that one or more hydrogens of the designated moiety are replaced with a suitable substituent, e.g., any of the substituents or groups described herein. Unless otherwise indicated, an "optionally substituted" group may have a suitable substituent at each substitutable position of the group, and when more than one position in any given structure may be substituted with more than one substituent selected from a specified group, the substituent may be either the same or different at every position. For example, in the term "optionally substituted C_1-C_6 alkyl-C_2-C_9 heteroaryl," the alkyl portion, the heteroaryl portion, or both, may be optionally substituted. Combinations of substituents envisioned by the present disclosure are preferably those that result in the formation of stable or chemically feasible compounds. The term "stable", as used herein, refers to compounds that are not substantially altered when subjected to conditions to allow for their production, detection, and, in certain embodiments, their recovery, purification, and use for one or more of the purposes disclosed herein.

Suitable monovalent substituents on a substitutable carbon atom of an "optionally substituted" group may be, independently, deuterium; halogen; —$(CH_2)_{0-4}R^{\circ}$; —$(CH_2)_{0-4}OR^{\circ}$; —$O(CH_2)_{0-4}R^{\circ}$; —O—$(CH_2)_{0-4}C(O)OR^{\circ}$; —$(CH_2)_{0-4}CH(OR^{\circ})_2$; —$(CH_2)_{0-4}SR^{\circ}$; —$(CH_2)_{0-4}Ph$, which may be substituted with R°; —$(CH_2)_{0-4}O(CH_2)_{0-1}Ph$ which may be substituted with R°; —CH═CHPh, which may be substituted with R°; —$(CH_2)_{0-4}O(CH_2)_{0-1}$-pyridyl which may be substituted with R°; 4-8 membered saturated or unsaturated heterocycloalkyl (e.g., pyridyl); 3-8 membered saturated or unsaturated cycloalkyl (e.g., cyclopropyl, cyclobutyl, or cyclopentyl); —NO_2; —CN; —N_3; —$(CH_2)_{0-4}N(R^{\circ})_2$; —$(CH_2)_{0-4}N(R^{\circ})C(O)R^{\circ}$; —$N(R^{\circ})C(S)R^{\circ}$; —$(CH_2)_{0-4}N(R^{\circ})C(O)NR^{\circ}_2$; —$N(R^{\circ})C(S)NR^{\circ}_2$; —$(CH_2)_{0-4}N(R^{\circ})C(O)OR^{\circ}$; —$N(R^{\circ})N(R^{\circ})C(O)R^{\circ}$; —$N(R^{\circ})N(R^{\circ})C(O)NR^{\circ}_2$; —$N(R^{\circ})N(R^{\circ})C(O)OR^{\circ}$; —$(CH_2)_{0-4}C(O)R^{\circ}$; —$C(S)R^{\circ}$; —$(CH_2)_{0-4}C(O)OR^{\circ}$; —$(CH_2)_{0-4}$—C(O)—$N(R^{\circ})_2$; —$(CH_2)_{0-4}$—C(O)—N($R^{\circ}$)—$S(O)_2$—$R^{\circ}$; —$C(NCN)NR^{\circ}_2$; —$(CH_2)_{0-4}C(O)SR^{\circ}$; —$(CH_2)_{0-4}C(O)OSiR^{\circ}_3$; —$(CH_2)_{0-4}OC(O)R^{\circ}$; —$OC(O)(CH_2)_{0-4}SR^{\circ}$; —SC(S)SR$^{\circ}$; —$(CH_2)_{0-4}SC(O)R^{\circ}$; —$(CH_2)_{0-4}C(O)NR^{\circ}_2$; —$C(S)NR^{\circ}_2$; —$C(S)SR^{\circ}$; —$(CH_2)_{0-4}OC(O)NR^{\circ}_2$; —C(O)N($OR^{\circ}$)$R^{\circ}$; —C(O)C(O)$R^{\circ}$; —$C(O)CH_2C(O)R^{\circ}$; —C($NOR^{\circ}$)$R^{\circ}$; —$(CH_2)_{0-4}SSR^{\circ}$; —$(CH_2)_{0-4}S(O)_2R^{\circ}$; —$(CH_2)_{0-4}S(O)_2OR^{\circ}$; —$(CH_2)_{0-4}OS(O)_2R^{\circ}$; —$S(O)_2NR^{\circ}_2$; —$(CH_2)_{0-4}S(O)R^{\circ}$; —N($R^{\circ}$)$S(O)_2NR^{\circ}_2$; —N($R^{\circ}$)$S(O)_2R^{\circ}$; —N($OR^{\circ}$)$R^{\circ}$; —C($NOR^{\circ}$)$NR^{\circ}_2$; —C(NH)$NR^{\circ}_2$; —$P(O)_2R^{\circ}$; —$P(O)R^{\circ}_2$; —$P(O)(OR^{\circ})_2$; —$OP(O)R^{\circ}_2$; —$OP(O)(OR^{\circ})_2$; —$OP(O)(OR^{\circ})R^{\circ}$, —$SiR^{\circ}_3$; —($C_{1-4}$ straight or branched alkylene)O—$N(R^{\circ})_2$; or —(C_{1-4} straight or branched alkylene)C(O)O—$N(R^{\circ})_2$, wherein each R° may be substituted as defined below and is independently hydrogen, —C_{1-6} aliphatic, —CH_2Ph, —$O(CH_2)_{0-1}Ph$, —CH_2-(5-6 membered heteroaryl ring), or a 3-6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or, notwithstanding the definition above, two independent occurrences of R°, taken together with their intervening atom(s), form a 3-12-membered saturated, partially unsaturated, or aryl mono- or bicyclic ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, which may be substituted as defined below.

Suitable monovalent substituents on R° (or the ring formed by taking two independent occurrences of R° together with their intervening atoms), may be, independently, halogen, —$(CH_2)_{0-2}R^{\bullet}$, -(halo$R^{\bullet}$), —$(CH_2)_{0-2}OH$, —$(CH_2)_{0-2}OR^{\bullet}$, —$(CH_2)_{0-2}CH(OR^{\bullet})_2$; —O(halo$R^{\bullet}$), —CN, —$N_3$, —$(CH_2)_{0-2}C(O)R^{\bullet}$, —$(CH_2)_{0-2}C(O)OH$, —$(CH_2)_{0-2}C(O)OR^{\bullet}$, —$(CH_2)_{0-2}SR^{\bullet}$, —$(CH_2)_{0-2}SH$, —$(CH_2)_{0-2}NH_2$, —$(CH_2)_{0-2}NHR^{\bullet}$, —$(CH_2)_{0-2}NR^{\bullet}_2$, —$NO_2$, —$SiR^{\bullet}_3$, —$OSiR^{\bullet}_3$, —$C(O)SR^{\bullet}$, —($C_{1-4}$ straight or branched alkylene)$C(O)OR^{\bullet}$, or —$SSR^{\bullet}$ wherein each $R^{\bullet}$ is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently selected from C_{1-4} aliphatic, —CH_2Ph, —$O(CH_2)_{0-1}Ph$, or a 5-6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Suitable divalent substituents on a saturated carbon atom of R° include ═O and ═S.

Suitable divalent substituents on a saturated carbon atom of an "optionally substituted" group include the following: ═O, ═S, ═NNR^{*}_2, ═NNHC(O)R^{*}, ═NNHC(O)OR^{*}, ═$NNHS(O)_2R^{*}$, ═NR^{*}, ═NOR^{*}, —$O(C(R^{*}_2))_{2-3}O$—, or —$S(C(R^{*}_2))_{2-3}S$—, wherein each independent occurrence of R^{*} is selected from hydrogen, C_{1-6} aliphatic which may be substituted as defined below, or an unsubstituted 5-6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Suitable divalent substituents that are

bound to vicinal substitutable carbons of an "optionally substituted" group include: —O(CR^*_2)$_{2-3}$O—, wherein each independent occurrence of R* is selected from hydrogen, C_{1-6} aliphatic which may be substituted as defined below, or an unsubstituted 5-6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

Suitable substituents on the aliphatic group of $R^•$ include halogen, —$R^•$, -(halo$R^•$), —OH, —$OR^•$, —O(halo$R^•$), —CN, —C(O)OH, —C(O)$OR^•$, —NH_2, —$NHR^•$, —$NR^•_2$, or —NO_2, wherein each $R^•$ is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently C_{1-4} aliphatic, —CH_2Ph, —O(CH_2)$_{0-1}$Ph, or a 5-6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

Suitable substituents on a substitutable nitrogen of an "optionally substituted" group include —$R^†$, —$NR^†_2$, —C(O)$R^†$, —C(O)$OR^†$, —C(O)C(O)$R^†$, —C(O)CH_2C(O)$R^†$, —S(O)$_2R^†$, —S(O)$_2NR^†_2$, —C(S)$NR^†_2$, —C(NH)$NR^†_2$, or —N($R^†$)S(O)$_2R^†$; wherein each $R^†$ is independently hydrogen, C_{1-6} aliphatic which may be substituted as defined below, unsubstituted —OPh, or an unsubstituted 3-6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur, or, notwithstanding the definition above, two independent occurrences of $R^†$, taken together with their intervening atom(s) form an unsubstituted 3-12-membered saturated, partially unsaturated, or aryl mono- or bicyclic ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur.

Suitable substituents on an aliphatic group of $R^†$ are independently halogen, —$R^•$, -(halo$R^•$), —OH, —$OR^•$, —O(halo$R^•$), —CN, —C(O)OH, —C(O)$OR^•$, —NH_2, —$NHR^•$, —$NR^•_2$, or —NO_2, wherein each $R^•$ is unsubstituted or where preceded by "halo" is substituted only with one or more halogens, and is independently C_{1-4} aliphatic, —CH_2Ph, —O(CH_2)$_{0-1}$Ph, or a 5-6-membered saturated, partially unsaturated, or aryl ring having 0-4 heteroatoms independently selected from nitrogen, oxygen, or sulfur. Suitable divalent substituents on a saturated carbon atom of $R^†$ include ═O and ═S.

The term "acetyl," as used herein, refers to the group —C(O)CH_3.

The term "alkoxy," as used herein, refers to a —O—C_1-C_{20} alkyl group, wherein the alkoxy group is attached to the remainder of the compound through an oxygen atom.

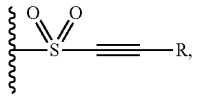
The term "alkyl," as used herein, refers to a saturated, straight or branched monovalent hydrocarbon group containing from 1 to 20 (e.g., from 1 to 10 or from 1 to 6) carbons. In some embodiments, an alkyl group is unbranched (i.e., is linear); in some embodiments, an alkyl group is branched. Alkyl groups are exemplified by, but not limited to, methyl, ethyl, n- and iso-propyl, n-, sec-, iso- and tert-butyl, and neopentyl.

The term "alkylene," as used herein, represents a saturated divalent hydrocarbon group derived from a straight or branched chain saturated hydrocarbon by the removal of two hydrogen atoms, and is exemplified by methylene, ethylene, isopropylene, and the like. The term "Cr-Cy alkylene" represents alkylene groups having between x and y carbons. Exemplary values for x are 1, 2, 3, 4, 5, and 6, and exemplary values for y are 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, or 20 (e.g., C_1-C_6, C_1-C_{10}, C_2-C_{20}, C_2-C_6, C_2-C_{10}, or C_2-C_{20} alkylene). In some embodiments, the alkylene can be further substituted with 1, 2, 3, or 4 substituent groups as defined herein.

The term "alkenyl," as used herein, represents monovalent straight or branched chain groups of, unless otherwise specified, from 2 to 20 carbons (e.g., from 2 to 6 or from 2 to 10 carbons) containing one or more carbon-carbon double bonds and is exemplified by ethenyl, 1-propenyl, 2-propenyl, 2-methyl-1-propenyl, 1-butenyl, and 2-butenyl. Alkenyls include both cis and trans isomers. The term "alkenylene," as used herein, represents a divalent straight or branched chain groups of, unless otherwise specified, from 2 to 20 carbons (e.g., from 2 to 6 or from 2 to 10 carbons) containing one or more carbon-carbon double bonds.

The term "alkynyl," as used herein, represents monovalent straight or branched chain groups from 2 to 20 carbon atoms (e.g., from 2 to 4, from 2 to 6, or from 2 to 10 carbons) containing a carbon-carbon triple bond and is exemplified by ethynyl, and 1-propynyl.

The term "alkynyl sulfone," as used herein, represents a group comprising the structure



wherein R is any chemically feasible substituent described herein.

The term "amino," as used herein, represents —N($R^•$)$_2$, e.g., —NH_2 and —N(CH_3)$_2$.

The term "aminoalkyl," as used herein, represents an alkyl moiety substituted on one or more carbon atoms with one or more amino moieties.

The term "amino acid," as described herein, refers to a molecule having a side chain, an amino group, and an acid group (e.g., —CO_2H or —SO_3H), wherein the amino acid is attached to the parent molecular group by the side chain, amino group, or acid group (e.g., the side chain). As used herein, the term "amino acid" in its broadest sense, refers to any compound or substance that can be incorporated into a polypeptide chain, e.g., through formation of one or more peptide bonds. In some embodiments, an amino acid has the general structure H_2N—C(H)(R)—COOH. In some embodiments, an amino acid is a naturally-occurring amino acid. In some embodiments, an amino acid is a synthetic amino acid; in some embodiments, an amino acid is a D-amino acid; in some embodiments, an amino acid is an L-amino acid. "Standard amino acid" refers to any of the twenty standard L-amino acids commonly found in naturally occurring peptides. Exemplary amino acids include alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, optionally substituted hydroxylnorvaline, isoleucine, leucine, lysine, methionine, norvaline, ornithine, phenylalanine, proline, pyrrolysine, selenocysteine, serine, taurine, threonine, tryptophan, tyrosine, and valine.

The term "aryl," as used herein, represents a monovalent monocyclic, bicyclic, or multicyclic ring system formed by carbon atoms, wherein the ring attached to the pendant group is aromatic. Examples of aryl groups are phenyl, naphthyl, phenanthrenyl, and anthracenyl. An aryl ring can be attached to its pendant group at any heteroatom or carbon ring atom that results in a stable structure and any of the ring atoms can be optionally substituted unless otherwise specified.

The term "C_0," as used herein, represents a bond. For example, part of the term —N(C(O)—(C_0-C_5 alkylene-H)—

includes —N(C(O)—(C_0 alkylene-H)—, which is also represented by —N(C(O)—H)—.

The terms "carbocyclic" and "carbocyclyl," as used herein, refer to a monovalent, optionally substituted C_3-C_{12} monocyclic, bicyclic, or tricyclic ring structure, which may be bridged, fused or spirocyclic, in which all the rings are formed by carbon atoms and at least one ring is non-aromatic. Carbocyclic structures include cycloalkyl, cycloalkenyl, and cycloalkynyl groups. Examples of carbocyclyl groups are cyclohexyl, cyclohexenyl, cyclooctynyl, 1,2-dihydronaphthyl, 1,2,3,4-tetrahydronaphthyl, fluorenyl, indenyl, indanyl, decalinyl, and the like. A carbocyclic ring can be attached to its pendant group at any ring atom that results in a stable structure and any of the ring atoms can be optionally substituted unless otherwise specified.

The term "carbonyl," as used herein, represents a C(O) group, which can also be represented as C═0.

The term "carboxyl," as used herein, means —CO_2H, (C═O)(OH), COOH, or C(O)OH or the unprotonated counterparts.

The term "cyano," as used herein, represents a —CN group.

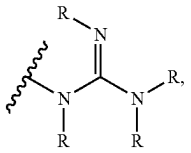
The term "cycloalkyl," as used herein, represents a monovalent saturated cyclic hydrocarbon group, which may be bridged, fused or spirocyclic having from three to eight ring carbons, unless otherwise specified, and is exemplified by cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and cycloheptyl.

The term "cycloalkenyl," as used herein, represents a monovalent, non-aromatic, saturated cyclic hydrocarbon group, which may be bridged, fused or spirocyclic having from three to eight ring carbons, unless otherwise specified, and containing one or more carbon-carbon double bonds.

The term "diastereomer," as used herein, means stereoisomers that are not mirror images of one another and are non-superimposable on one another.

The term "enantiomer," as used herein, means each individual optically active form of a compound of the invention, having an optical purity or enantiomeric excess (as determined by methods standard in the art) of at least 80% (i.e., at least 90% of one enantiomer and at most 10% of the other enantiomer), preferably at least 90% and more preferably at least 98%.

The term "guanidinyl," refers to a group having the structure:



wherein each R is, independently, any chemically feasible substituent described herein.

The term "guanidinoalkyl alkyl," as used herein, represents an alkyl moiety substituted on one or more carbon atoms with one or more guanidinyl moieties.

The term "haloacetyl," as used herein, refers to an acetyl group wherein at least one of the hydrogens has been replaced by a halogen.

The term "haloalkyl," as used herein, represents an alkyl moiety substituted on one or more carbon atoms with one or more of the same of different halogen moieties.

The term "halogen," as used herein, represents a halogen selected from bromine, chlorine, iodine, or fluorine.

The term "heteroalkyl" as used herein, refers to an "alkyl" group, as defined herein, in which at least one carbon atom has been replaced with a heteroatom (e.g., an O, N, or S atom). The heteroatom may appear in the middle or at the end of the radical.

The term "heteroaryl," as used herein, represents a monovalent, monocyclic or polycyclic ring structure that contains at least one fully aromatic ring: i.e., they contain 4n+2 pi electrons within the monocyclic or polycyclic ring system and contains at least one ring heteroatom selected from N, O, or S in that aromatic ring. Exemplary unsubstituted heteroaryl groups are of 1 to 12 (e.g., 1 to 11, 1 to 10, 1 to 9, 2 to 12, 2 to 11, 2 to 10, or 2 to 9) carbons. The term "heteroaryl" includes bicyclic, tricyclic, and tetracyclic groups in which any of the above heteroaromatic rings is fused to one or more, aryl or carbocyclic rings, e.g., a phenyl ring, or a cyclohexane ring. Examples of heteroaryl groups include, but are not limited to, pyridyl, pyrazolyl, benzooxazolyl, benzoimidazolyl, benzothiazolyl, imidazolyl, thiazolyl, quinolinyl, tetrahydroquinolinyl, and 4-azaindolyl. A heteroaryl ring can be attached to its pendant group at any ring atom that results in a stable structure and any of the ring atoms can be optionally substituted unless otherwise specified. In some embodiment, the heteroaryl is substituted with 1, 2, 3, or 4 substituents groups.

The term "heterocycloalkyl," as used herein, represents a monovalent monocyclic, bicyclic or polycyclic ring system, which may be bridged, fused or spirocyclic, wherein at least one ring is non-aromatic and wherein the non-aromatic ring contains one, two, three, or four heteroatoms independently selected from the group consisting of nitrogen, oxygen, and sulfur. The 5-membered ring has zero to two double bonds, and the 6- and 7-membered rings have zero to three double bonds. Exemplary unsubstituted heterocycloalkyl groups are of 1 to 12 (e.g., 1 to 11, 1 to 10, 1 to 9, 2 to 12, 2 to 11, 2 to 10, or 2 to 9) carbons. The term "heterocycloalkyl" also represents a heterocyclic compound having a bridged multicyclic structure in which one or more carbons or heteroatoms bridges two non-adjacent members of a monocyclic ring, e.g., a quinuclidinyl group. The term "heterocycloalkyl" includes bicyclic, tricyclic, and tetracyclic groups in which any of the above heterocyclic rings is fused to one or more aromatic, carbocyclic, heteroaromatic, or heterocyclic rings, e.g., an aryl ring, a cyclohexane ring, a cyclohexene ring, a cyclopentane ring, a cyclopentene ring, a pyridine ring, or a pyrrolidine ring. Examples of heterocycloalkyl groups are pyrrolidinyl, piperidinyl, 1,2,3,4-tetrahydroquinolinyl, decahydroquinolinyl, dihydropyrrolopyridine, and decahydronapthyridinyl. A heterocycloalkyl ring can be attached to its pendant group at any ring atom that results in a stable structure and any of the ring atoms can be optionally substituted unless otherwise specified.

The term "hydroxy," as used herein, represents a —OH group.

The term "hydroxyalkyl," as used herein, represents an alkyl moiety substituted on one or more carbon atoms with one or more —OH moieties.

The term "isomer," as used herein, means any tautomer, stereoisomer, atropiosmer, enantiomer, or diastereomer of any compound of the invention. It is recognized that the compounds of the invention can have one or more chiral centers or double bonds and, therefore, exist as stereoisomers, such as double-bond isomers (i.e., geometric E/Z isomers) or diastereomers (e.g., enantiomers (i.e., (+) or (−)) or cis/trans isomers). According to the invention, the chemical structures depicted herein, and therefore the compounds of the invention, encompass all the corresponding stereoi-

somers, that is, both the stereomerically pure form (e.g., geometrically pure, enantiomerically pure, or diastereomerically pure) and enantiomeric and stereoisomeric mixtures, e.g., racemates. Enantiomeric and stereoisomeric mixtures of compounds of the invention can typically be resolved into their component enantiomers or stereoisomers by well-known methods, such as chiral-phase gas chromatography, chiral-phase high performance liquid chromatography, crystallizing the compound as a chiral salt complex, or crystallizing the compound in a chiral solvent. Enantiomers and stereoisomers can also be obtained from stereomerically or enantiomerically pure intermediates, reagents, and catalysts by well-known asymmetric synthetic methods.

As used herein, the term "linker" refers to a divalent organic moiety connecting moiety B to moiety W in a compound of Formula I, such that the resulting compound is capable of achieving an IC50 of 2 uM or less in the Ras-RAF disruption assay protocol provided in the Examples below, and provided here:

The purpose of this biochemical assay is to measure the ability of test compounds to facilitate ternary complex formation between a nucleotide-loaded Ras isoform and cyclophilin A; the resulting ternary complex disrupts binding to a $BRAF^{RBD}$ construct, inhibiting Ras signaling through a RAF effector.

In assay buffer containing 25 mM HEPES pH 7.3, 0.002% Tween20, 0.1% BSA, 100 mM NaCl and 5 mM $MgCl_2$, tagless Cyclophilin A, His6-K-Ras-GMPPNP (or other Ras variant), and GST-$BRAF^{RBD}$ are combined in a 384-well assay plate at final concentrations of 25 μM, 12.5 nM and 50 nM, respectively. Compound is present in plate wells as a 10-point 3-fold dilution series starting at a final concentration of 30 μM. After incubation at 25° C. for 3 hours, a mixture of Anti-His Eu—W1024 and anti-GST allophycocyanin is then added to assay sample wells at final concentrations of 10 nM and 50 nM, respectively, and the reaction incubated for an additional 1.5 hours. TR-FRET signal is read on a microplate reader (Ex 320 nm, Em 665/615 nm). Compounds that facilitate disruption of a Ras:RAF complex are identified as those eliciting a decrease in the TR-FRET ratio relative to DMSO control wells.

In some embodiments, the linker comprises 20 or fewer linear atoms. In some embodiments, the linker comprises 15 or fewer linear atoms. In some embodiments, the linker comprises 10 or fewer linear atoms. In some embodiments, the linker has a molecular weight of under 500 g/mol. In some embodiments, the linker has a molecular weight of under 400 g/mol. In some embodiments, the linker has a molecular weight of under 300 g/mol. In some embodiments, the linker has a molecular weight of under 200 g/mol. In some embodiments, the linker has a molecular weight of under 100 g/mol. In some embodiments, the linker has a molecular weight of under 50 g/mol.

As used herein, a "monovalent organic moiety" is less than 500 kDa. In some embodiments, a "monovalent organic moiety" is less than 400 kDa. In some embodiments, a "monovalent organic moiety" is less than 300 kDa. In some embodiments, a "monovalent organic moiety" is less than 200 kDa. In some embodiments, a "monovalent organic moiety" is less than 100 kDa. In some embodiments, a "monovalent organic moiety" is less than 50 kDa. In some embodiments, a "monovalent organic moiety" is less than 25 kDa. In some embodiments, a "monovalent organic moiety" is less than 20 kDa. In some embodiments, a "monovalent organic moiety" is less than 15 kDa. In some embodiments, a "monovalent organic moiety" is less than 10 kDa. In some embodiments, a "monovalent organic moiety" is less than 1 kDa. In some embodiments, a "monovalent organic moiety" is less than 500 g/mol. In some embodiments, a "monovalent organic moiety" ranges between 500 g/mol and 500 kDa.

The term "stereoisomer," as used herein, refers to all possible different isomeric as well as conformational forms which a compound may possess (e.g., a compound of any formula described herein), in particular all possible stereochemically and conformationally isomeric forms, all diastereomers, enantiomers or conformers of the basic molecular structure, including atropisomers. Some compounds of the present invention may exist in different tautomeric forms, all of the latter being included within the scope of the present invention.

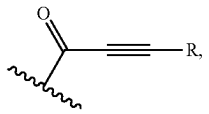
The term "sulfonyl," as used herein, represents an —$S(O)_2$— group.

The term "thiocarbonyl," as used herein, refers to a —C(S)— group.

The term "vinyl ketone," as used herein, refers to a group comprising a carbonyl group directly connected to a carbon-carbon double bond.

The term "vinyl sulfone," as used herein, refers to a group comprising a sulfonyl group directed connected to a carbon-carbon double bond.

The term "ynone," as used herein, refers to a group comprising the structure,



wherein R is any chemically feasible substituent described herein.

Those of ordinary skill in the art, reading the present disclosure, will appreciate that certain compounds described herein may be provided or utilized in any of a variety of forms such as, for example, salt forms, protected forms, pro-drug forms, ester forms, isomeric forms (e.g., optical or structural isomers), isotopic forms, etc. In some embodiments, reference to a particular compound may relate to a specific form of that compound. In some embodiments, reference to a particular compound may relate to that compound in any form. In some embodiments, for example, a preparation of a single stereoisomer of a compound may be considered to be a different form of the compound than a racemic mixture of the compound; a particular salt of a compound may be considered to be a different form from another salt form of the compound; a preparation containing one conformational isomer ((Z) or (E)) of a double bond may be considered to be a different form from one containing the other conformational isomer ((E) or (Z)) of the double bond; a preparation in which one or more atoms is a different isotope than is present in a reference preparation may be considered to be a different form.

DETAILED DESCRIPTION

Compounds

Provided herein are Ras inhibitors. The approach described herein entails formation of a high affinity three-component complex, or conjugate, between a synthetic ligand and two intracellular proteins which do not interact under normal physiological conditions: the target protein of interest (e.g., Ras), and a widely expressed cytosolic chaperone (presenter protein) in the cell (e.g., cyclophilin A).

More specifically, in some embodiments, the inhibitors of Ras described herein induce a new binding pocket in Ras by driving formation of a high affinity tri-complex, or conjugate, between the Ras protein and the widely expressed cytosolic chaperone, cyclophilin A (CYPA). Without being bound by theory, the inventors believe that one way the inhibitory effect on Ras is effected by compounds of the invention and the complexes, or conjugates, they form is by steric occlusion of the interaction site between Ras and downstream effector molecules, such as RAF, which are required for propagating the oncogenic signal.

Without being bound by theory, the inventors postulate that both covalent and non-covalent interactions of a compound of the present invention with Ras and the chaperone protein (e.g., cyclophilin A) may contribute to the inhibition of Ras activity. In some embodiments, a compound of the present invention forms a covalent adduct with a side chain of a Ras protein (e.g., a sulfhydryl side chain of the cysteine at position 12 or 13 of a mutant Ras protein). Covalent adducts may also be formed with other side chains of Ras. In addition, or alternatively, non-covalent interactions may be at play: for example, van der Waals, hydrophobic, hydrophilic and hydrogen bond interactions, and combinations thereof, may contribute to the ability of the compounds of the present invention to form complexes and act as Ras inhibitors. Accordingly, a variety of Ras proteins may be inhibited by compounds of the present invention (e.g., K-Ras, N-Ras, H-Ras, and mutants thereof at positions 12, 13 and 61, such as G12C, G12D, G12V, G12S, G13C, G13D, and Q61L, and others described herein).

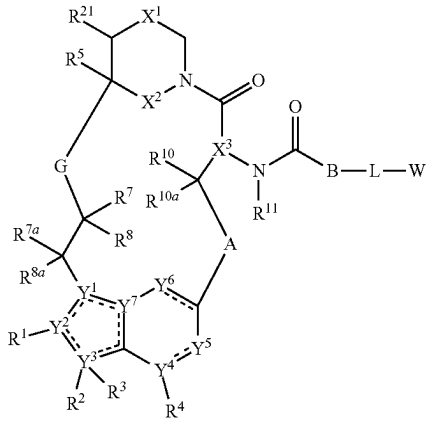
Methods of determining covalent adduct formation are known in the art. One method of determining covalent adduct formation is to perform a "cross-linking" assay, such as under these conditions (Note—the following protocol describes a procedure for monitoring cross-linking of K-Ras G12C (GMP-PNP) to a compound of the invention. This protocol may also be executed substituting other Ras proteins or nucleotides).

The purpose of this biochemical assay is to measure the ability of test compounds to covalently label nucleotide-loaded K-Ras isoforms. In assay buffer containing 12.5 mM HEPES pH 7.4, 75 mM NaCl, 1 mM $MgCl_2$, 1 mM BME, 5 μM Cyclophilin A and 2 μM test compound, a 5 μM stock of GMP-PNP-loaded K-Ras (1-169) G12C is diluted 10-fold to yield a final concentration of 0.5 μM; with final sample volume being 100 μL.

The sample is incubated at 25° C. for a time period of up to 24 hours prior to quenching by the addition of 10 μL of 5% Formic Acid. Quenched samples are centrifuged at 15000 rpm for 15 minutes in a benchtop centrifuge before injecting a 10 μL aliquot onto a reverse phase C_4 column and eluting into the mass spectrometer with an increasing acetonitrile gradient in the mobile phase. Analysis of raw data may be carried out using Waters MassLynx MS software, with % bound calculated from the deconvoluted protein peaks for labeled and unlabeled K-Ras.

Accordingly, provided herein is a compound, or pharmaceutically acceptable salt thereof, having the structure of Formula I:

Formula I



wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is —N(H or CH_3)C(O)—(CH_2)— where the amino nitrogen is bound to the carbon atom of —CH(R^{10})—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 10-membered heteroarylene;

B is absent, —CH(R^9)—, >C=C$R^9R^{9'}$, or >C$R^9R^{9'}$ where the carbon is bound to the carbonyl carbon of —N(R^{11})C(O)—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, —C(O)O—CH(R^6)— where C is bound to —C(R^7R^8)—, —C(O)NH—CH(R^6)— where C is bound to —C(R^7R^8)—, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, a haloacetal, or an alkynyl sulfone;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or $S(O)_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 is CH, CH_2, or N;

Y^6 is C(O), CH, CH_2, or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6

to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl, or

R^1 and R^2 combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

R^2 is absent, hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C{=}CR^{7'}R^{8'}$; C═N(OH), C═N(O—C_1-C_3 alkyl), C═O, C═S, C═NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{7a} and R^{8a} are, independently, hydrogen, halo, optionally substituted C_1-C_3 alkyl, or combine with the carbon to which they are attached to form a carbonyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is H, F, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl, or

R^9 and L combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

$R^{9'}$ is hydrogen or optionally substituted C_1-C_6 alkyl; or

R^9 and $R^{9'}$, combined with the atoms to which they are attached, form a 3 to 6-membered cycloalkyl or a 3 to 6-membered heterocycloalkyl;

R^{10} is hydrogen, halo, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl;

R^{10a} is hydrogen or halo;

R^{11} is hydrogen or C_1-C_3 alkyl; and

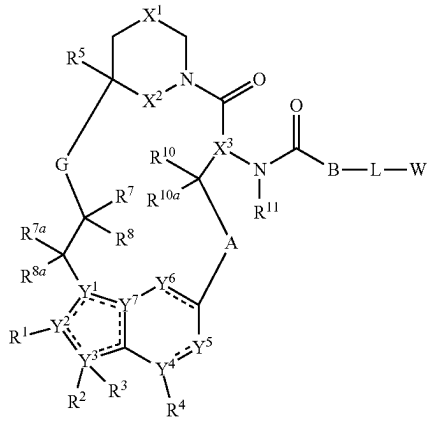
R^{21} is hydrogen or C_1-C_3 alkyl (e.g., methyl).

In some embodiments, R^9 is H, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl.

In some embodiments, R^{21} is hydrogen.

In some embodiments, provided herein is a compound, or pharmaceutically acceptable salt thereof, having the structure of Formula Ia:

Formula Ia



wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is —N(H or CH_3)C(O)—(CH_2)— where the amino nitrogen is bound to the carbon atom of —CH(R^{10})—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 10-membered heteroarylene;

B is —CH(R^9)— or >C═C$R^9R^{9'}$ where the carbon is bound to the carbonyl carbon of —N(R^{11})C(O)—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, —C(O)O—CH(R^6)— where C is bound to —C(R^7R^8)—, —C(O)NH—CH(R^6)— where C is bound to —C(R^7R^8)—, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, a haloacetal, or an alkynyl sulfone;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or $S(O)_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 is CH, CH_2, or N;

Y^6 is C(O), CH, CH_2, or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl, or

R^1 and R^2 combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

R^2 is absent, hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C{=}CR^{7'}R^{8'}$; C═N(OH), C═N(O—C_1-C_3 alkyl), C═O, C═S, C═NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{7a} and R^{8a} are, independently, hydrogen, halo, optionally substituted C_1-C_3 alkyl, or combine with the carbon to which they are attached to form a carbonyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl, or

R^9 and L combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

$R^{9'}$ is hydrogen or optionally substituted C_1-C_6 alkyl;

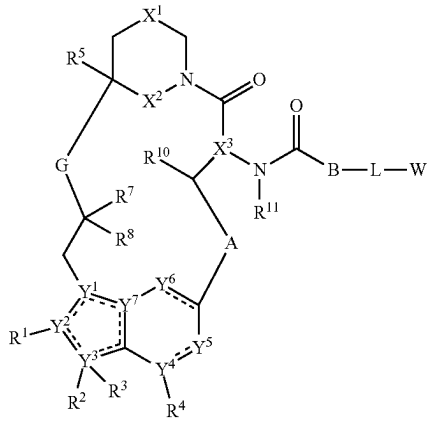
R^{10} is hydrogen, halo, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl;

R^{10a} is hydrogen or halo; and

R^{11} is hydrogen or C_1-C_3 alkyl.

In some embodiments, the disclosure features a compound, or pharmaceutically acceptable salt thereof, of structural Formula Ib:

Formula Ib



wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is —N(H or CH_3)C(O)—(CH_2)— where the amino nitrogen is bound to the carbon atom of —CH(R^{10})—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is —CH(R^9)— where the carbon is bound to the carbonyl carbon of —N(R^{11})C(O)—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, —C(O)O—CH(R^6)— where C is bound to —C(R^7R^8)—, —C(O)NH—CH(R^6)— where C is bound to —C(R^7R^8)—, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, a haloacetal, or an alkynyl sulfone;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or $S(O)_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substi-

tuted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 and Y^6 are, independently, CH or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

R^2 is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form C═CR$^{7'}$R$^{8'}$; C═N(OH), C═N(O—C_1-C_3 alkyl), C═O, C═S, C═NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

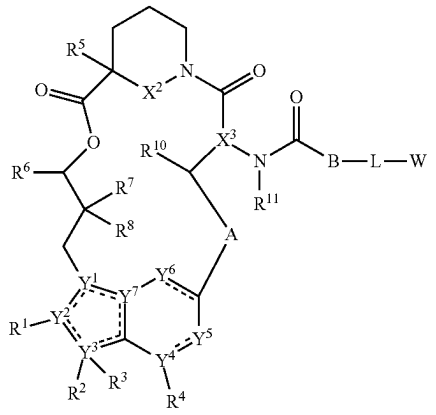
R^{10} is hydrogen, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl; and

R^{11} is hydrogen or C_1-C_3 alkyl.

In some embodiments of compounds of the present invention, G is optionally substituted C_1-C_4 heteroalkylene.

In some embodiments, a compound having the structure of Formula Ic is provided, or a pharmaceutically acceptable salt thereof:

Formula Ic



wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is —N(H or CH_3)C(O)—(CH_2)— where the amino nitrogen is bound to the carbon atom of —CH(R^{10})—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is —CH(R^9)— where the carbon is bound to the carbonyl carbon of —N(R^{11})C(O)—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, or an alkynyl sulfone;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 and Y^6 are, independently, CH or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

R^2 is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally

substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C{=}CR^{7'}R^{8'}$; C═N(OH), C═N(O—C_1-C_3 alkyl), C═O, C═S, C═NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

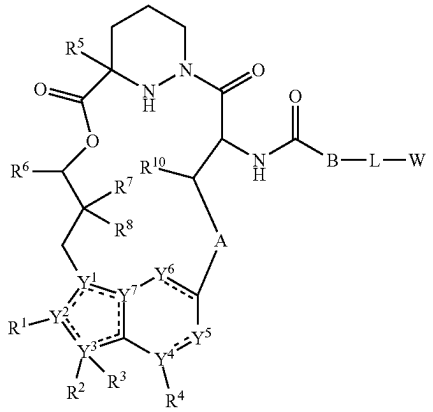
R^{10} is hydrogen, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl; and

R^{11} is hydrogen or C_1-C_3 alkyl.

In some embodiments of compounds of the present invention, X^2 is NH. In some embodiments, X^3 is CH. In some embodiments, R^{11} is hydrogen. In some embodiments, R^{11} is C_1-C_3 alkyl. In some embodiments, R^{11} is methyl.

In some embodiments, a compound of the present invention has the structure of Formula Id, or a pharmaceutically acceptable salt thereof:

Formula Id



wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is —N(H or CH_3)C(O)—(CH_2)— where the amino nitrogen is bound to the carbon atom of —CH(R^{10})—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is —CH(R^9)— where the carbon is bound to the carbonyl carbon of —NHC(O)—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, or an alkynyl sulfone;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 and Y^6 are, independently, CH or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

R^2 is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C{=}CR^{7'}R^{8'}$; C═N(OH), C═N(O—C_1-C_3 alkyl), C═O, C═S, C═NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl; and

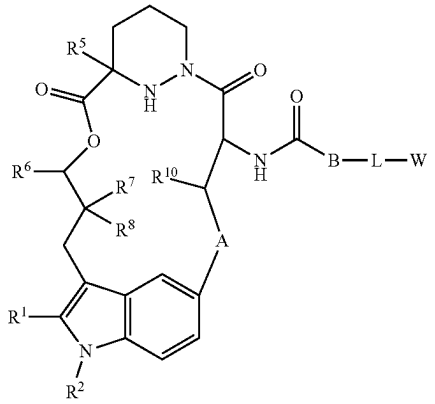
R^{10} is hydrogen, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl.

In some embodiments of a compound of the present invention, X^1 is optionally substituted C_1-C_2 alkylene. In some embodiments, X^1 is methylene. In some embodiments, X^1 is methylene substituted with a C_1-C_6 alkyl group or a halogen. In some embodiments, X^1 is —CH(Br)—. In some embodiments, X^1 is —CH(CH_3)—. In some embodiments, R^5 is hydrogen. In some embodiments, R^5 is C_1-C_4 alkyl optionally substituted with halogen. In some embodiments, R^5 is methyl. In some embodiments, Y^4 is C. In some embodiments, R^4 is hydrogen. In some embodiments, Y^5 is CH.

In some embodiments, Y^6 is CH. In some embodiments, Y^1 is C. In some embodiments, Y^2 is C. In some embodiments, Y^3 is N. In some embodiments, R^3 is absent. In some embodiments, Y^7 is C.

In some embodiments, a compound of the present invention has the structure of Formula Ie, or a pharmaceutically acceptable salt thereof:

Formula Ie



wherein A is —N(H or CH_3)C(O)—(CH_2)— where the amino nitrogen is bound to the carbon atom of —CH(R^{10})—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is —CH(R^9)— where the carbon is bound to the carbonyl carbon of —NHC(O)—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, or an alkynyl sulfone;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

R^2 is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to

14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form C═$CR^{7'}R^{8'}$; C═N(OH), C═N(O—C_1-C_3 alkyl), C═O, C═S, C═NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

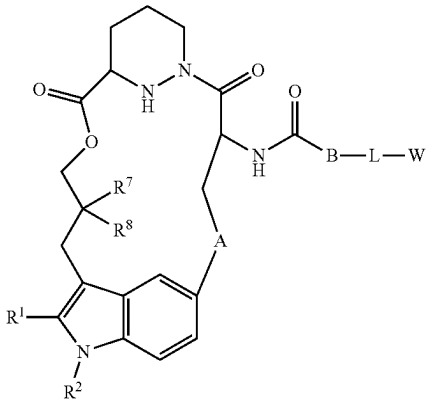
R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl; and

R^{10} is hydrogen, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl.

In some embodiments of a compound of the present invention, R^6 is hydrogen. In some embodiments, R^2 is hydrogen, cyano, optionally substituted C_1-C_6 alkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 6-membered heterocycloalkyl. In some embodiments, R^2 is optionally substituted C_1-C_6 alkyl. In some embodiments, R_2 is fluoroalkyl. In some embodiments, R^2 is ethyl. In some embodiments, R_2 is —CH_2CF_3. In some embodiments, R_2 is C_2-C_6 alkynyl. In some embodiments, R_2 is —CHC≡CH. In some embodiments, R_2 is —$CH_2C{\equiv}CCH_3$. In some embodiments, R^7 is optionally substituted C_1-C_3 alkyl. In some embodiments, R^7 is C_1-C_3 alkyl. In some embodiments, R^8 is optionally substituted C_1-C_3 alkyl. In some embodiments, R^8 is C_1-C_3 alkyl.

In some embodiments, a compound of the present invention has the structure of Formula If, or a pharmaceutically acceptable salt thereof:

Formula If



wherein A is —N(H or CH_3)C(O)—(CH_2)— where the amino nitrogen is bound to the carbon atom of —CH(R^{10})—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is —CH(R^9)— where the carbon is bound to the carbonyl carbon of —NHC(O)—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, or an alkynyl sulfone;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

R^2 is C_1-C_6 alkyl or 3 to 6-membered cycloalkyl;

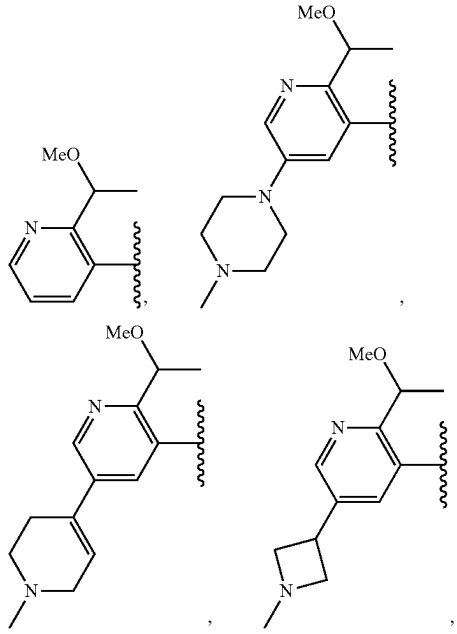
R^7 is C_1-C_3 alkyl;

R^8 is C_1-C_3 alkyl; and

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl.

In some embodiments of a compound of the present invention, R^1 is optionally substituted 6 to 10-membered aryl, optionally substituted 3 to 6-membered cycloalkenyl, or optionally substituted 5 to 10-membered heteroaryl. In some embodiments, R^1 is optionally substituted 6-membered aryl, optionally substituted 6-membered cycloalkenyl, or optionally substituted 6-membered heteroaryl.

In some embodiments of a compound of the present invention, R_1 is



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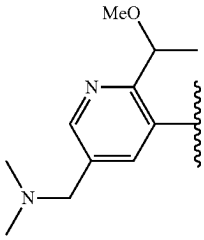
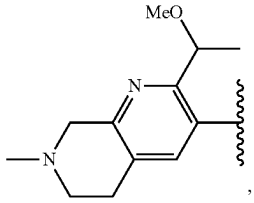
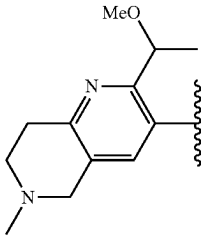
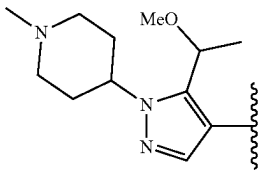
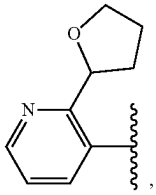






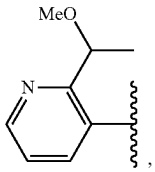


or

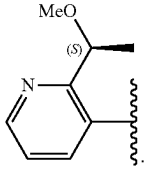


or a stereoisomer (e.g., atropisomer) thereof.

In some embodiments of a compound of the present invention, R_1 is

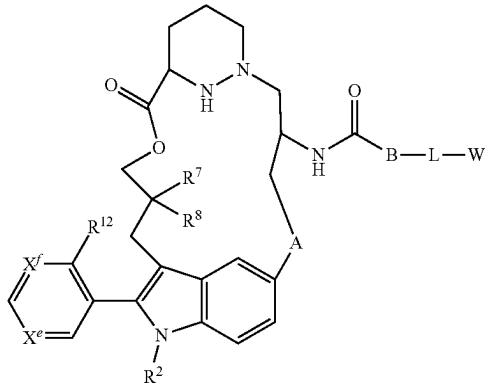


or a stereoisomer (e.g., atropisomer) thereof. In some embodiments of a compound of the present invention, R_1 is



In some embodiments, a compound of the present invention has the structure of Formula Ig, or a pharmaceutically acceptable salt thereof:

Formula Ig



wherein A is —N(H or CH_3)C(O)—(CH_2)— where the amino nitrogen is bound to the carbon atom of —CH(R^{10})—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is —CH(R^9)— where the carbon is bound to the carbonyl carbon of —NHC(O)—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, or an alkynyl sulfone;

R^2 is C_1-C_6 alkyl, C_1-C_6 fluoroalkyl, or 3 to 6-membered cycloalkyl;

R^7 is C_1-C_3 alkyl;

R^8 is C_1-C_3 alkyl; and

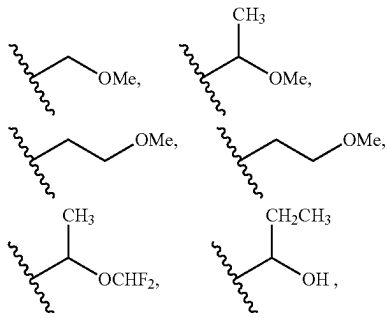
R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl

X^e and X^f are, independently, N or CH; and

R^{12} is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, or optionally substituted 3 to 6-membered heterocycloalkylene.

In some embodiments of a compound of the present invention, X^e is N and X^f is CH. In some embodiments, X^e is CH and X^f is N.

In some embodiments of a compound of the present invention, R^{12} is optionally substituted C_1-C_6 heteroalkyl. In some embodiments, R^{12} is



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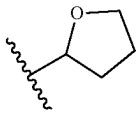
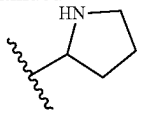
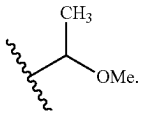


, or



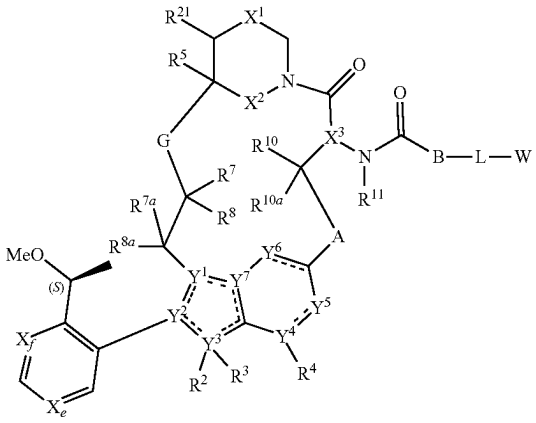
.

In some embodiments, R^{12}



In some embodiments, a compound of the present invention has the structure of Formula VI, or a pharmaceutically acceptable salt thereof:

Formula VI



wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is —N(H or CH_3)C(O)—(CH_2)— where the amino nitrogen is bound to the carbon atom of —CH(R^{10})—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene (e.g., phenyl or phenol), or optionally substituted 5 to 10-membered heteroarylene;

B is absent, —CH(R^9)—, >C═C$R^9R^{9'}$, or >C$R^9R^{9'}$ where the carbon is bound to the carbonyl carbon of —N(R^{11})C(O)—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, —C(O)O—CH(R^6)— where C is bound to —C(R^7R^8)—, —C(O)NH—CH(R^6)— where C is bound to —C(R^7R^8)—, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, a haloacetal, or an alkynyl sulfone;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or $S(O)_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 is CH, CH_2, or N;

Y^6 is C(O), CH, CH_2, or N;

R^2 is absent, hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form C═C$R^{7'}R^{8'}$; C═N(OH), C═N(O—C_1-C_3 alkyl), C═O, C═S, C═NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{7a} and R^{8a} are, independently, hydrogen, halo, optionally substituted C_1-C_3 alkyl, or combine with the carbon to which they are attached to form a carbonyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is H, F, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl; or

R^9 and L combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

$R^{9'}$ is hydrogen or optionally substituted C_1-C_6 alkyl; or

R^9 and $R^{9'}$, combined with the atoms to which they are attached, form a 3 to 6-membered cycloalkyl or a 3 to 6-membered heterocycloalkyl;

R^{10} is hydrogen, halo, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl;

R^{10a} is hydrogen or halo;

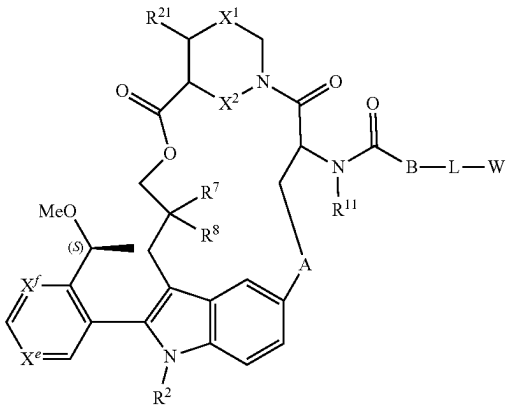
R^{11} is hydrogen or C_1-C_3 alkyl;

R^{21} is hydrogen or C_1-C_3 alkyl (e.g., methyl); and

X^e and X^f are, independently, N or CH.

In some embodiments, a compound of the present invention has the structure of Formula VIa, or a pharmaceutically acceptable salt thereof:

Formula VIa



wherein A optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene (e.g., phenyl or phenol), or optionally substituted 5 to 6-membered heteroarylene;

B is —CH(R^9)— where the carbon is bound to the carbonyl carbon of —NHC(O)—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, or an alkynyl sulfone;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or $S(O)_n$;

X^2 is O or NH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

R^2 is C_1-C_6 alkyl, C_1-C_6 fluoroalkyl, or 3 to 6-membered cycloalkyl;

R^7 is C_1-C_3 alkyl;

R^8 is C_1-C_3 alkyl; and

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

X^e and X^f are, independently, N or CH;

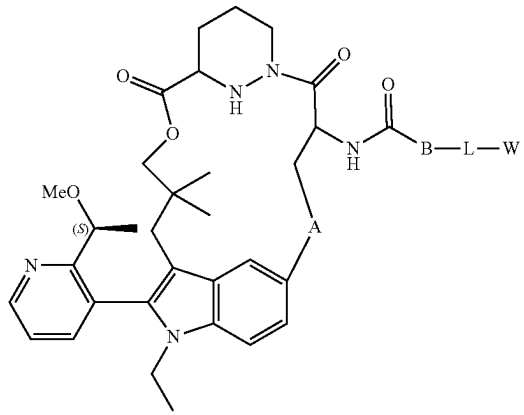
R^{11} is hydrogen or C_1-C_3 alkyl; and

R^{21} is hydrogen or C_1-C_3 alkyl.

In some embodiments of a compound of the present invention, X^e is N and X^f is CH. In some embodiments, X^e is CH and X^f is N.

In some embodiments, a compound of the present invention has the structure of Formula VIb, or a pharmaceutically acceptable salt thereof:

Formula VIb



wherein A optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene (e.g., phenyl or phenol), or optionally substituted 5 to 6-membered heteroarylene;

B is —CH(R^9)— where the carbon is bound to the carbonyl carbon of —NHC(O)—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

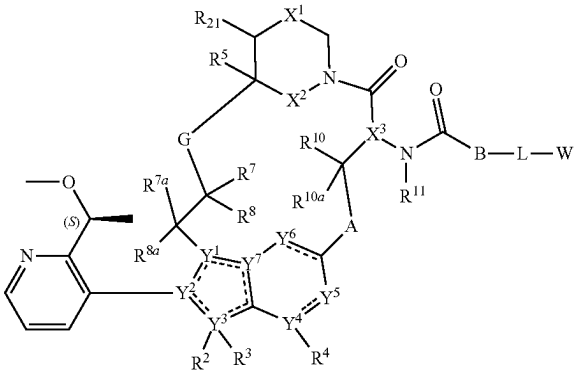
R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

L is absent or a linker; and

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, or an alkynyl sulfone. In some embodiments of a compound of the present invention, A is optionally substituted 6-membered arylene.

In some embodiments, a compound of the present invention has the structure of Formula VIc (corresponding for Formula BB of FIG. **1**A and FIG. **1**B), or a pharmaceutically acceptable salt thereof:

Formula VIc



wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is —N(H or CH_3)C(O)—(CH_2)— where the amino nitrogen is bound to the carbon atom of —CH(R^{10})—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene (e.g., phenyl or phenol), or optionally substituted 5 to 10-membered heteroarylene;

B is absent, —CH(R^9)—, >C=C$R^9R^{9'}$, or >C$R^9R^{9'}$ where the carbon is bound to the carbonyl carbon of —N(R^{11})C(O)—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, —C(O)O—CH(R^6)— where C is bound to —C(R^7R^8)—, —C(O)NH—CH(R^6)— where C is bound to —C(R^7R^8)—, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, a haloacetal, or an alkynyl sulfone;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or $S(O)_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 is CH, CH_2, or N;

Y^6 is C(O), CH, CH_2, or N;

R^2 is absent, hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form C=C$R^{7'}R^{8'}$; C=N(OH), C=N(O—C_1-C_3 alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{7a} and R^{8a} are, independently, hydrogen, halo, optionally substituted C_1-C_3 alkyl, or combine with the carbon to which they are attached to form a carbonyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is H, F, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl; or

R^9 and L combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

$R^{9'}$ is hydrogen or optionally substituted C_1-C_6 alkyl; or

R^9 and $R^{9'}$, combined with the atoms to which they are attached, form a 3 to 6-membered cycloalkyl or a 3 to 6-membered heterocycloalkyl;

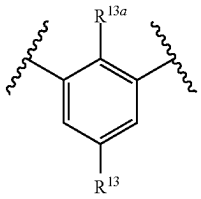
R^{10} is hydrogen, halo, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl;

R^{10a} is hydrogen or halo;

R^{11} is hydrogen or C_1-C_3 alkyl; and

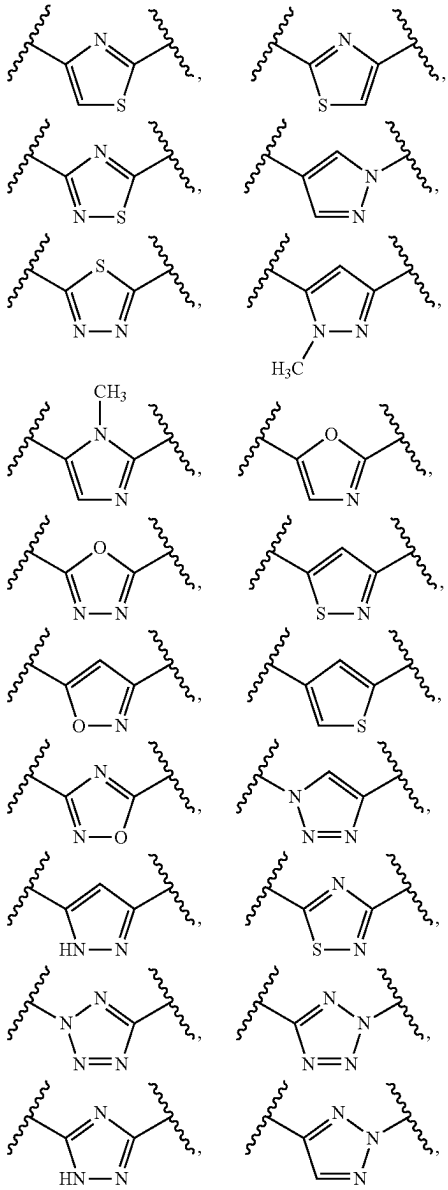
R^{21} is hydrogen or C_1-C_3 alkyl (e.g., methyl).

In some embodiments, A has the structure:



wherein R^{13} is hydrogen, halo, hydroxy, amino, optionally substituted C_1-C_6 alkyl, or optionally substituted C_1-C_6 heteroalkyl; and R^{13a} is hydrogen or halo. In some embodiments, R^{13} is hydrogen. In some embodiments, R^{13} and R^{13a} are each hydrogen. In some embodiments, R^{13} is hydroxy, methyl, fluoro, or difluoromethyl.

In some embodiments, A is optionally substituted 5 to 6-membered heteroarylene. In some embodiments, A is:



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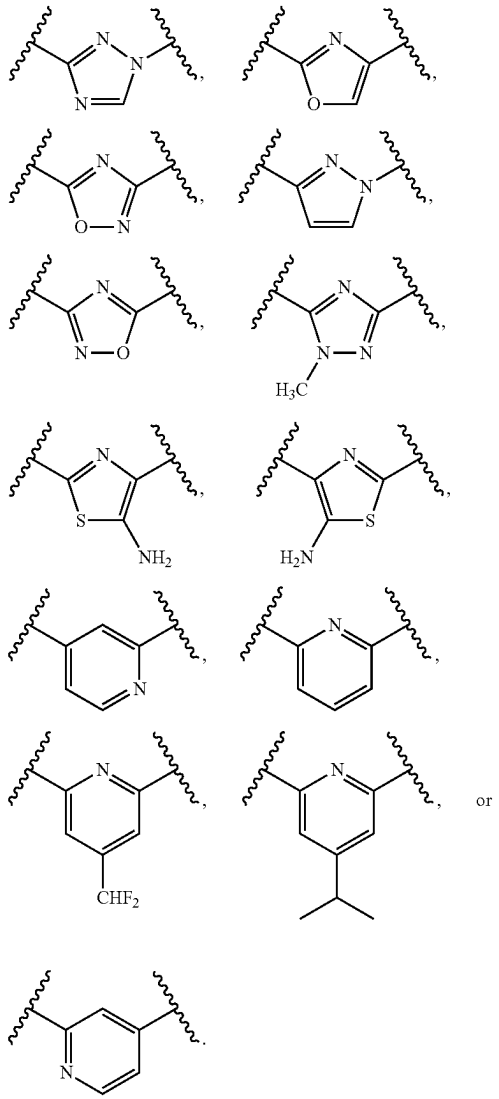
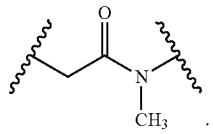
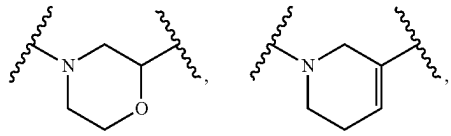


or

In some embodiments, A is optionally substituted C_1-C_4 heteroalkylene. In some embodiments, A is:



In some embodiments, A is optionally substituted 3 to 6-membered heterocycloalkylene. In some embodiments, A is:



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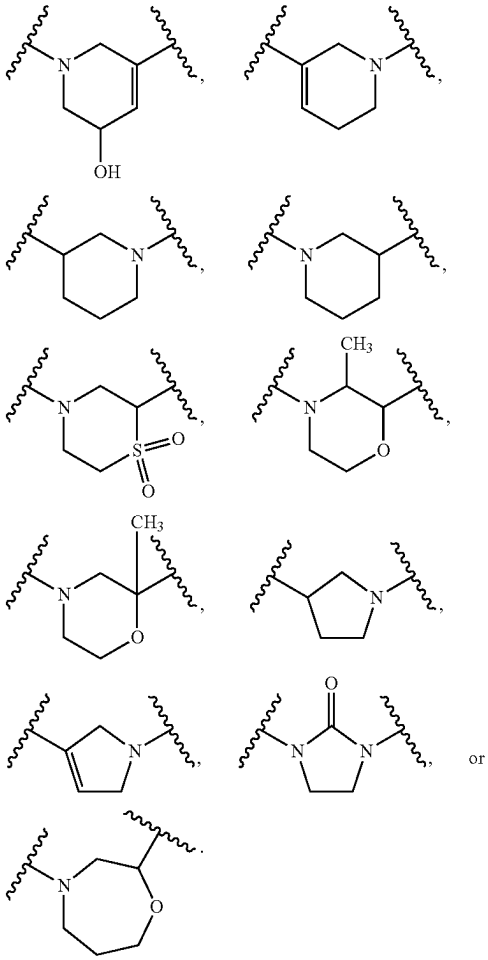
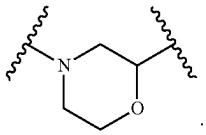
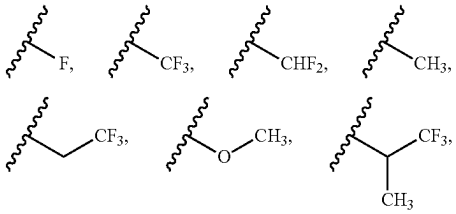


In some embodiments, A is



In some embodiments of a compound of the present invention, B is —CHR^9—. In some embodiments, R^9 is H, F, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl. In some embodiments, R^9 is:



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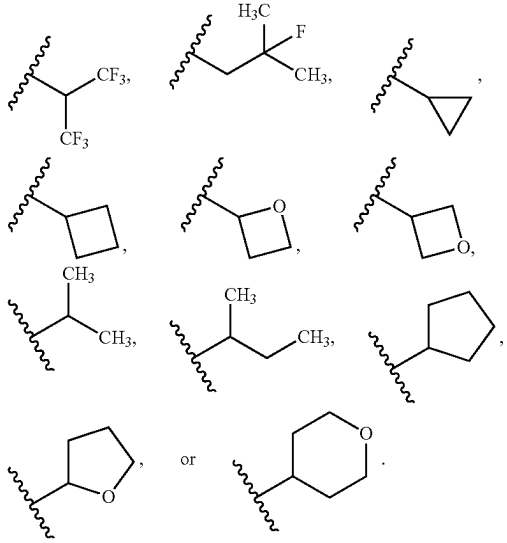
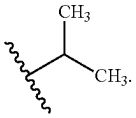
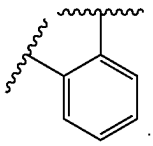


In some embodiments, R^9 is:



In some embodiments, R^9 is H, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl.

In some embodiments of a compound of the present invention, B is optionally substituted 6-membered arylene. In some embodiments, B is 6-membered arylene. In some embodiments, B is:



In some embodiments of a compound of the present invention, R^7 is methyl.

In some embodiments of a compound of the present invention, R^8 is methyl.

In some embodiments, R^{21} is hydrogen.

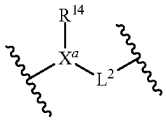
In some embodiments of a compound of the present invention, the linker is the structure of Formula II:

$$A^1\text{-}(B^1)_f\text{—}(C^1)_g\text{—}(B^2)_h\text{-}(D^1)\text{-}(B^3)_i\text{—}(C^2)_j\text{—}(B^4)_k\text{-}A^2 \qquad \text{Formula II}$$

where A^1 is a bond between the linker and B; A^2 is a bond between W and the linker; B^1, B^2, B^3, and B^4 each, independently, is selected from optionally substituted C_1-C_2 alkylene, optionally substituted C_1-C_3 heteroalkylene, O, S, and NR^N; R^N is hydrogen, optionally substituted C_{1-4} alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted C_1-C_7 heteroalkyl; C^1 and C^2

are each, independently, selected from carbonyl, thiocarbonyl, sulphonyl, or phosphoryl; f, g, h, i, j, and k are each, independently, 0 or 1; and D^1 is optionally substituted C_1-C_{10} alkylene, optionally substituted C_2-C_{10} alkenylene, optionally substituted C_2-C_{10} alkynylene, optionally substituted 3 to 14-membered heterocycloalkylene, optionally substituted 5 to 10-membered heteroarylene, optionally substituted 3 to 8-membered cycloalkylene, optionally substituted 6 to 10-membered arylene, optionally substituted C_2-C_{10} polyethylene glycolene, or optionally substituted C_1-C_{10} heteroalkylene, or a chemical bond linking A^1-$(B^1)_f$—$(C^1)_g$—$(B^2)_h$— to —$(B^3)_i$—$(C^2)_j$—$(B^4)_k$-A^2. In some embodiments, the linker is acyclic. In some embodiments, linker has the structure of Formula IIa:

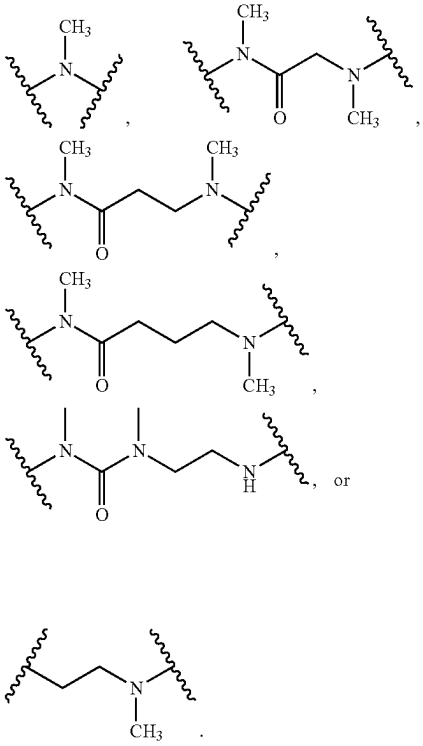
Formula IIa



wherein X^a is absent or N;

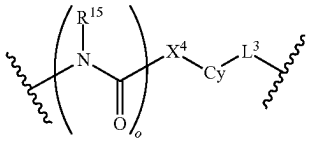
R^{14} is absent, hydrogen or optionally substituted C_1-C_6 alkyl; and

L^2 is absent, —SO_2—, optionally substituted C_1-C_4 alkylene or optionally substituted C_1-C_4 heteroalkylene, wherein at least one of X^a, R^{14}, or L^2 is present. In some embodiments, the linker has the structure:



In some embodiments, the linker is or comprises a cyclic moiety. In some embodiments, the linker has the structure of Formula IIb:

Formula IIb



wherein o is 0 or 1;

R^{15} is hydrogen or optionally substituted C_1-C_6 alkyl, optionally substituted 3 to 8-membered cycloalkylene, or optionally substituted 3 to 8-membered heterocycloalkylene;

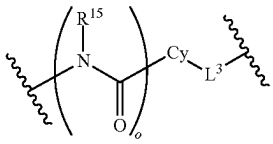
X^4 is absent, optionally substituted C_1-C_4 alkylene, O, NCH_3, or optionally substituted C_1-C_4 heteroalkylene;

Cy is optionally substituted 3 to 8-membered cycloalkylene, optionally substituted 3 to 8-membered heterocycloalkylene, optionally substituted 6-10 membered arylene, or optionally substituted 5 to 10-membered heteroarylene; and

L^3 is absent, —SO_2—, optionally substituted C_1-C_4 alkylene or optionally substituted C_1-C_4 heteroalkylene.

In some embodiments, the linker has the structure of Formula IIb-1:

Formula IIb-1



wherein o is 0 or 1;

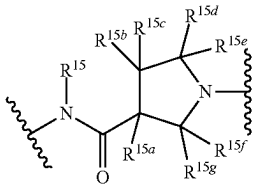
R^{15} is hydrogen or optionally substituted C_1-C_6 alkyl, optionally substituted 3 to 8-membered cycloalkylene, or optionally substituted 3 to 8-membered heterocycloalkylene;

Cy is optionally substituted 3 to 8-membered cycloalkylene, optionally substituted 3 to 8-membered heterocycloalkylene, optionally substituted 6-10 membered arylene, or optionally substituted 5 to 10-membered heteroarylene; and

L^3 is absent, —SO_2—, optionally substituted C_1-C_4 alkylene or optionally substituted C_1-C_4 heteroalkylene.

In some embodiments, the linker has the structure of Formula IIc:

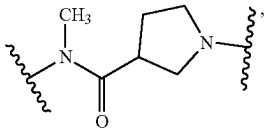
Formula IIc

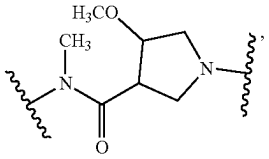


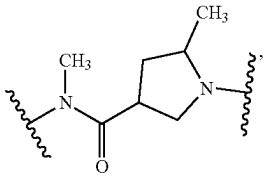
wherein R^{15} is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted 3 to 8-membered cycloalkylene, or optionally substituted 3 to 8-membered heterocycloalkylene; and

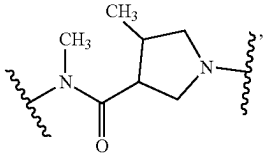
R^{15a}, R^{15b}, R^{15c}, R^{15d}, R^{15e}, R^{15f}, and R^{15g} are, independently, hydrogen, halo, hydroxy, cyano, amino, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 alkoxy, or, or R^{15b} and R^{15d} combine with the carbons to which they are attached to form an optionally substituted 3 to 8-membered cycloalkylene, or optionally substituted 3 to 8-membered heterocycloalkylene.

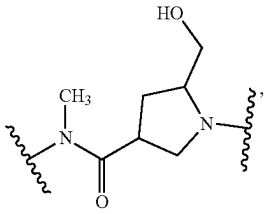
In some embodiments, the linker has the structure:

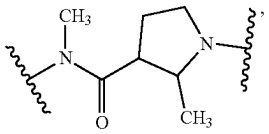


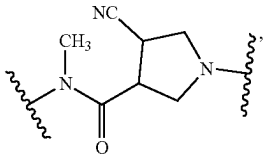


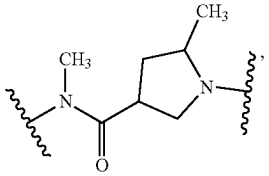


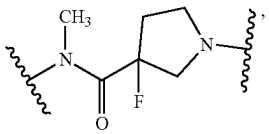












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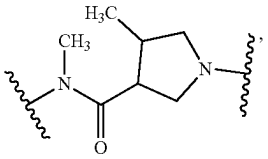

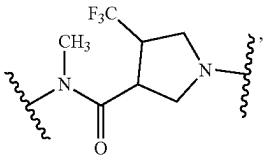

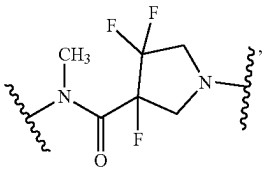

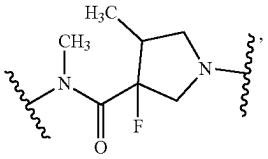

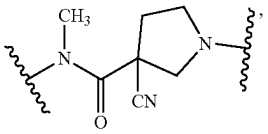

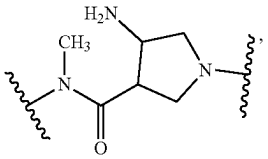

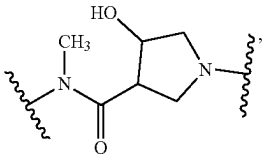

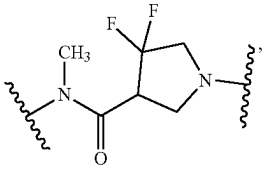

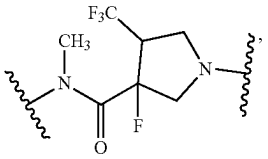

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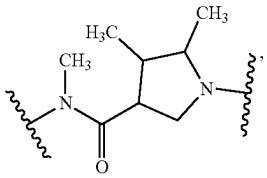

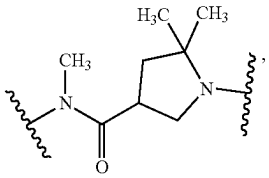

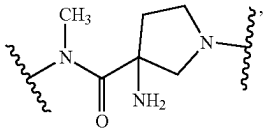

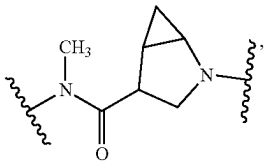

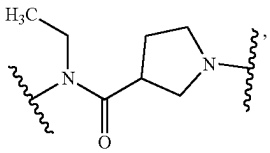

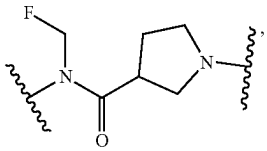

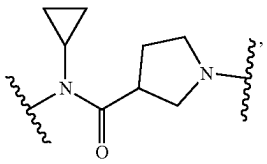

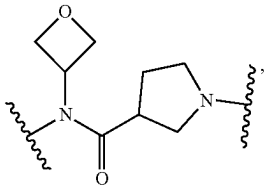

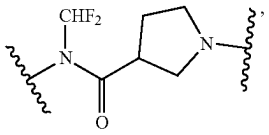

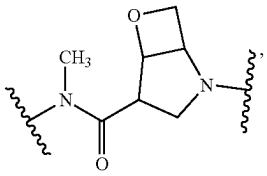

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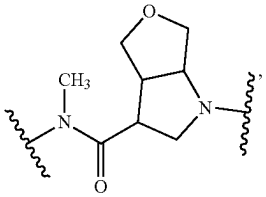

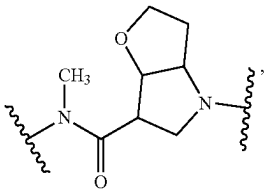

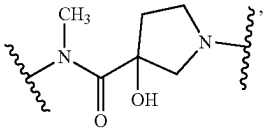

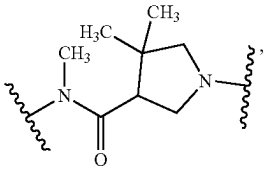

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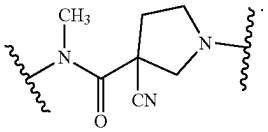
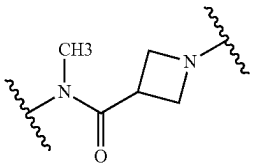
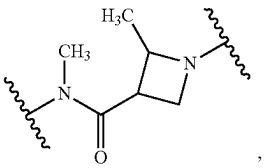


In some embodiments, the linker has the structure:





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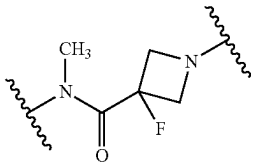

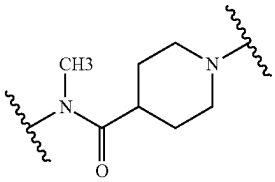

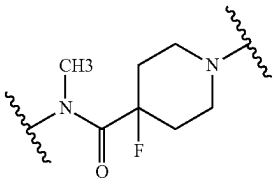

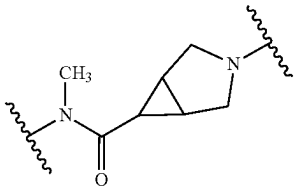

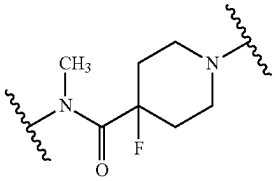

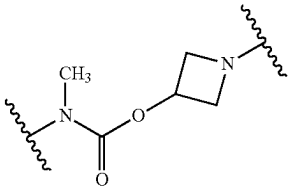

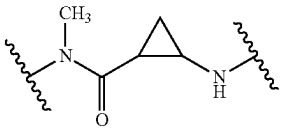

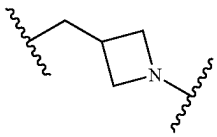

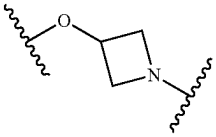

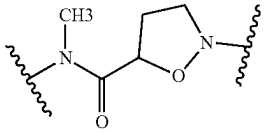

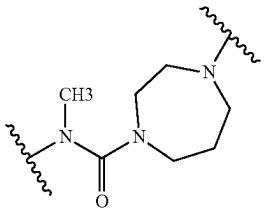

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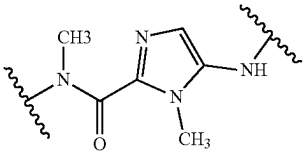

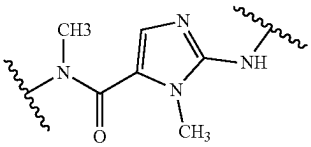

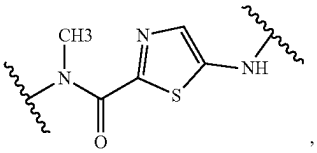

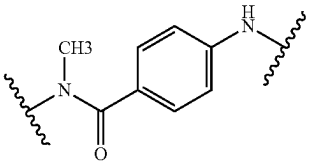

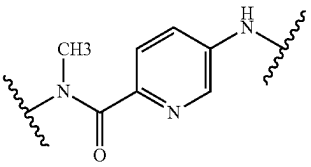

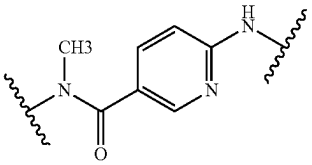

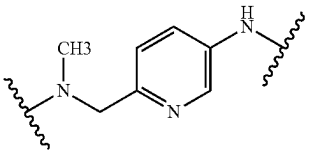

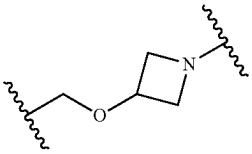

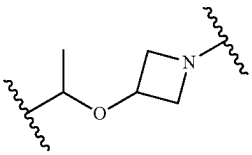

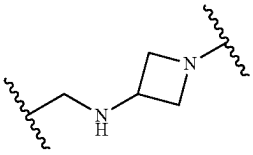

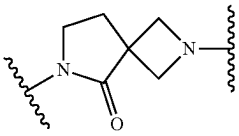

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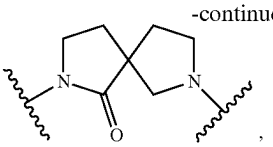

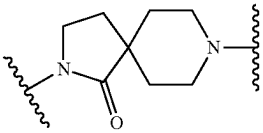

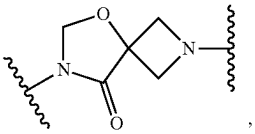

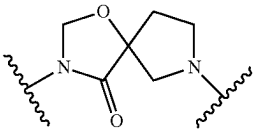

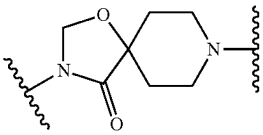

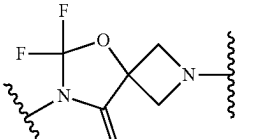

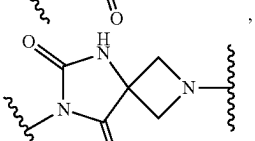

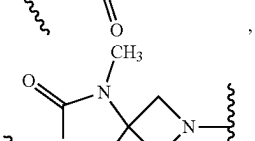

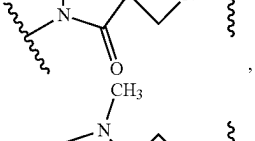

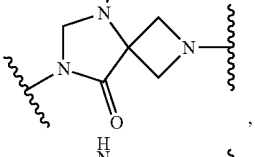

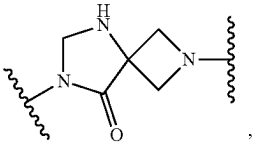

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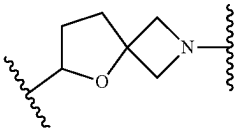

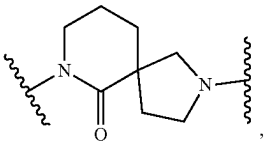

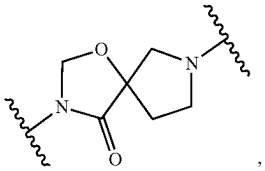

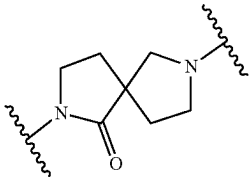

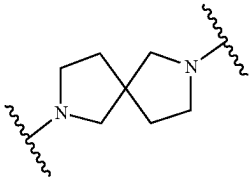

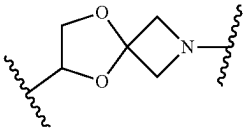

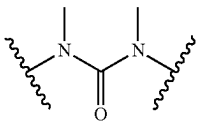

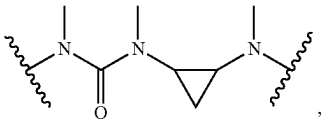

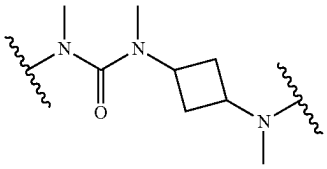

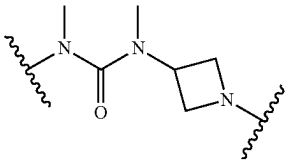

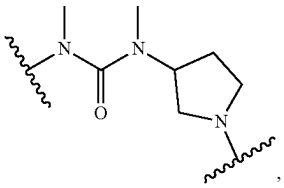

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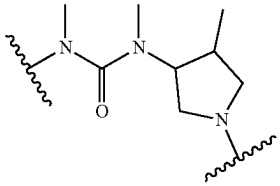

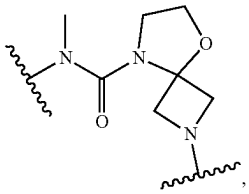

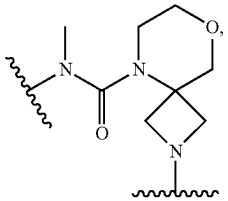

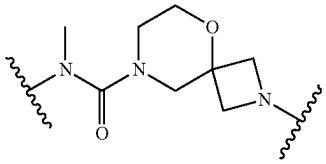

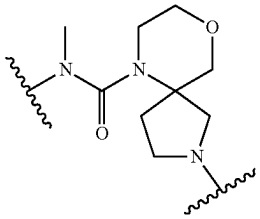

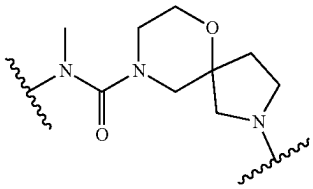

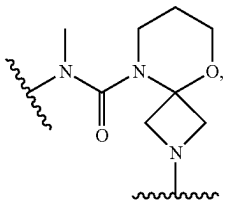

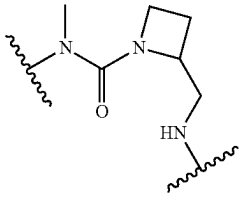

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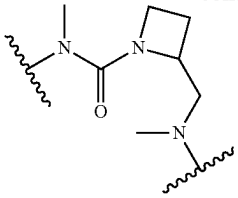

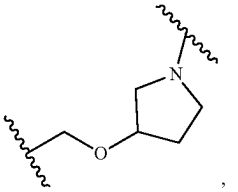

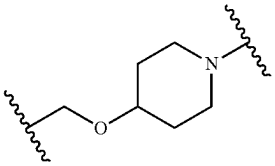

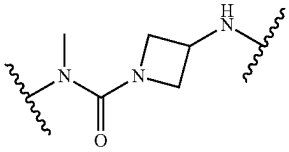

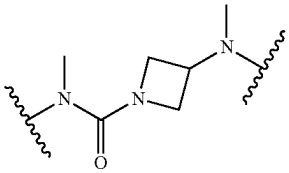

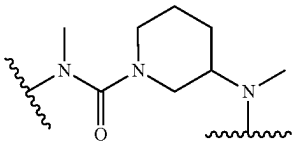

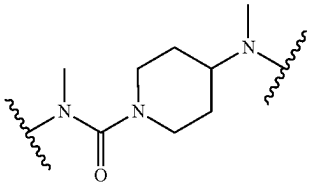

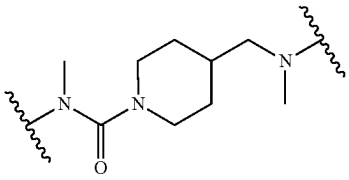

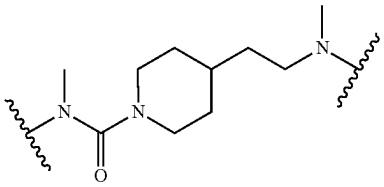

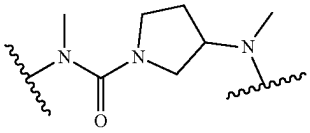

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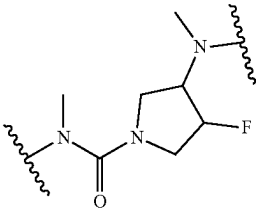

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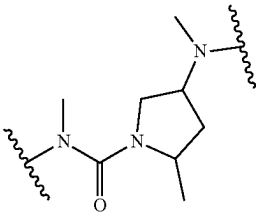

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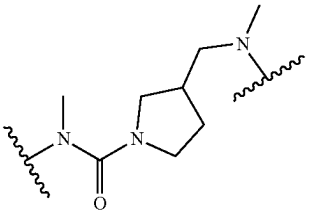

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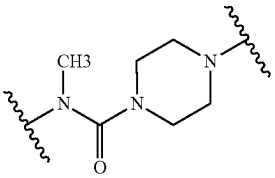

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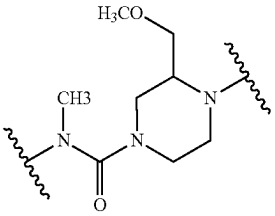

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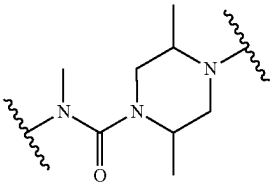

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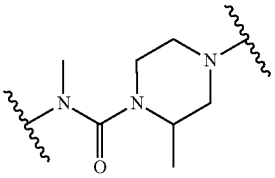

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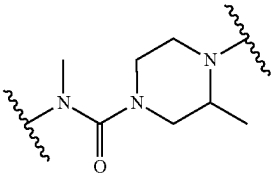

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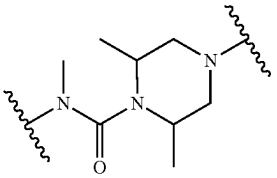

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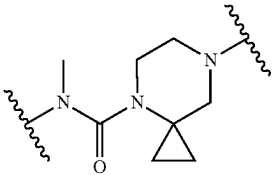

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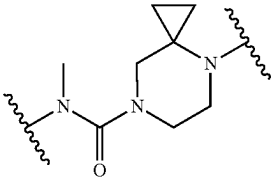

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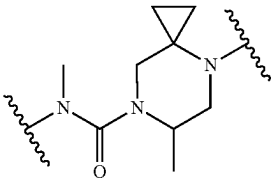

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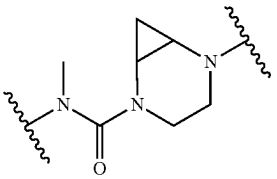

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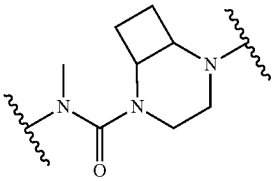

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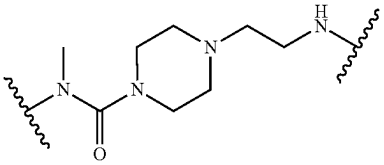

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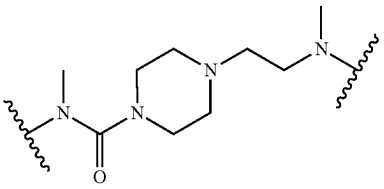

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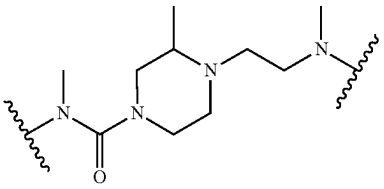

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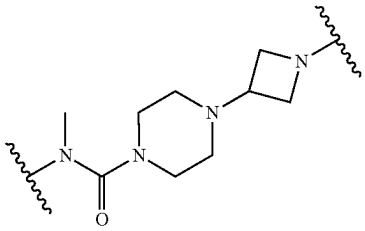

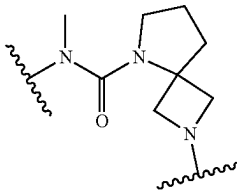

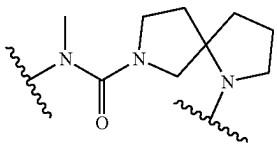

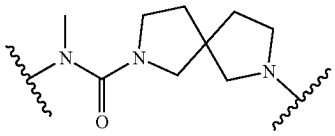

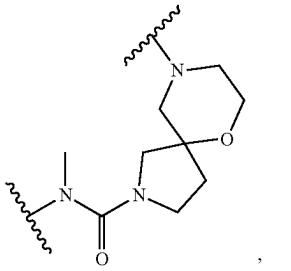

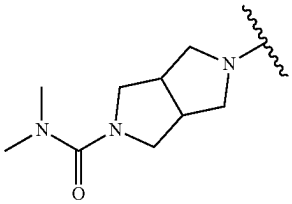

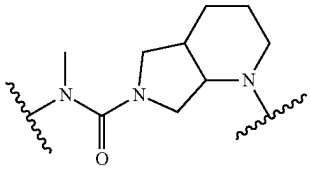

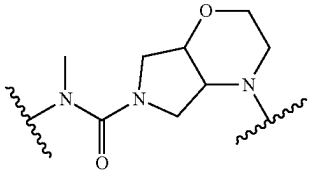

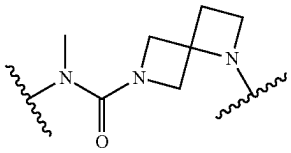

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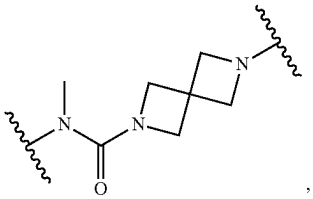

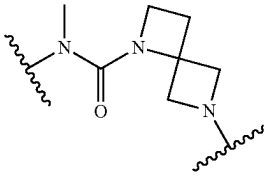

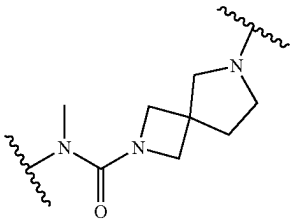

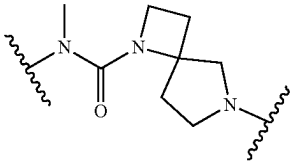

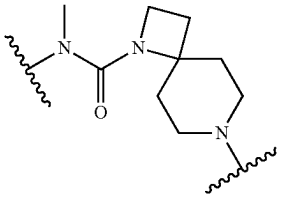

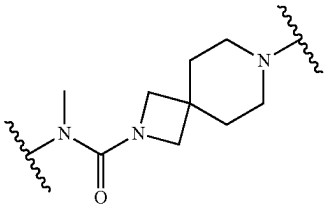

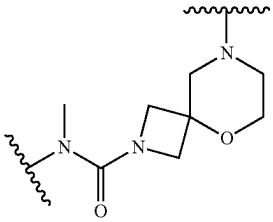

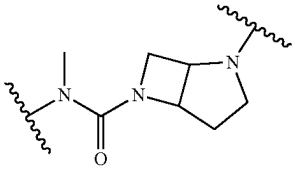

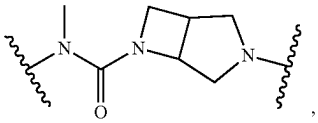

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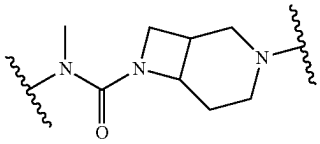

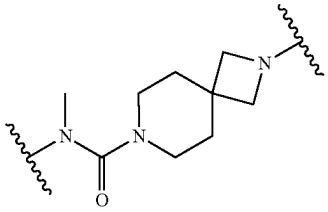

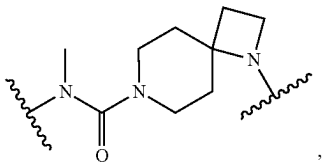

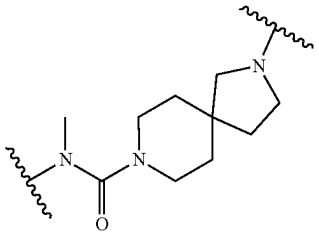

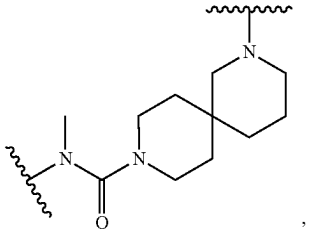

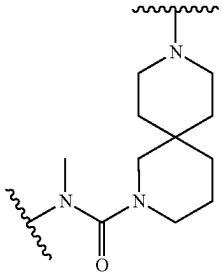

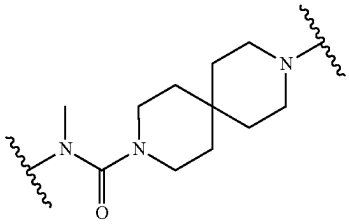

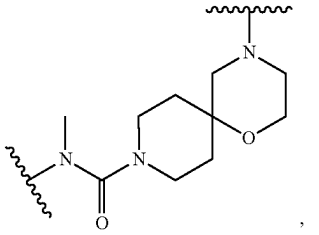

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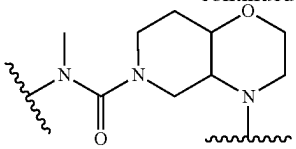

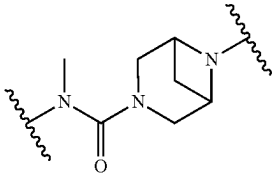

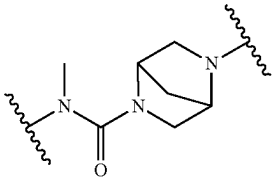

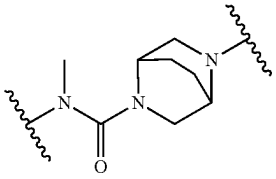

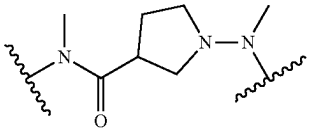

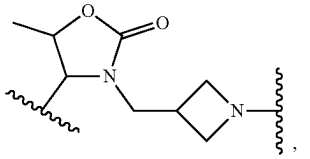

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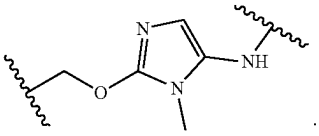
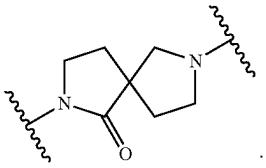
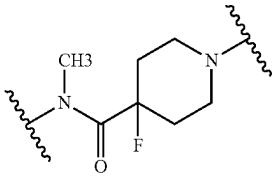


In some embodiments, the linker has the structure

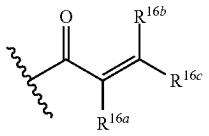


In some embodiments, the linker has the structure

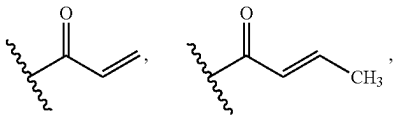


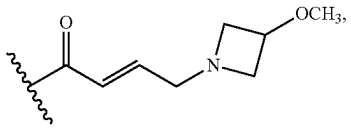
In some embodiments of a compound of the present invention, W is a cross-linking group comprising a vinyl ketone. In some embodiments, W has the structure of Formula IIIa:

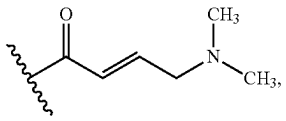
Formula IIIa

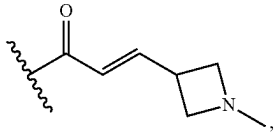


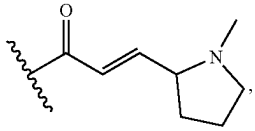
wherein R^{16a}, R^{16b}, and R^{16c} are, independently, hydrogen, —CN, halogen, or —C_1-C_3 alkyl optionally substituted with one or more substituents independently selected from —OH, —O—C_1-C_3 alkyl, —NH_2, —NH(C_1-C_3 alkyl), —N(C_1-C_3 alkyl)$_2$, or a 4 to 7-membered saturated heterocycloalkyl. In some embodiments, W is:

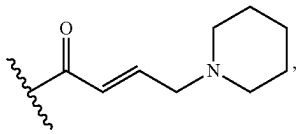


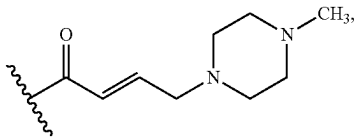


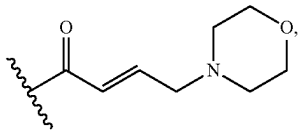












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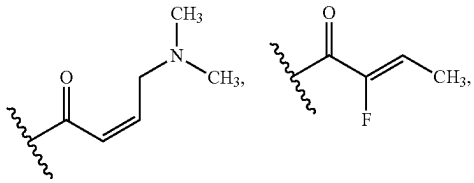
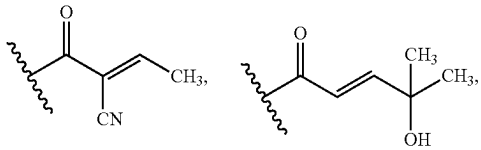
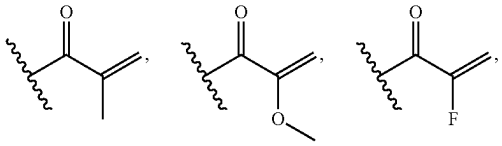
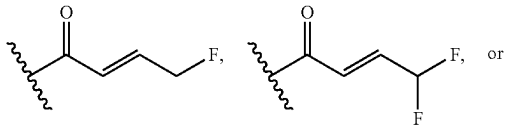
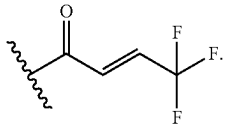




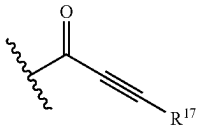




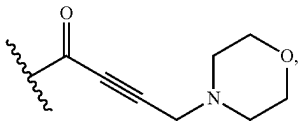


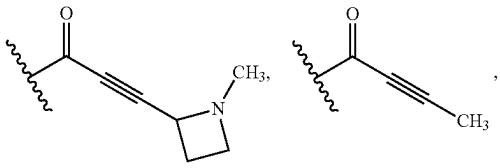
In some embodiments, W is a cross-linking group comprising an ynone. In some embodiments, W has the structure of Formula IIIb:

Formula IIIb



wherein R^{17} is hydrogen, —C_1-C_3 alkyl optionally substituted with one or more substituents independently selected from —OH, —O—C_1-C_3 alkyl, —NH_2, —NH(C_1-C_3 alkyl), —N(C_1-C_3 alkyl)$_2$, or a 4 to 7-membered saturated heterocycloalkyl, or a 4 to 7-membered saturated heterocycloalkyl. In some embodiments, W is:





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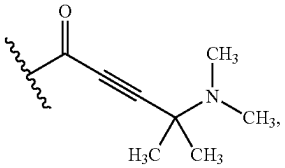

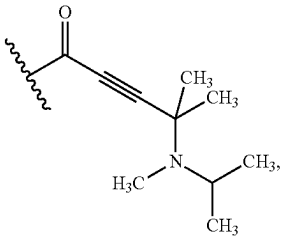

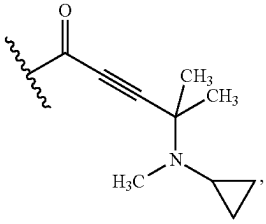

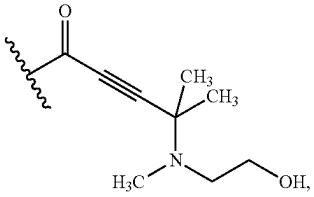

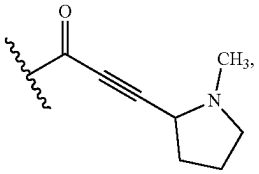

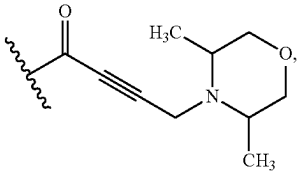

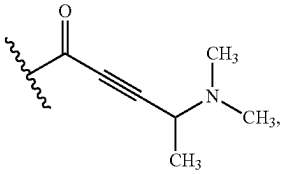

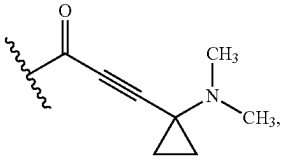

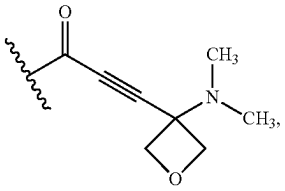

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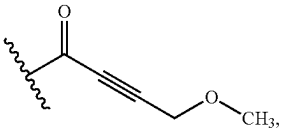

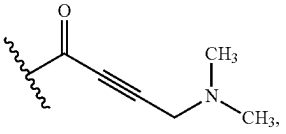

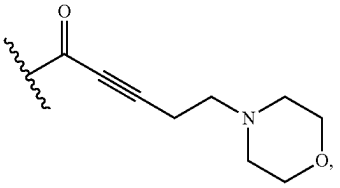

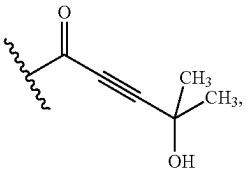

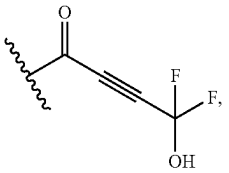

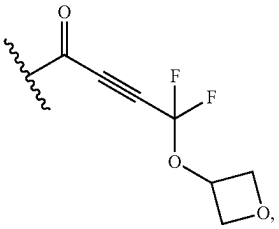

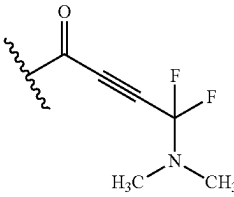

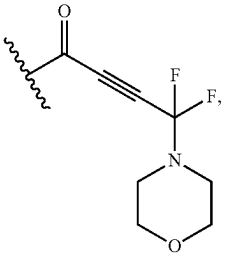

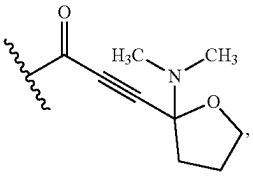

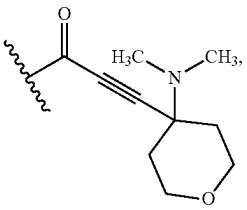

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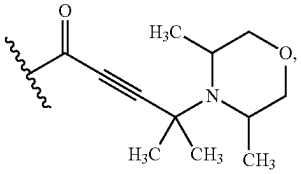

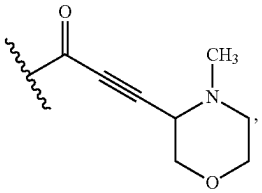

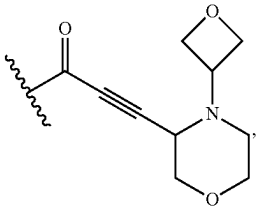

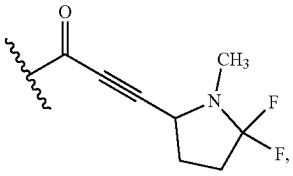

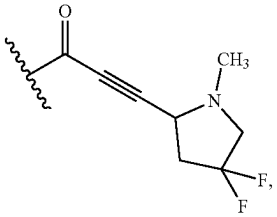

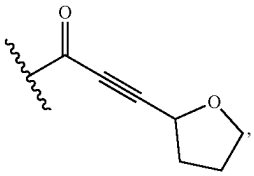

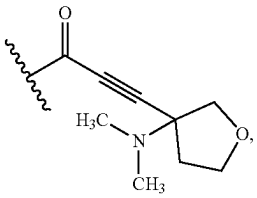

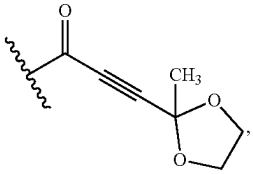

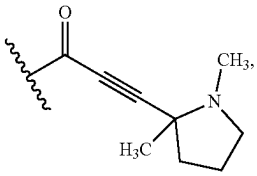

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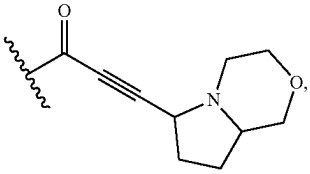
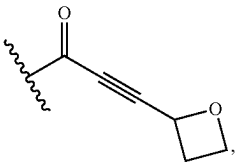
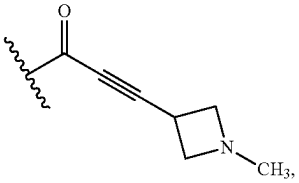
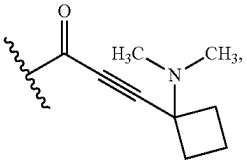
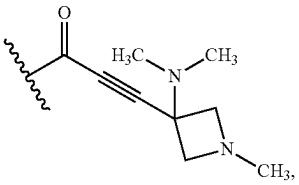
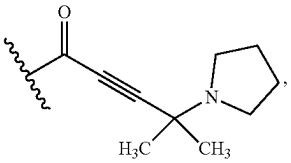


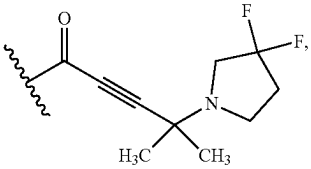


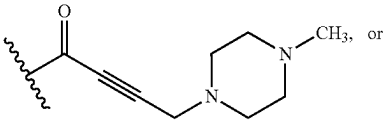


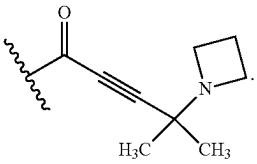




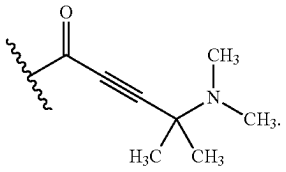






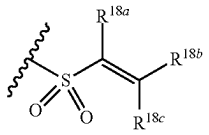


In some embodiments, W is

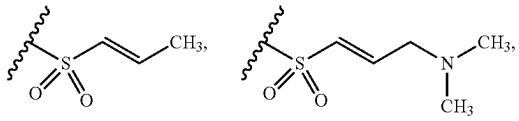


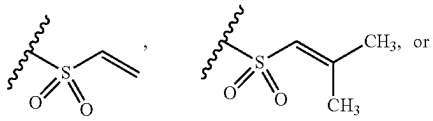
In some embodiments, W is a cross-linking group comprising a vinyl sulfone. In some embodiments, W has the structure of Formula IIIc:

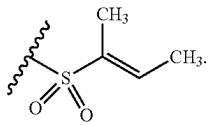
Formula IIIc



wherein R^{18a}, R^{18b}, and R^{18c} are, independently, hydrogen, —CN, or —C_1-C_3 alkyl optionally substituted with one or more substituents independently selected from —OH, —O—C_1-C_3 alkyl, —NH_2, —NH(C_1-C_3 alkyl), —N(C_1-C_3 alkyl)$_2$, or a 4 to 7-membered saturated heterocycloalkyl. In some embodiments, W is:

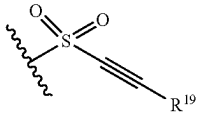




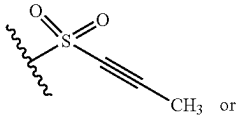


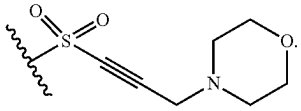
In some embodiments, W is a cross-linking group comprising an alkynyl sulfone. In some embodiments, W has the structure of Formula IIId:

Formula IIId



wherein R^{19} is hydrogen, —C_1-C_3 alkyl optionally substituted with one or more substituents independently selected from —OH, —O—C_1-C_3 alkyl, —NH_2, —NH(C_1-C_3 alkyl), —N(C_1-C_3 alkyl)$_2$, or a 4 to 7-membered saturated heterocycloalkyl, or a 4 to 7-membered saturated heterocycloalkyl. In some embodiments, W is:

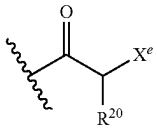




In some embodiments, W has the structure of Formula IIIe:

wherein X^e is a halogen; and

Formula IIIe



R^{20} is hydrogen, —C_1-C_3 alkyl optionally substituted with one or more substituents independently selected from —OH, —O—C_1-C_3 alkyl, —NH_2, —NH(C_1-C_3 alkyl), —N(C_1-C_3 alkyl)$_2$, or a 4 to 7-membered saturated heterocycloalkyl. In some embodiments, W is haloacetal. In some embodiments, W is not haloacetal.

In some embodiments, a compound of the present invention is selected from Table 1, or a pharmaceutically acceptable salt or stereoisomer thereof. In some embodiments, a compound of the present invention is selected from Table 1, or a pharmaceutically acceptable salt or atropisomer thereof.

TABLE 1

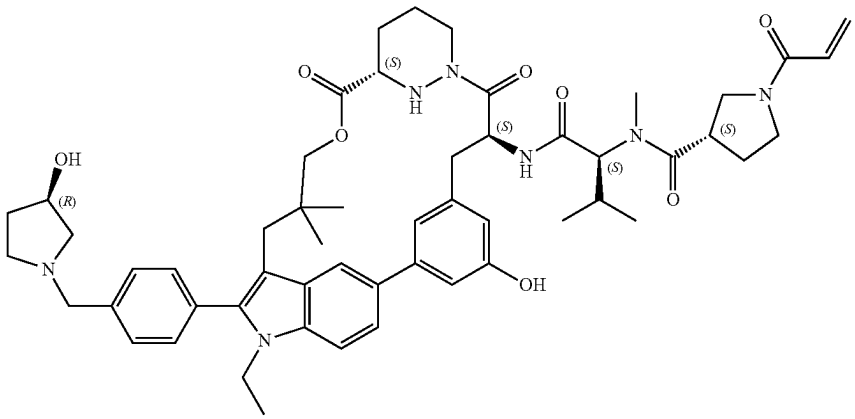
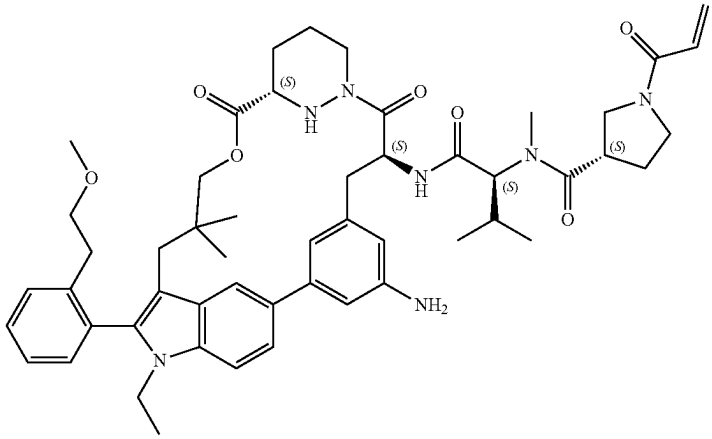
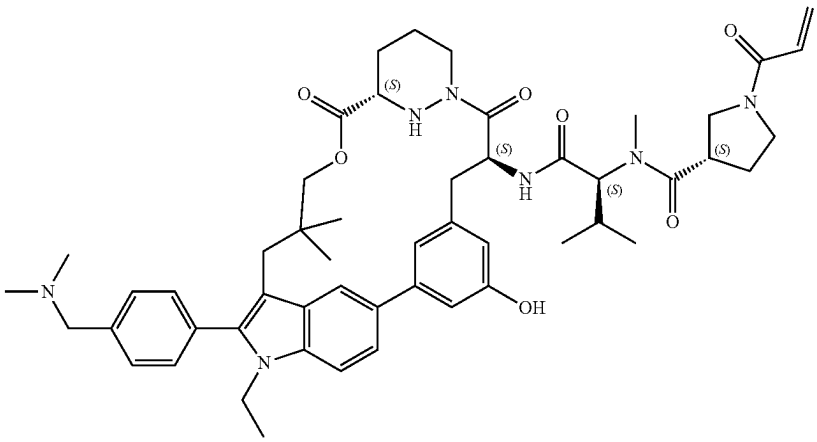
Certain Compounds of the Present Invention	
Ex#	Structure
A1	
A2	
A3	

TABLE 1-continued

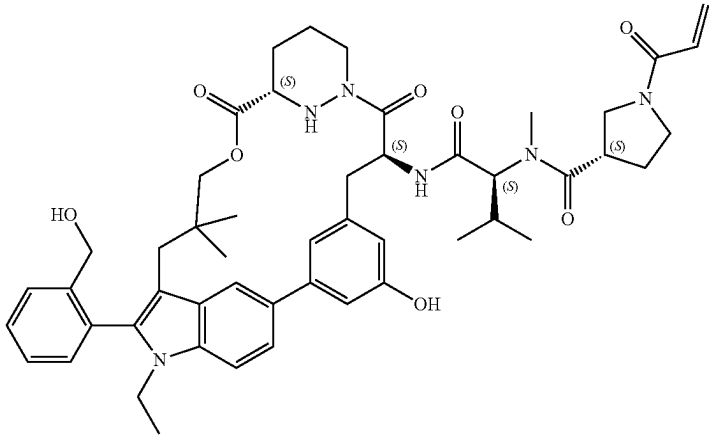
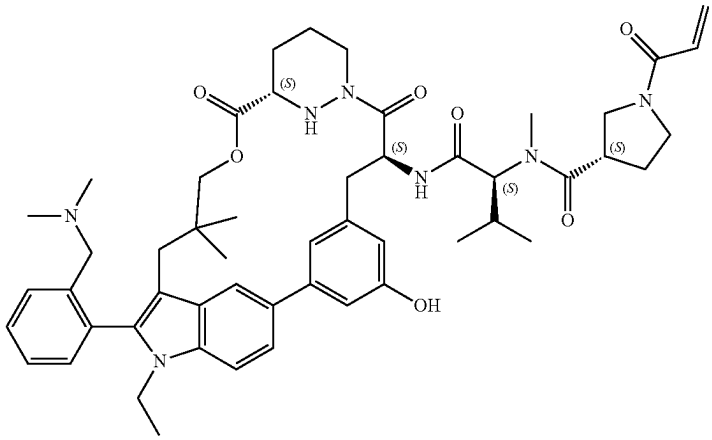
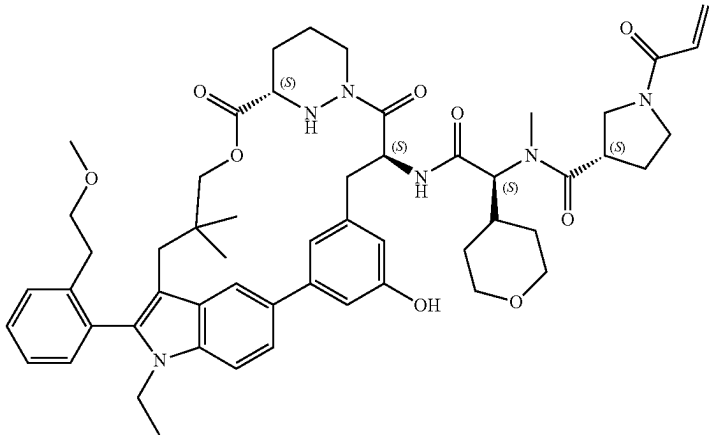
Certain Compounds of the Present Invention	
Ex#	Structure
A4	
A5	
A6	

TABLE 1-continued

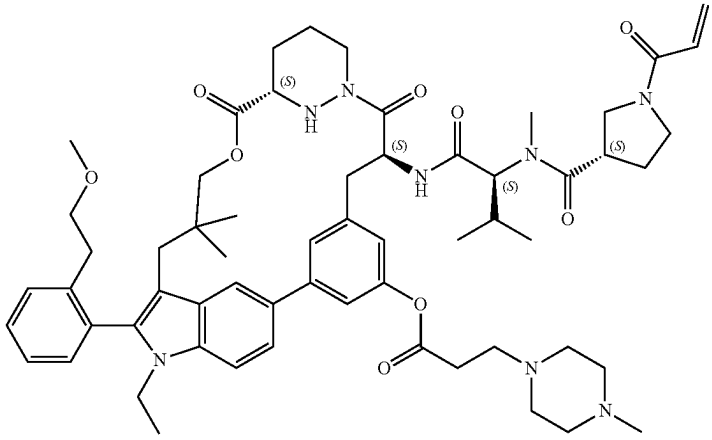
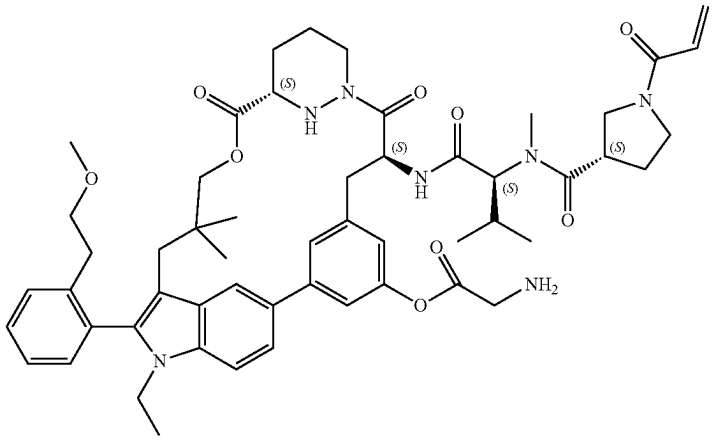
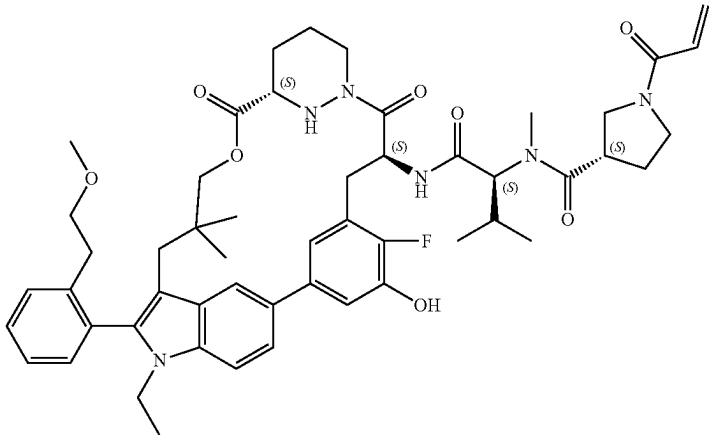
Certain Compounds of the Present Invention	
Ex#	Structure
A7	
A8	
A9	

TABLE 1-continued

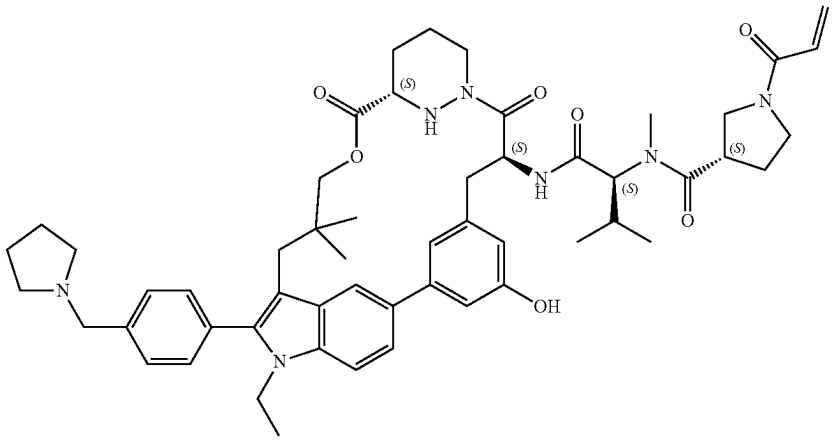
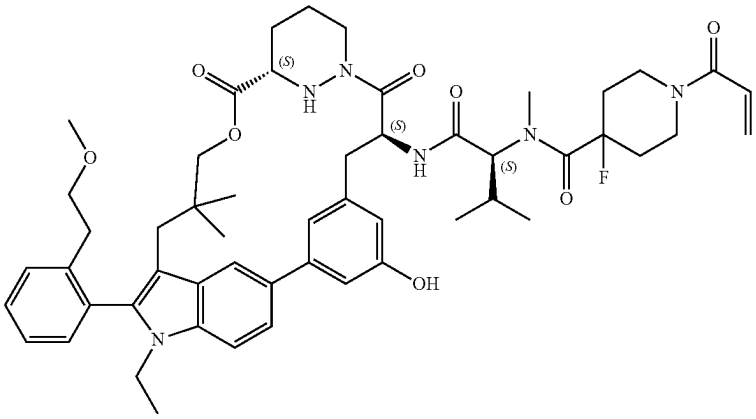
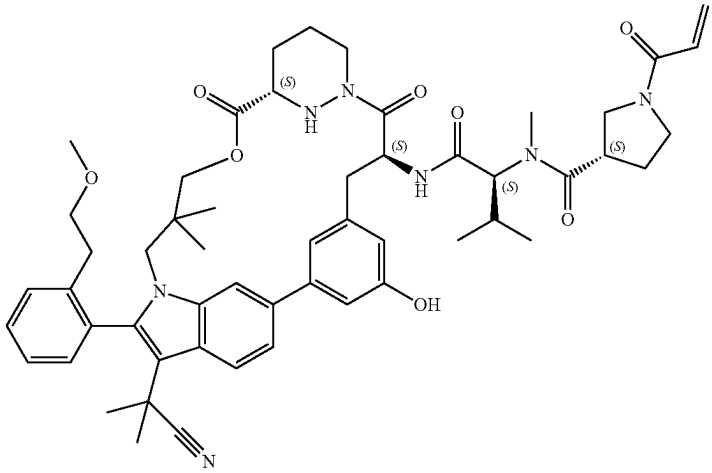
Certain Compounds of the Present Invention	
Ex#	Structure
A10	
A11	
A12	

TABLE 1-continued

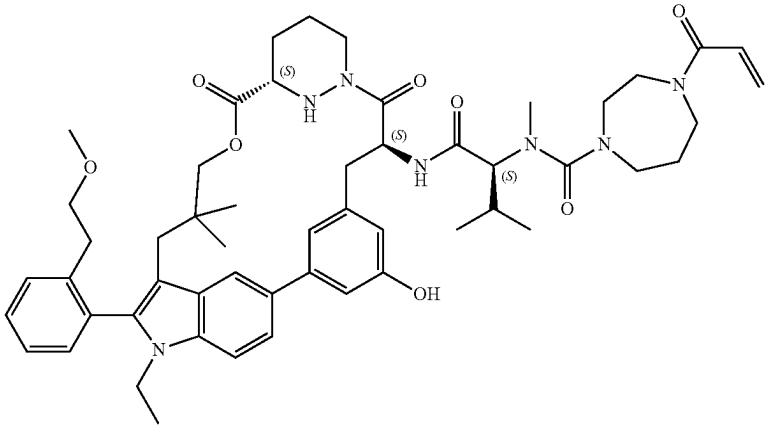
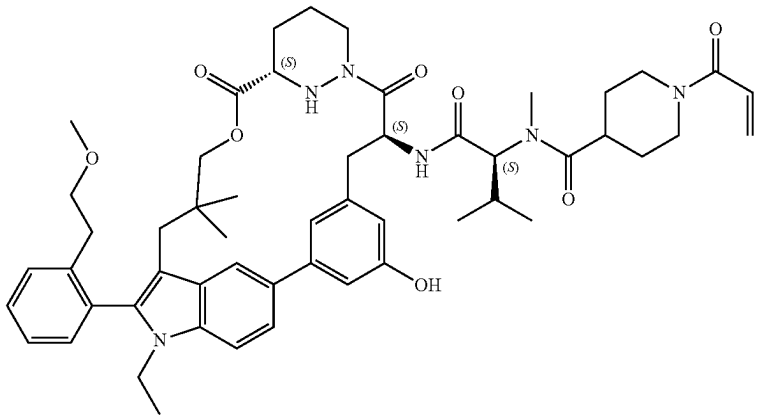
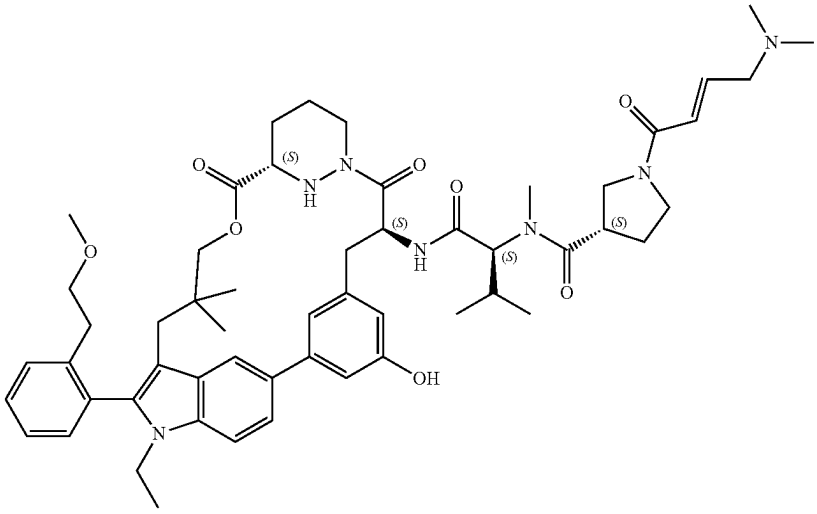
Certain Compounds of the Present Invention	
Ex#	Structure
A13	
A14	
A15	

TABLE 1-continued

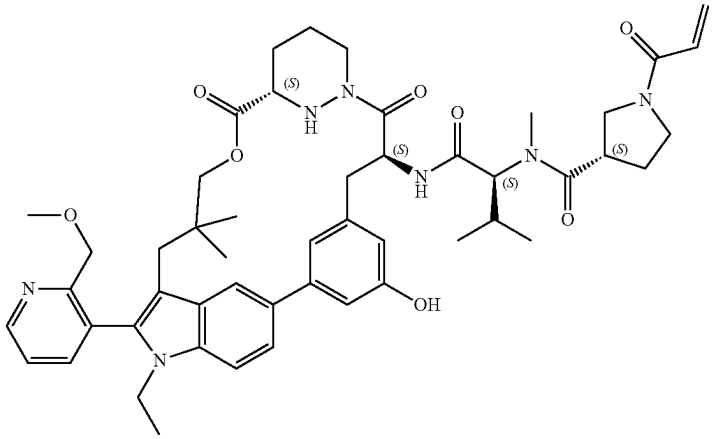
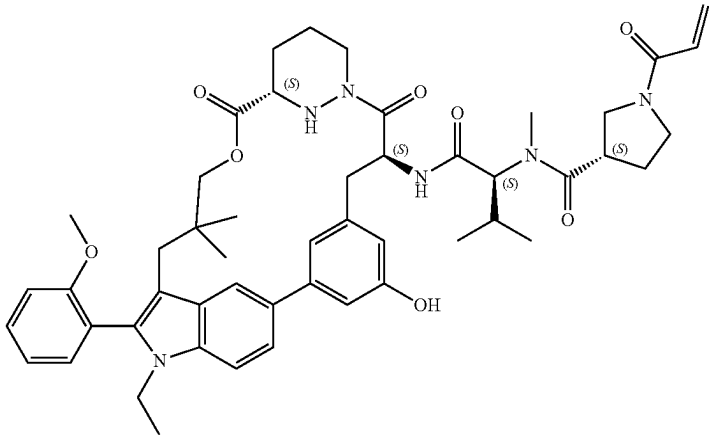
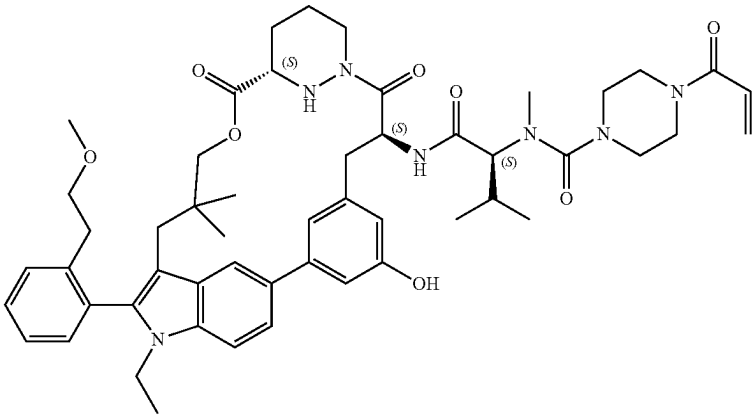
Certain Compounds of the Present Invention	
Ex#	Structure
A16	
A17	
A18	

TABLE 1-continued

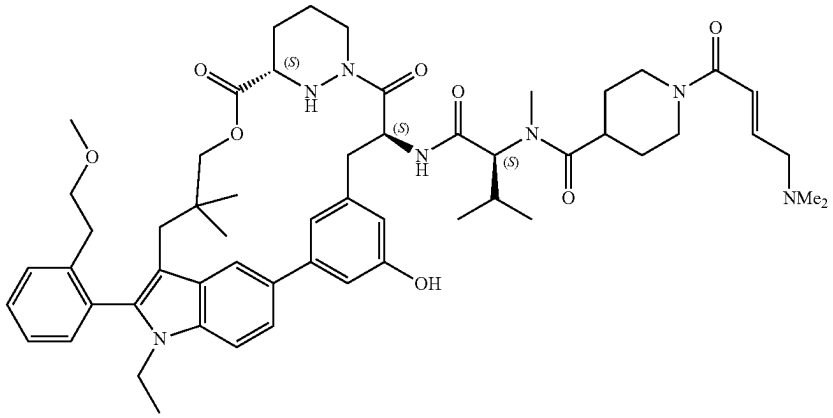
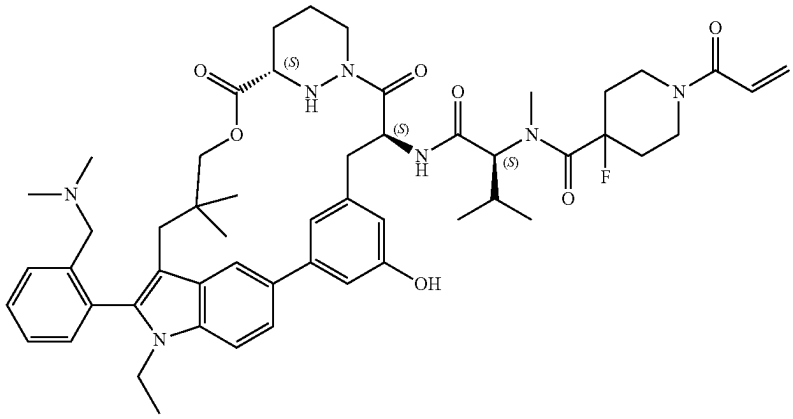
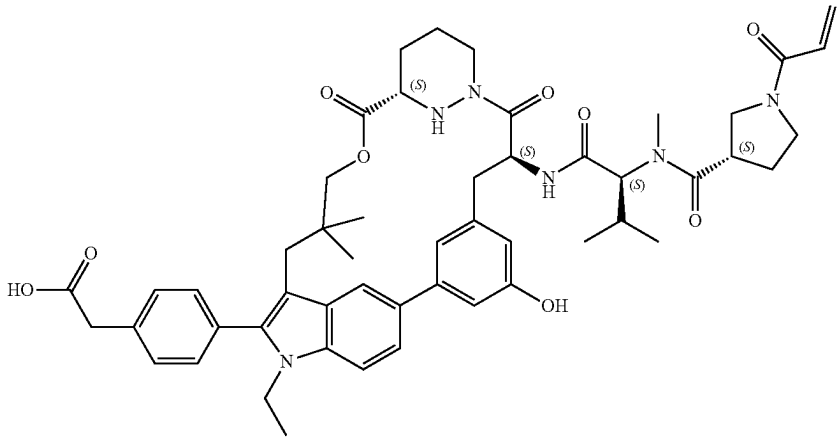
Certain Compounds of the Present Invention	
Ex#	Structure
A19	
A20	
A21	

TABLE 1-continued

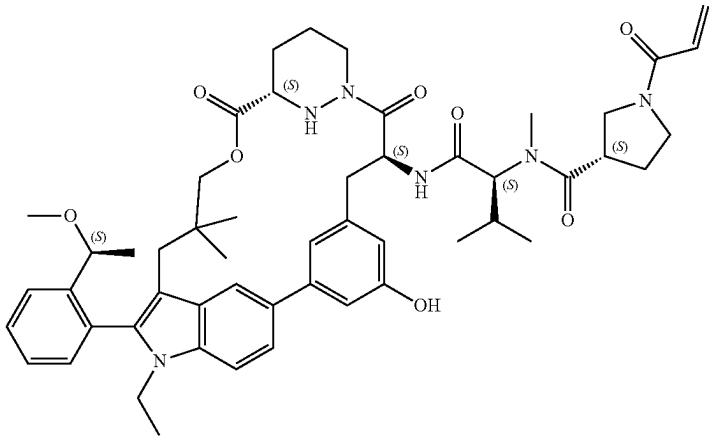
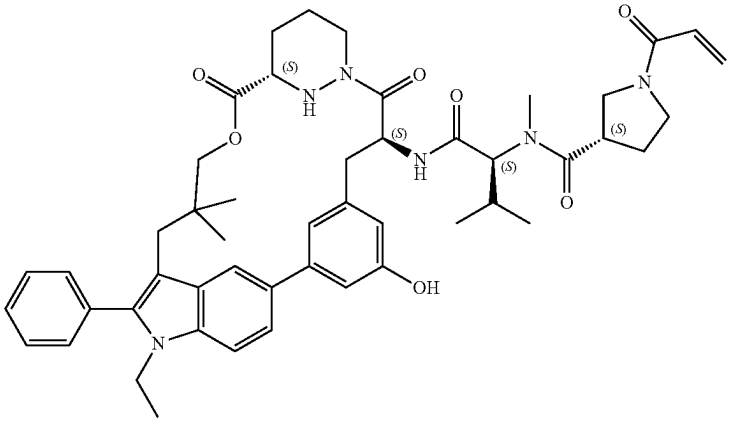
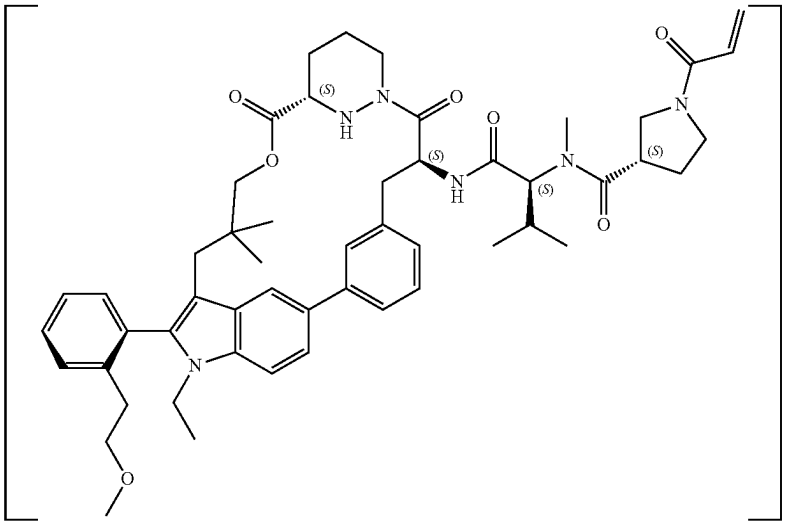
Certain Compounds of the Present Invention	
Ex#	Structure
A22	
A23	
A24	

TABLE 1-continued

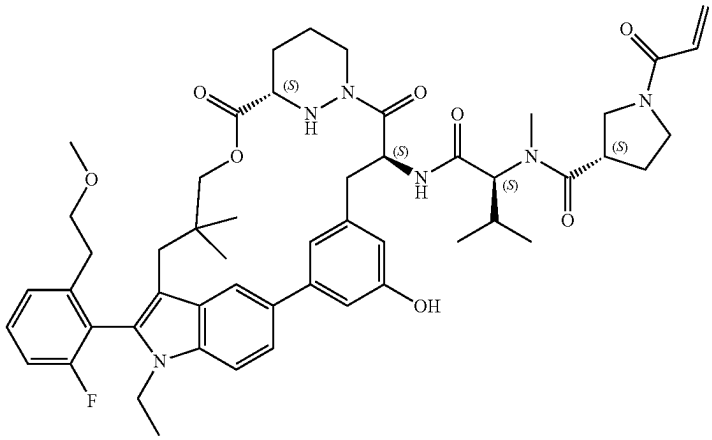
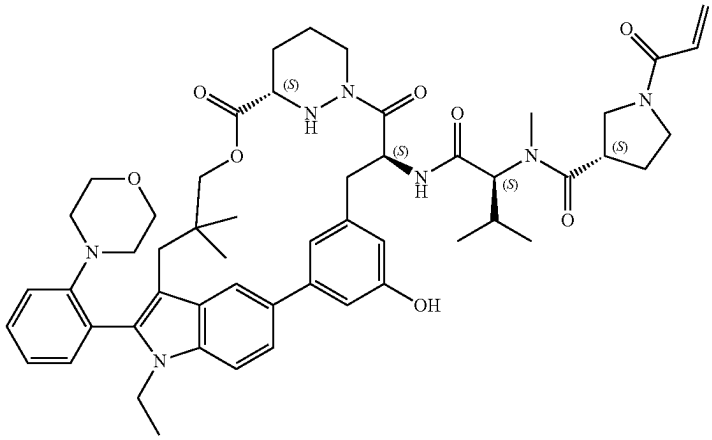
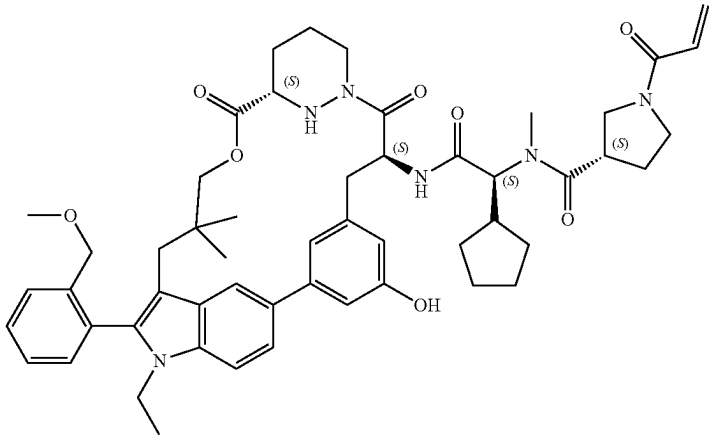
Certain Compounds of the Present Invention	
Ex#	Structure
A25	
A26	
A27	

TABLE 1-continued

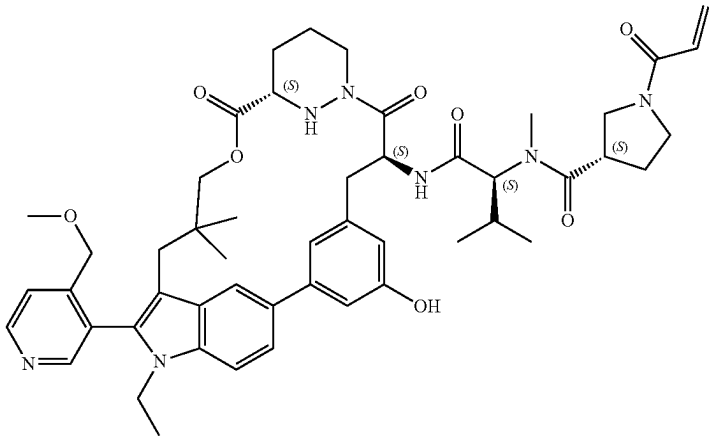
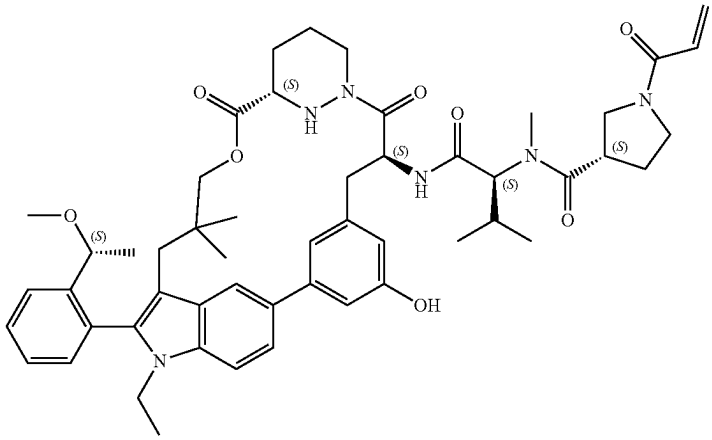
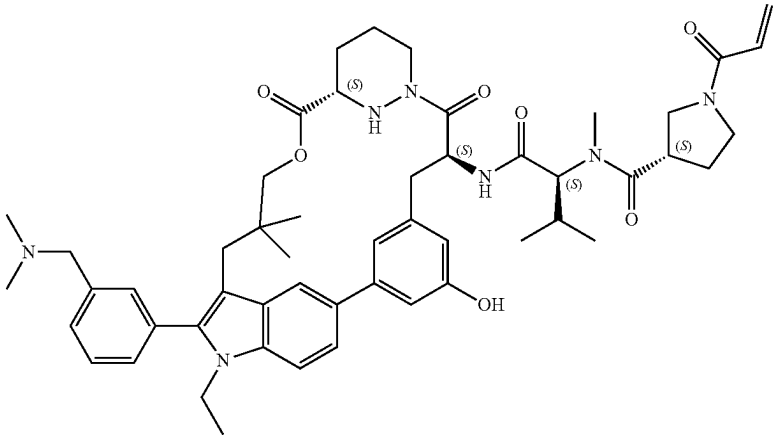
Certain Compounds of the Present Invention	
Ex#	Structure
A28	
A29	
A30	

TABLE 1-continued

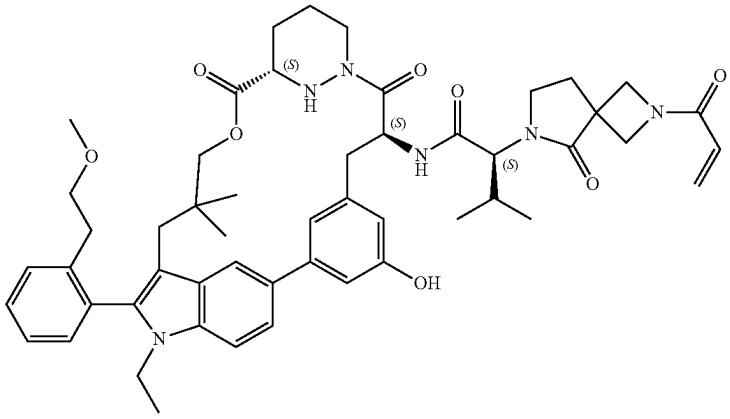
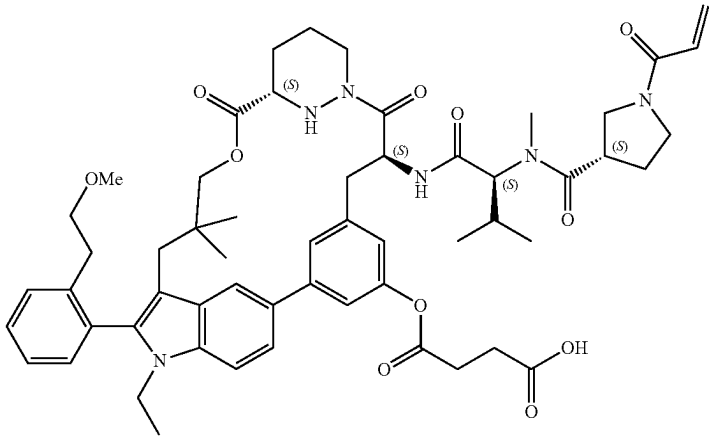
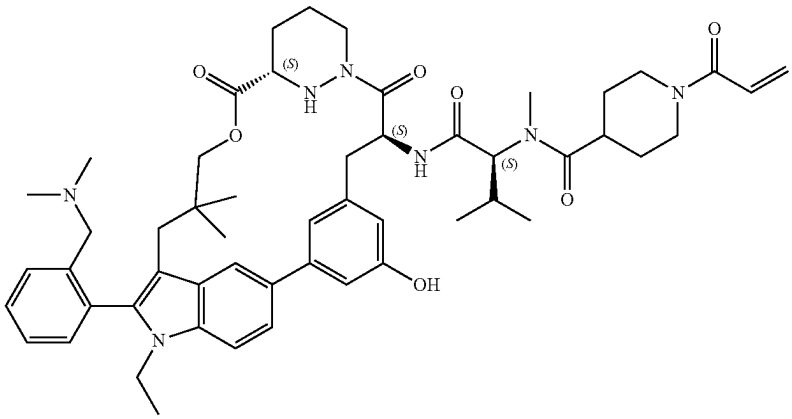
Certain Compounds of the Present Invention	
Ex#	Structure
A31	
A32	
A33	

TABLE 1-continued

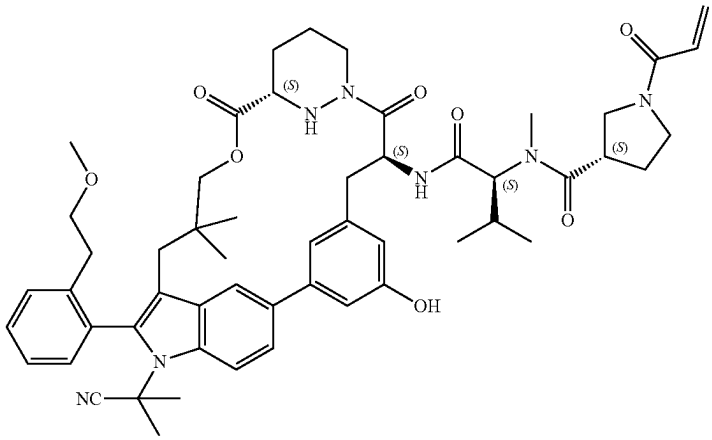
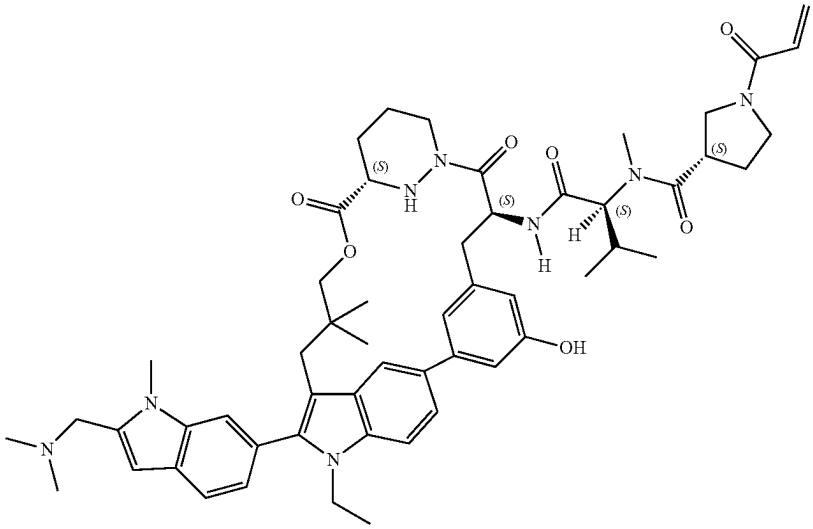
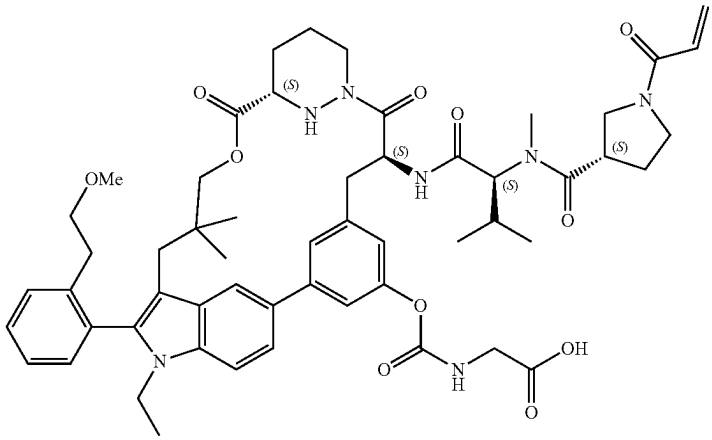
Certain Compounds of the Present Invention	
Ex#	Structure
A34	
A35	
A36	

TABLE 1-continued

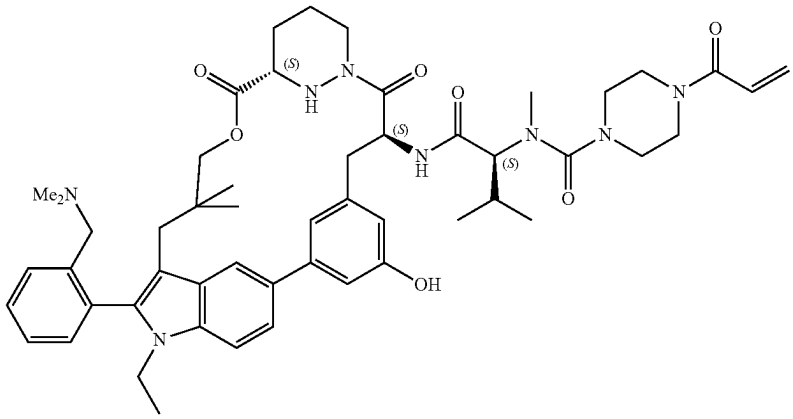
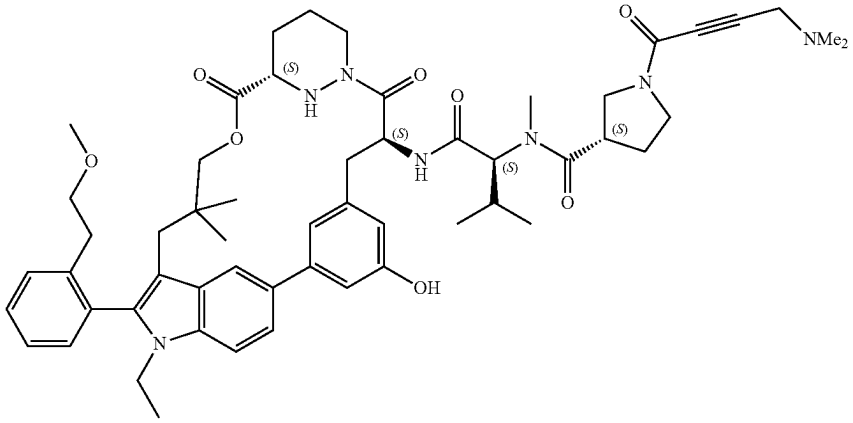
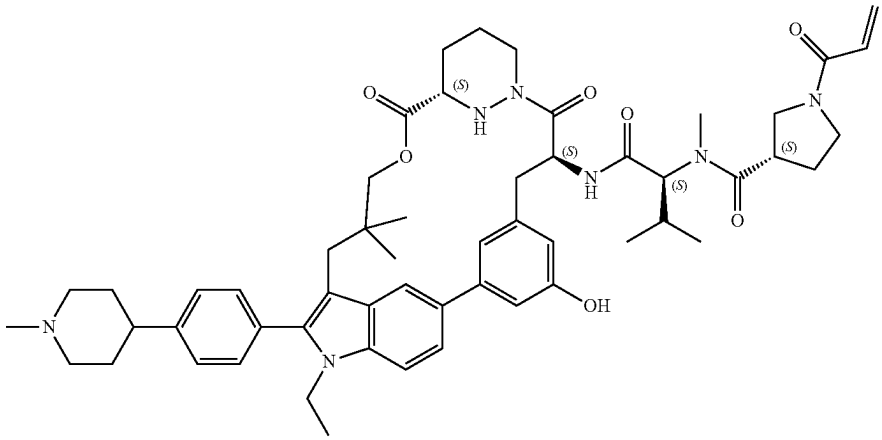
Certain Compounds of the Present Invention	
Ex#	Structure
A37	
A38	
A39	

TABLE 1-continued

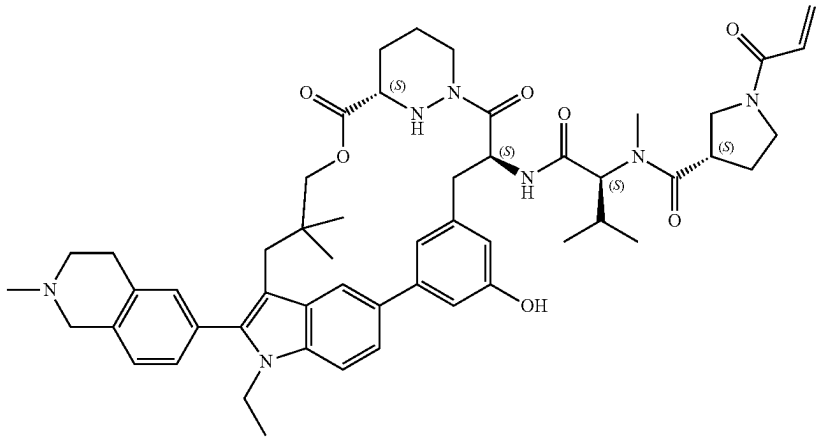
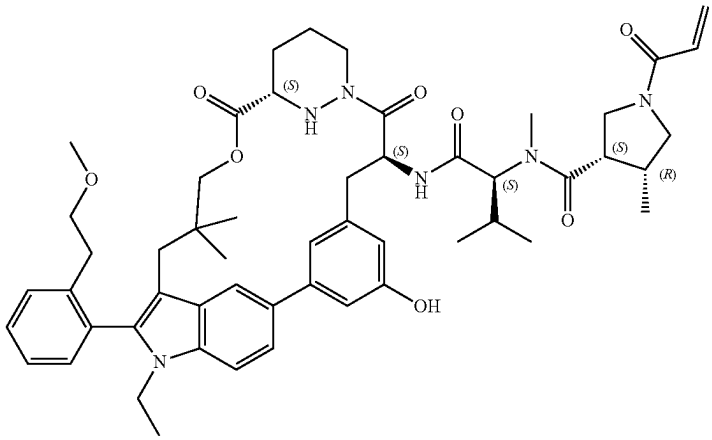
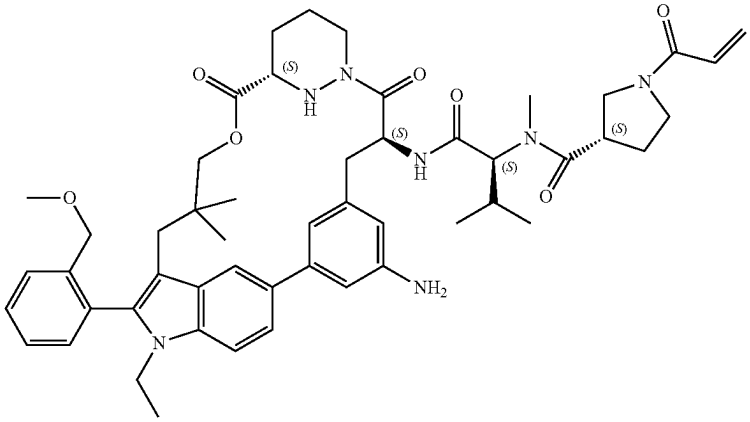
Certain Compounds of the Present Invention	
Ex#	Structure
A40	
A41	
A42	

TABLE 1-continued

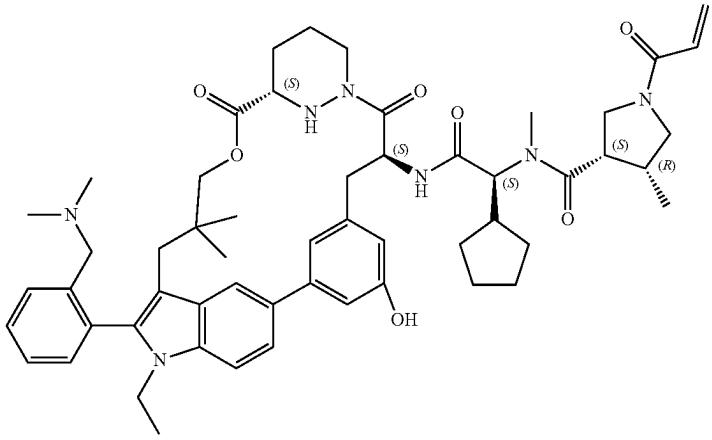
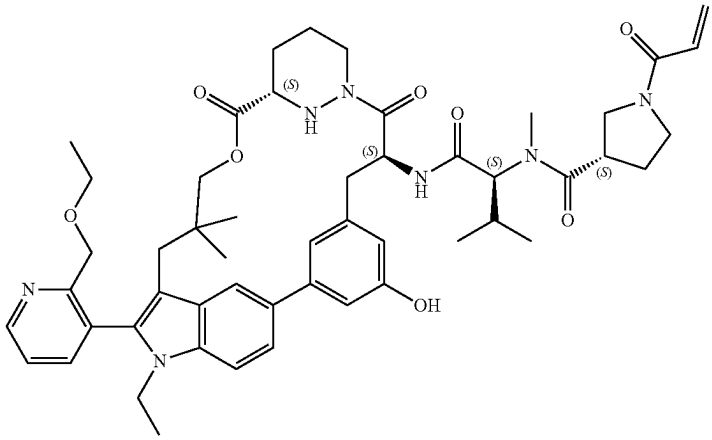
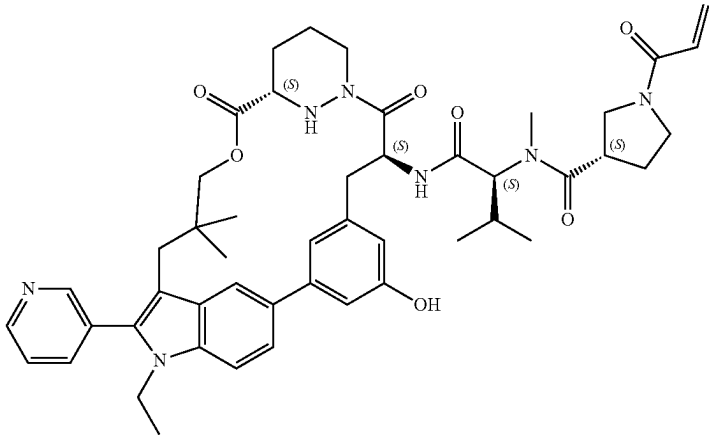
Certain Compounds of the Present Invention	
Ex#	Structure
A43	
A44	
A45	

TABLE 1-continued

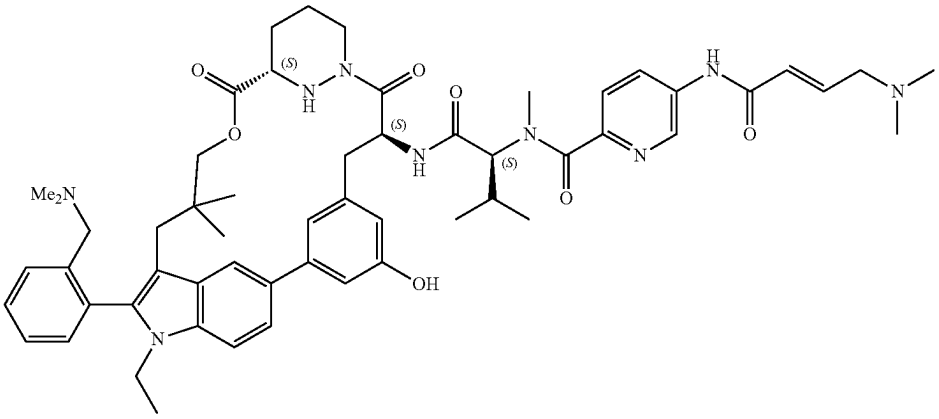
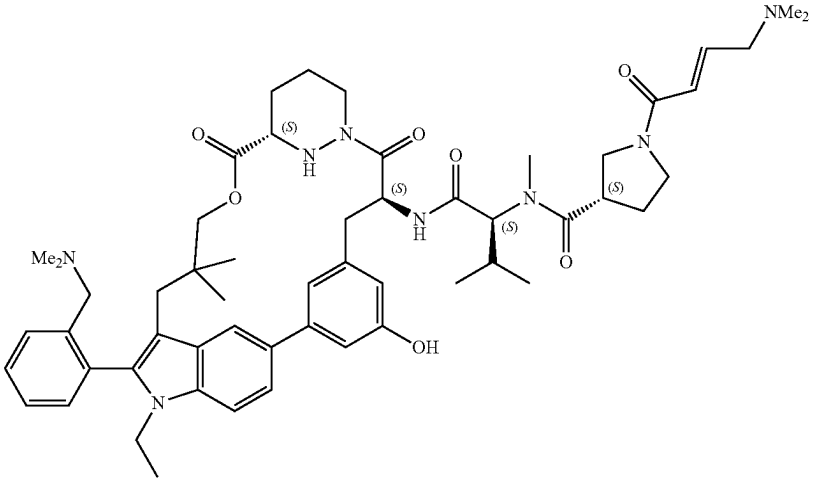
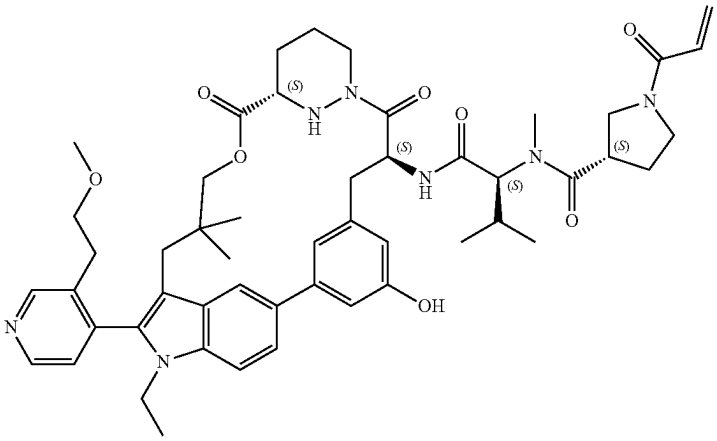
Certain Compounds of the Present Invention	
Ex#	Structure
A46	
A47	
A48	

TABLE 1-continued

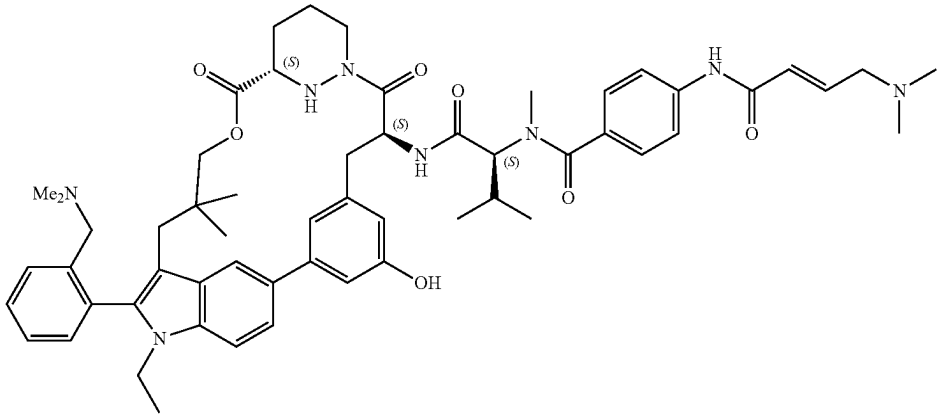
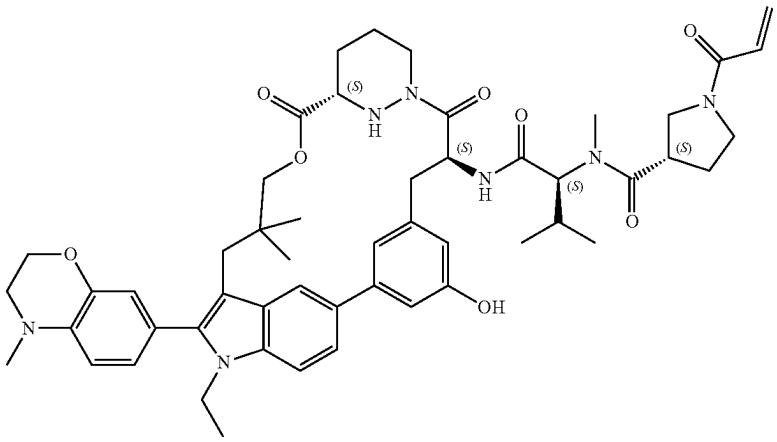
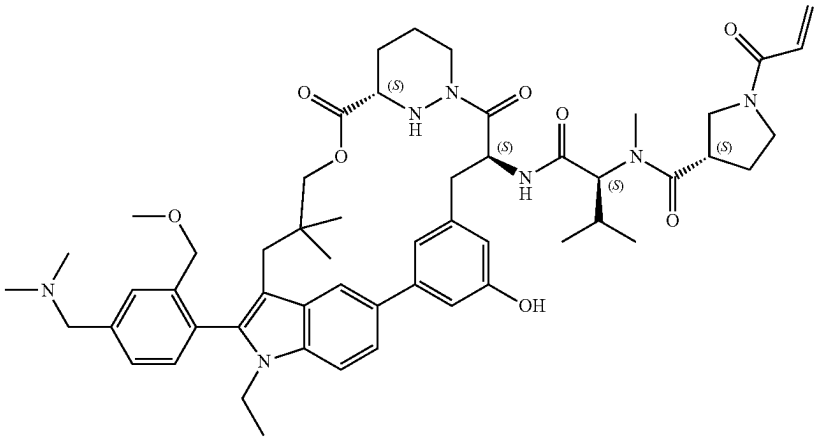
Certain Compounds of the Present Invention	
Ex#	Structure
A49	
A50	
A51	

TABLE 1-continued

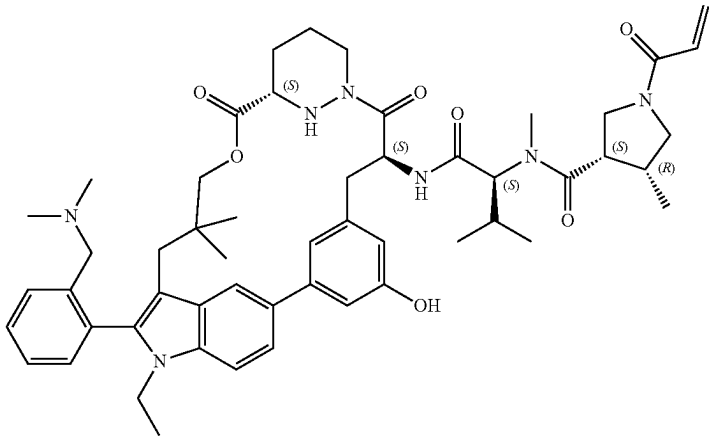
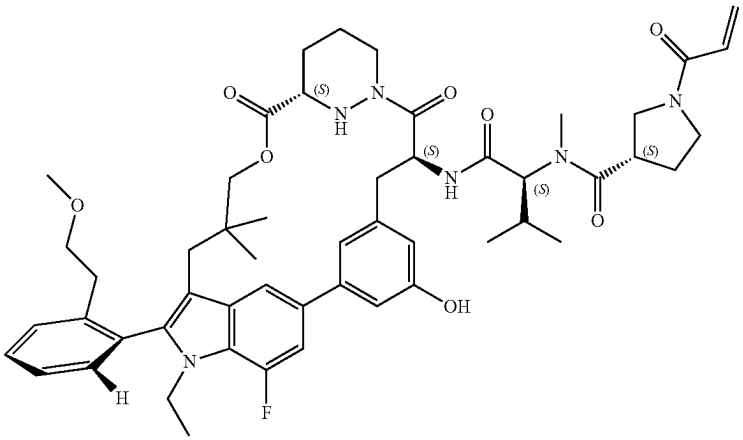
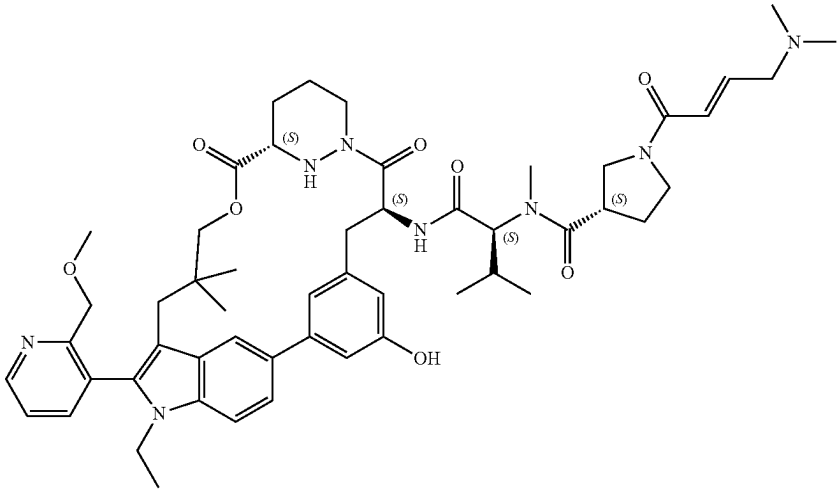
Certain Compounds of the Present Invention	
Ex#	Structure
A52	
A53	
A54	

TABLE 1-continued

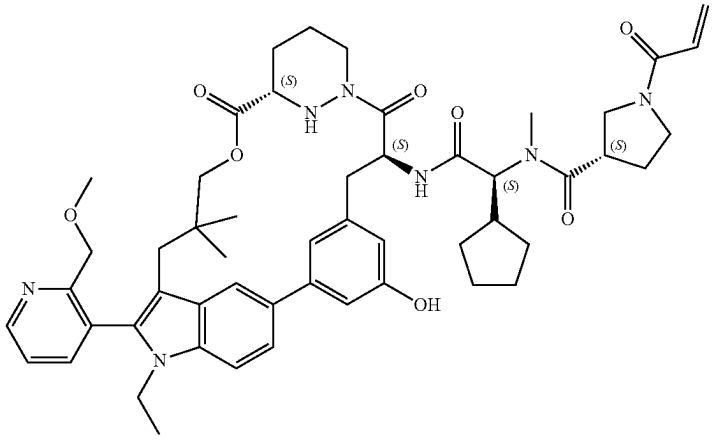
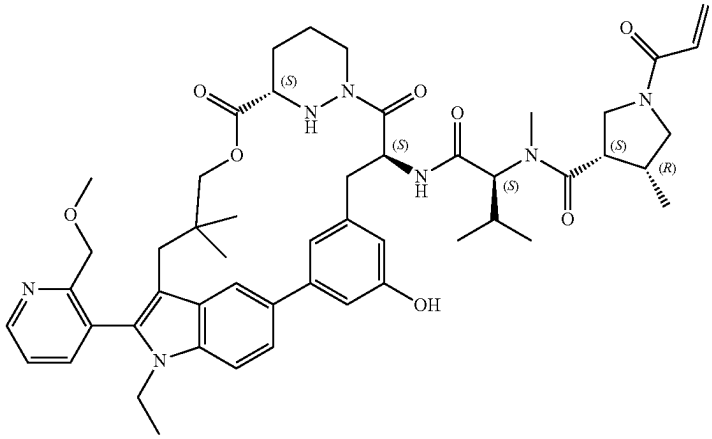
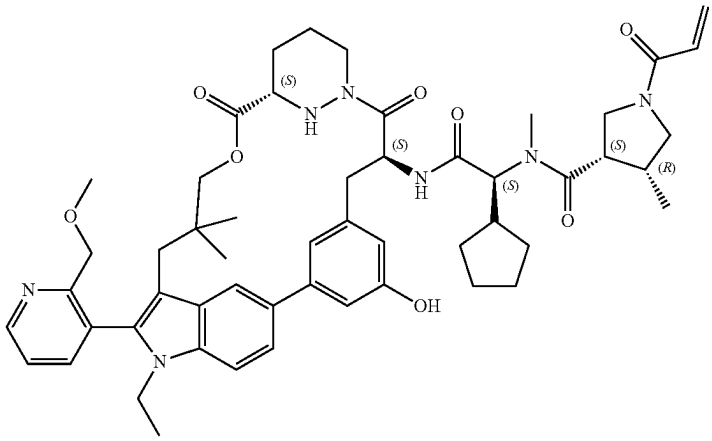
Certain Compounds of the Present Invention	
Ex#	Structure
A55	
A56	
A57	

TABLE 1-continued

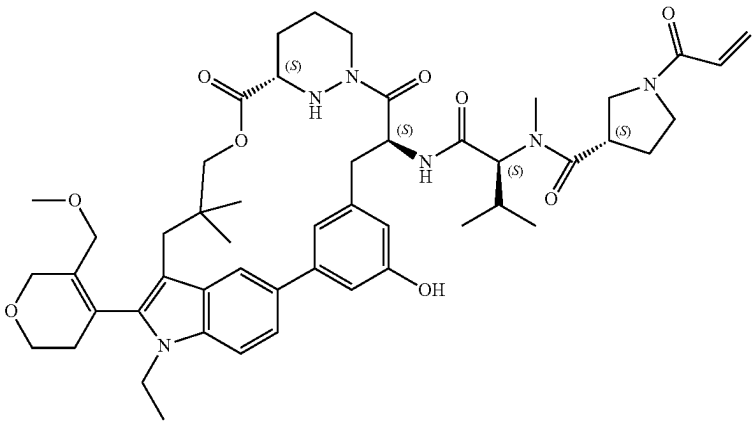
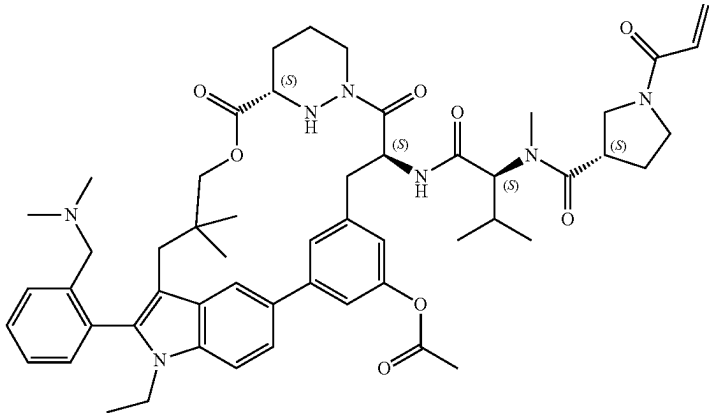
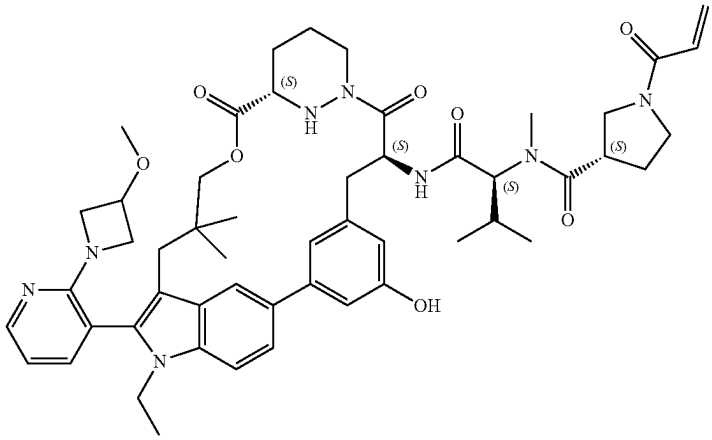
Certain Compounds of the Present Invention	
Ex#	Structure
A58	
A59	
A60	

TABLE 1-continued

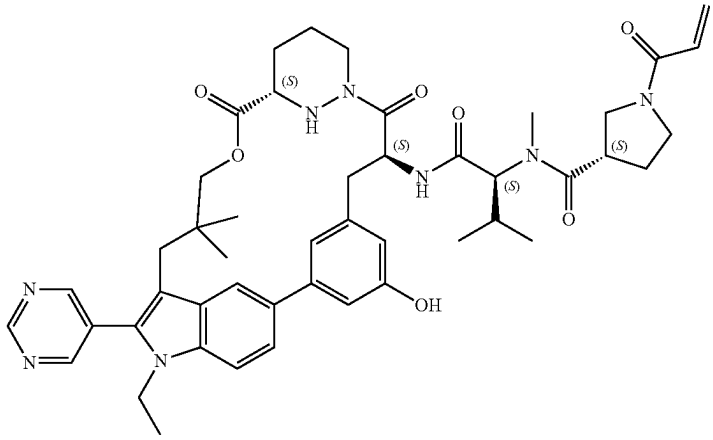
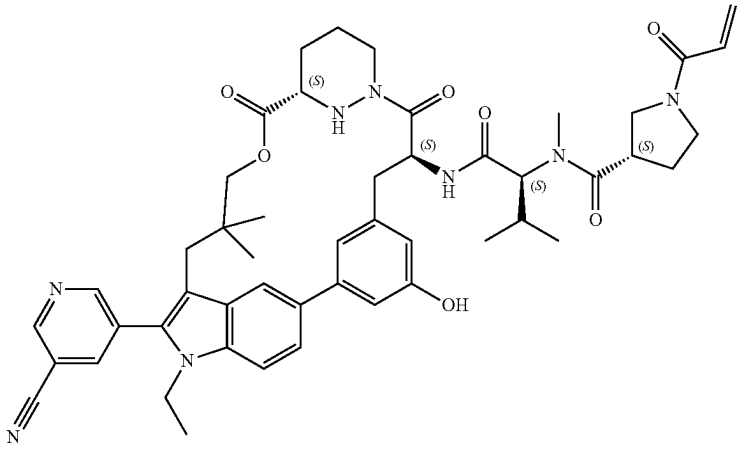
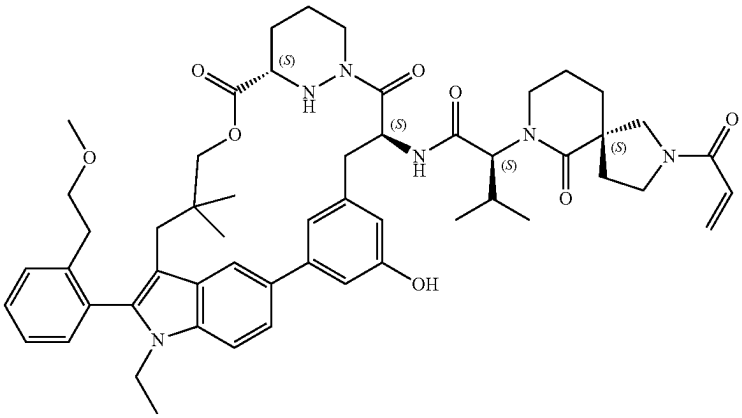
Certain Compounds of the Present Invention	
Ex#	Structure
A61	
A62	
A63	

TABLE 1-continued

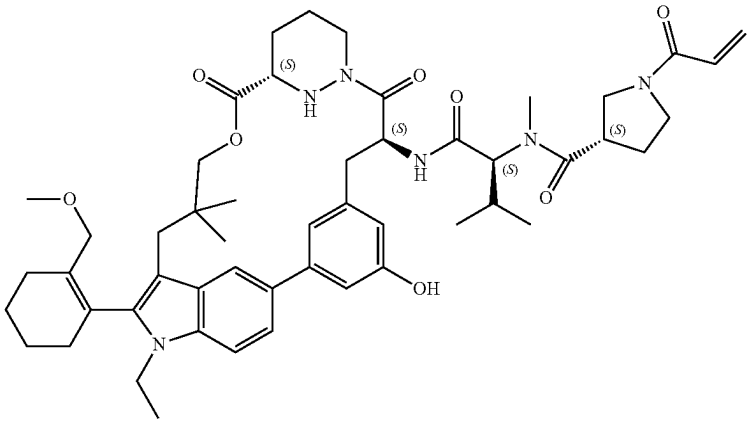
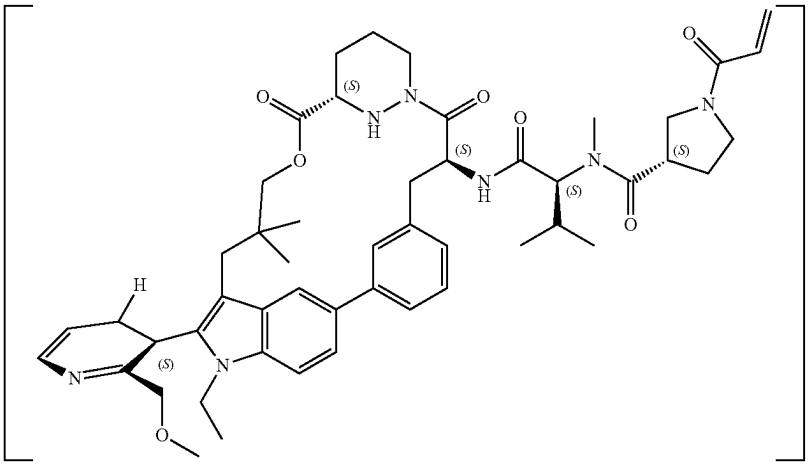
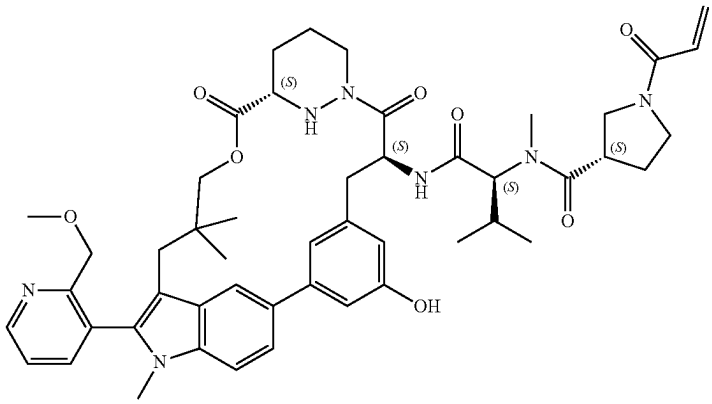
Certain Compounds of the Present Invention	
Ex#	Structure
A64	
A65	
A66	

TABLE 1-continued

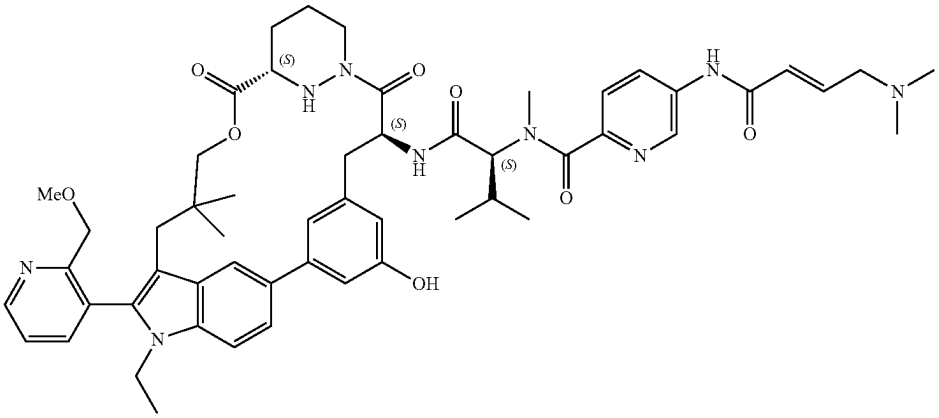
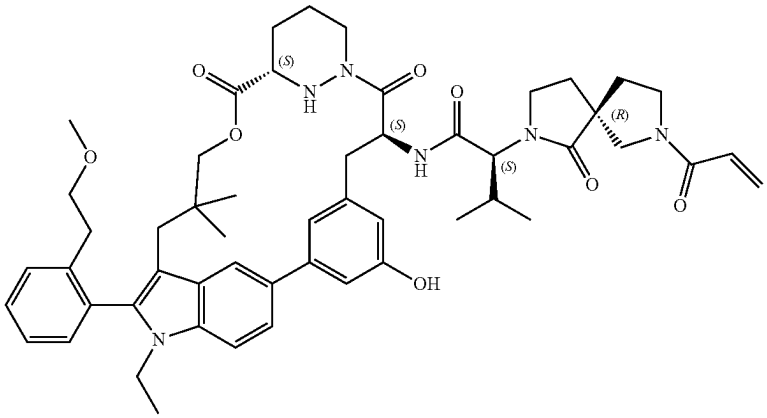
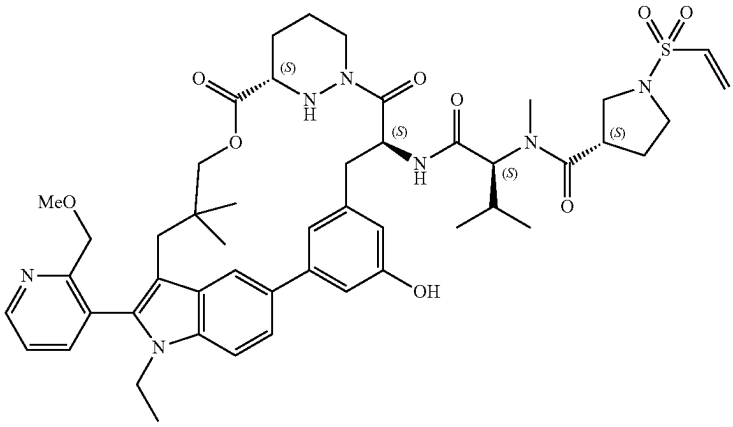
Certain Compounds of the Present Invention	
Ex#	Structure
A67	
A68	
A69	

TABLE 1-continued

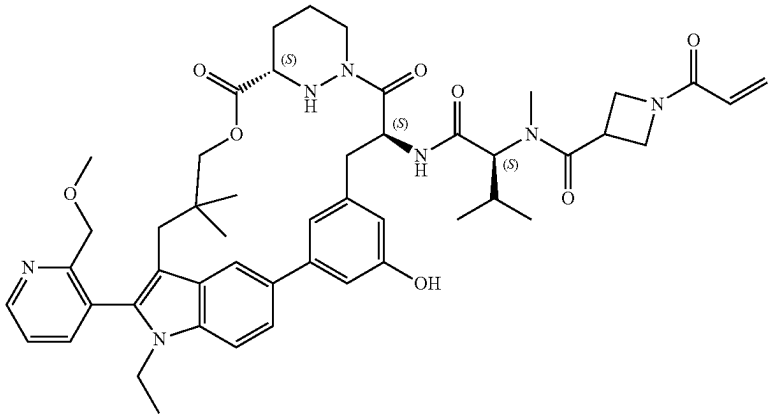
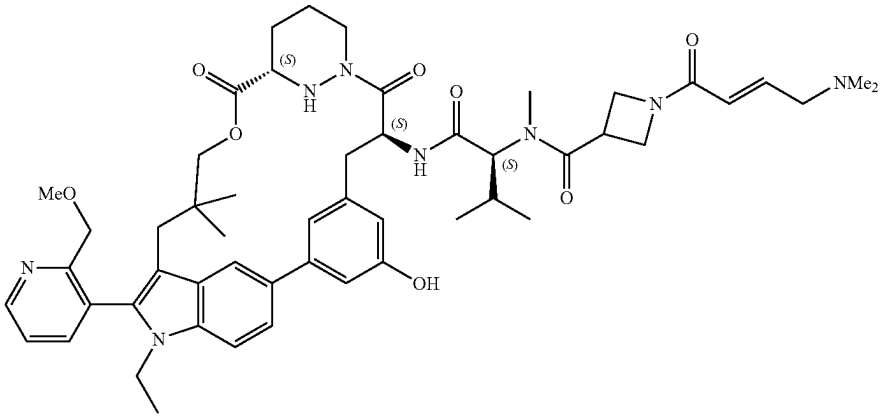
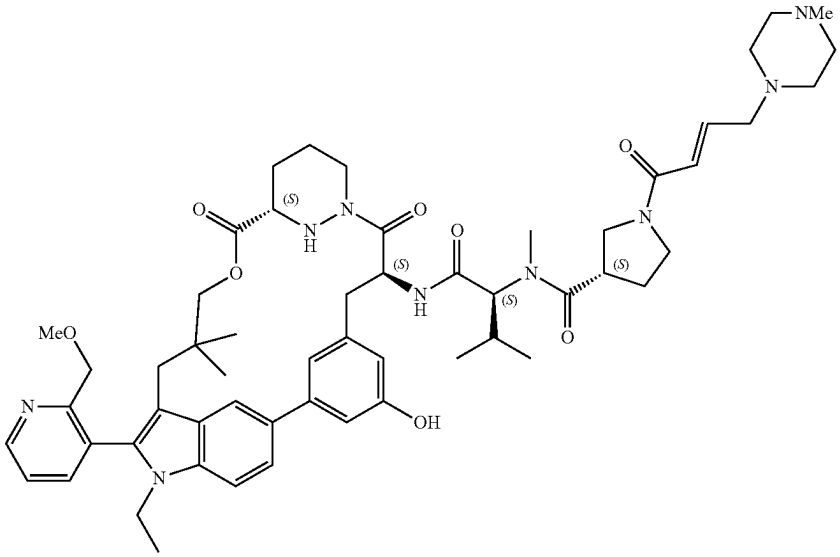
Certain Compounds of the Present Invention	
Ex#	Structure
A70	
A71	
A72	

TABLE 1-continued

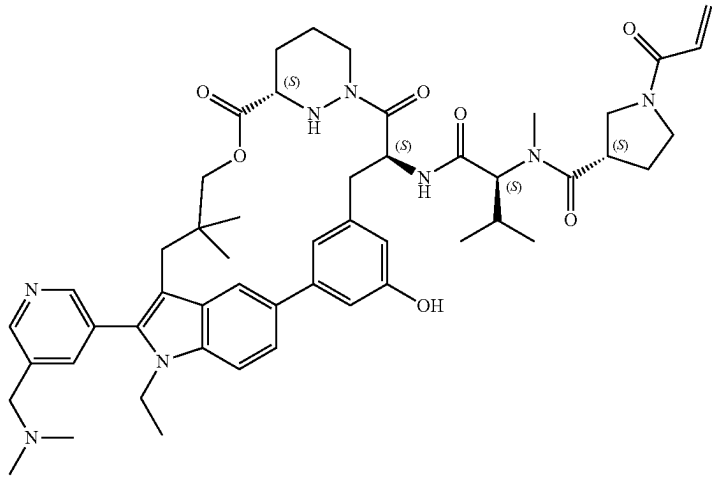
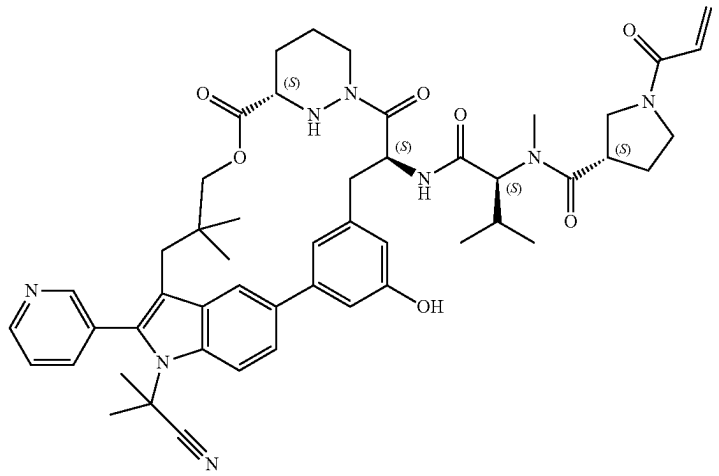
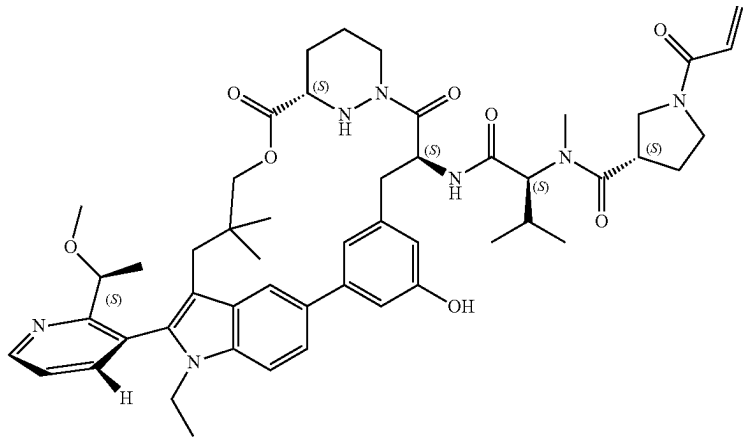
Certain Compounds of the Present Invention	
Ex#	Structure
A73	
A74	
A75	

TABLE 1-continued

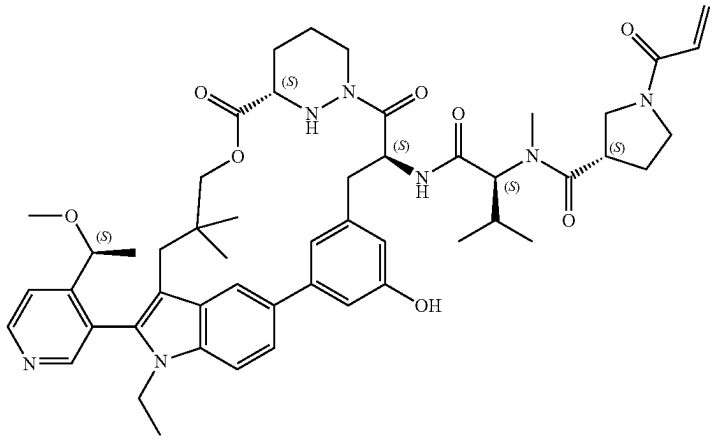
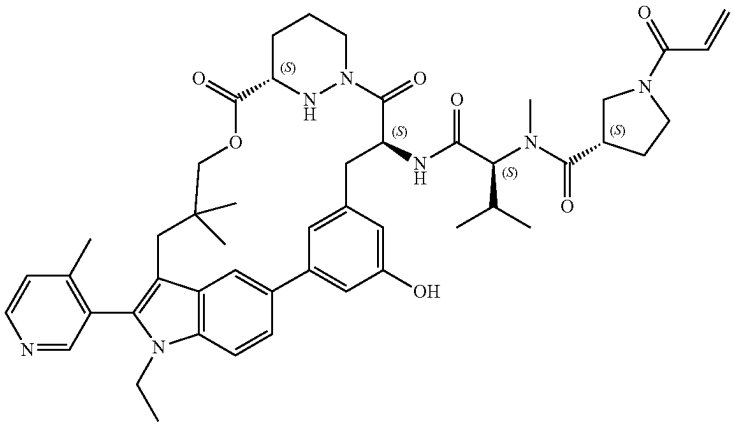
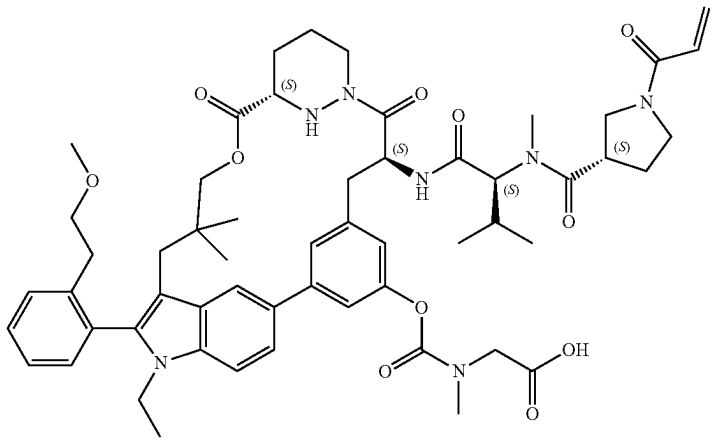
Certain Compounds of the Present Invention	
Ex#	Structure
A76	
A77	
A78	

TABLE 1-continued

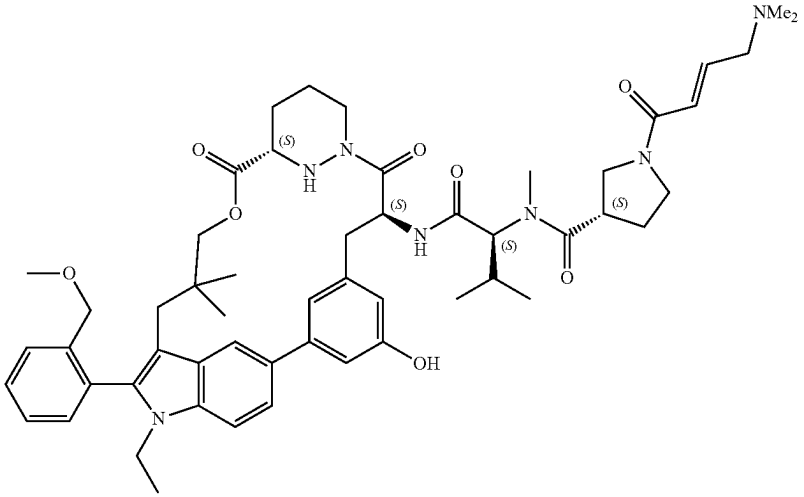
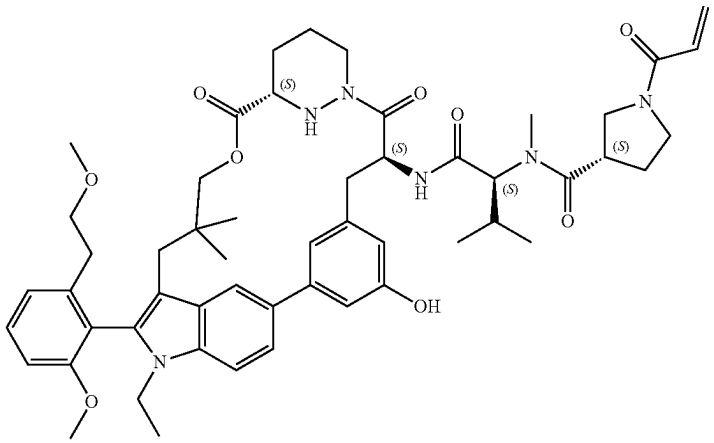
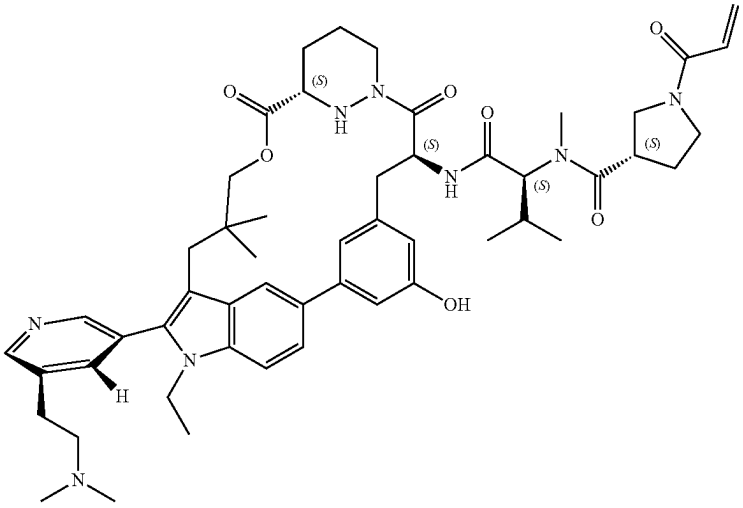
Certain Compounds of the Present Invention	
Ex#	Structure
A79	
A80	
A81	

TABLE 1-continued

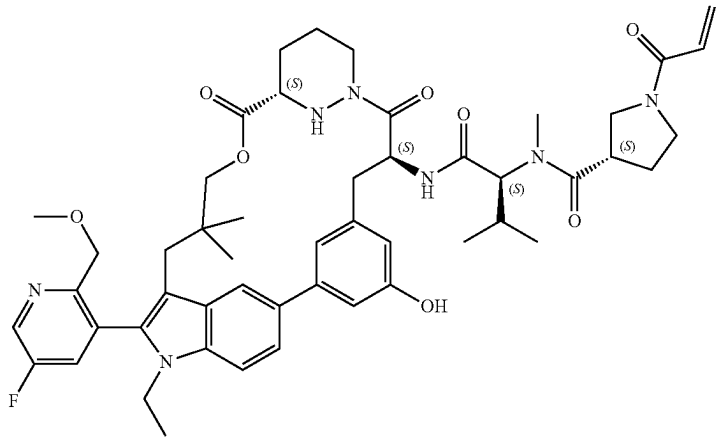
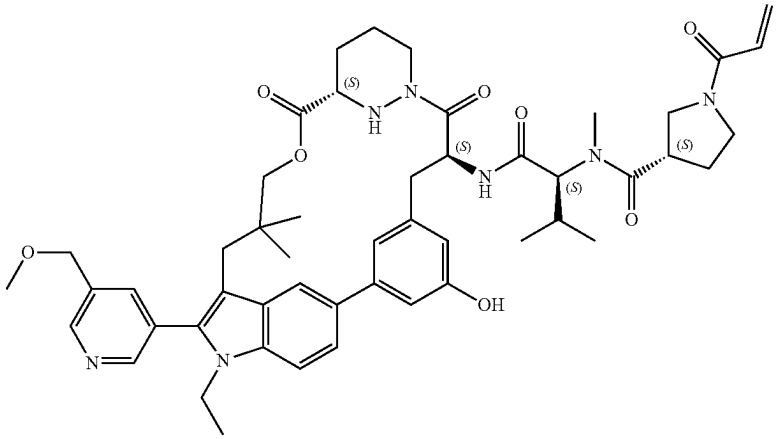
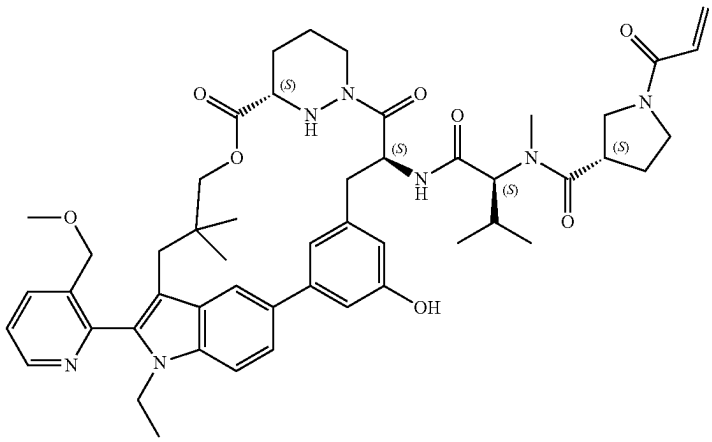
Certain Compounds of the Present Invention	
Ex#	Structure
A82	
A83	
A84	

TABLE 1-continued

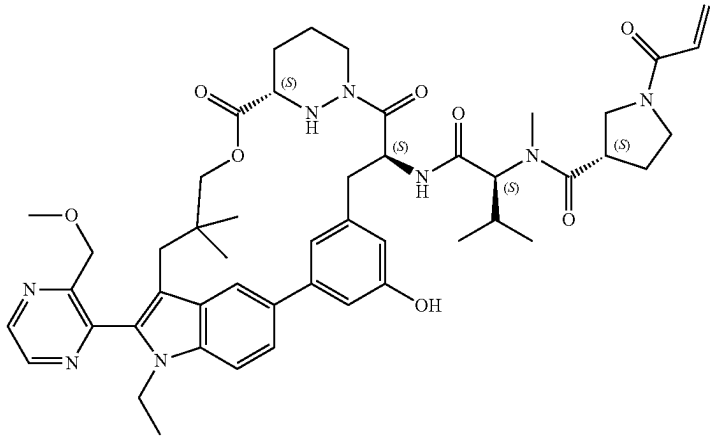
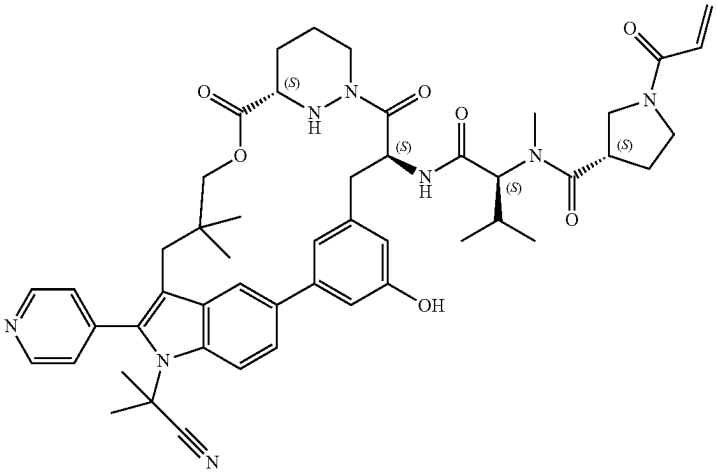
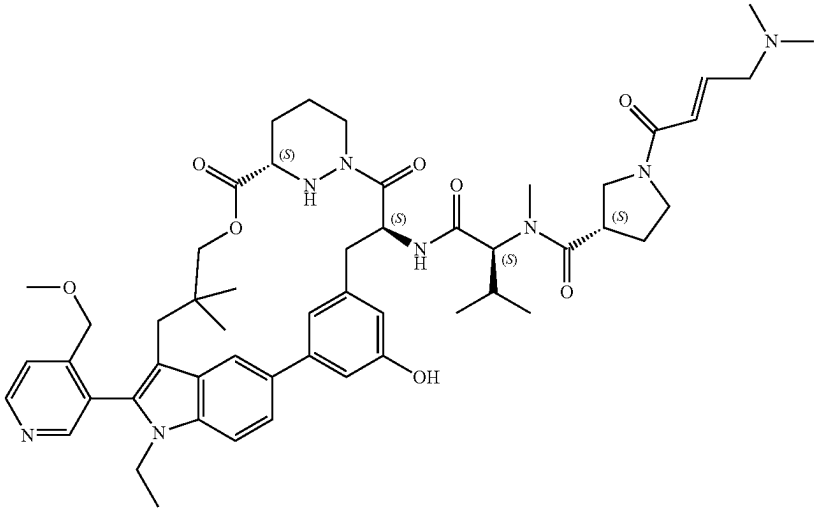
Certain Compounds of the Present Invention	
Ex#	Structure
A85	
A86	
A87	

TABLE 1-continued

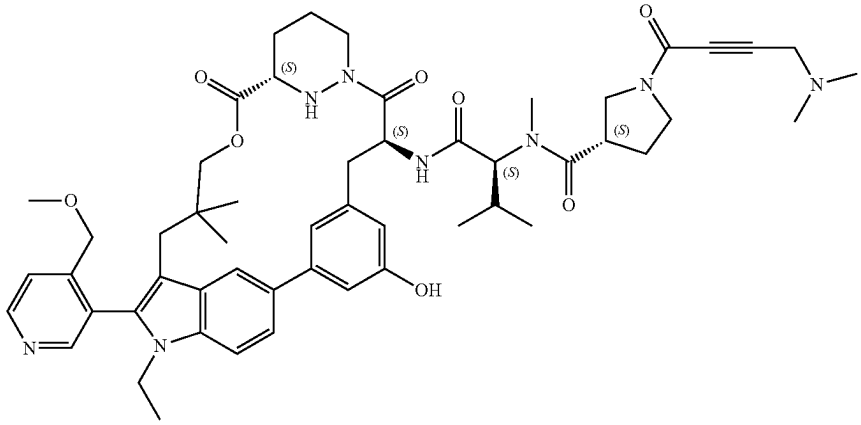
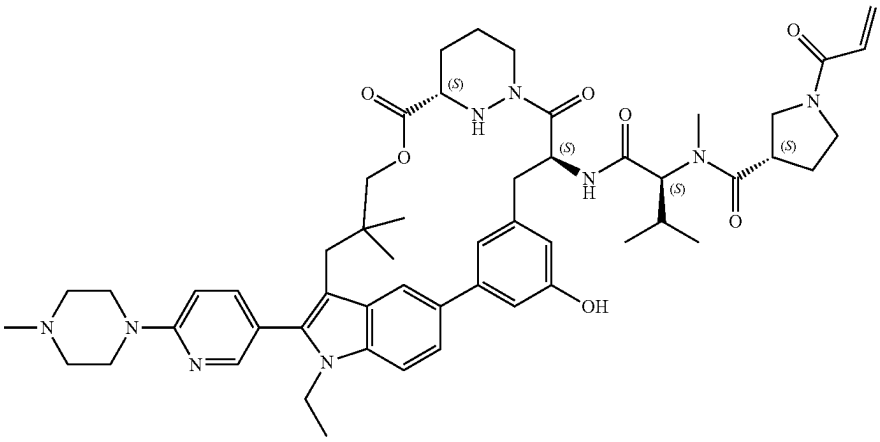
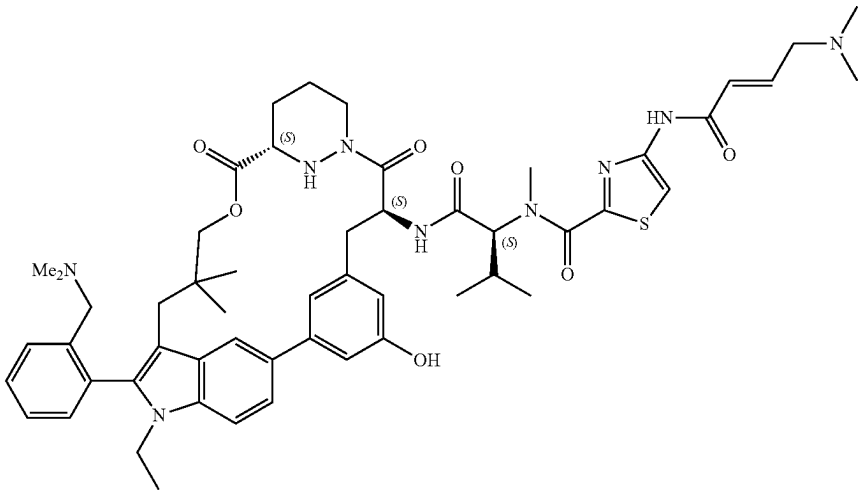
Certain Compounds of the Present Invention	
Ex#	Structure
A88	
A89	
A90	

TABLE 1-continued

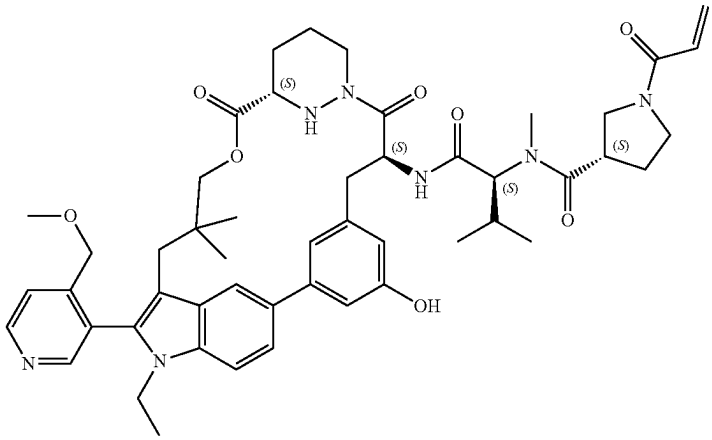
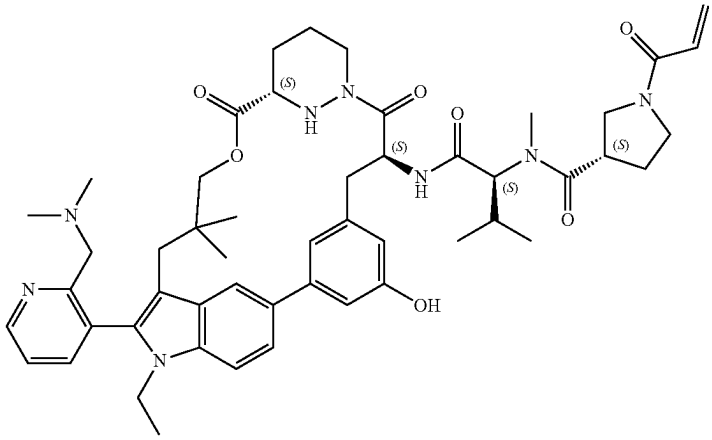
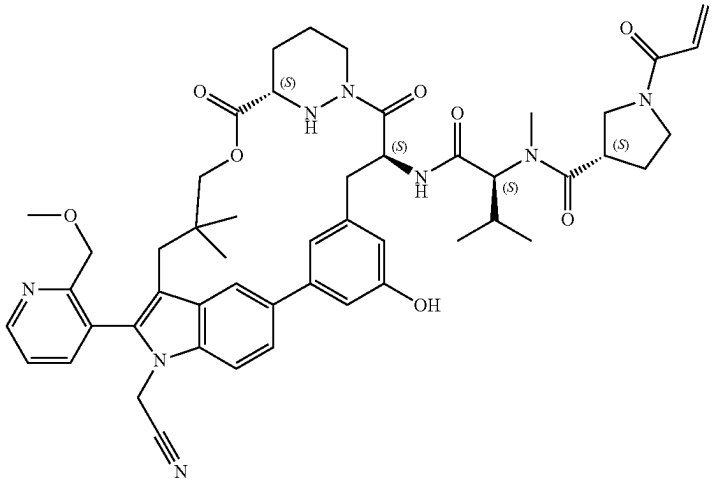
Certain Compounds of the Present Invention	
Ex#	Structure
A91	
A92	
A93	

TABLE 1-continued

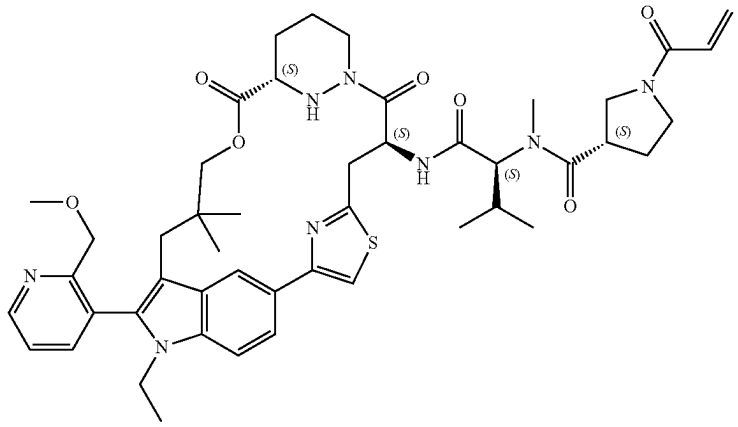
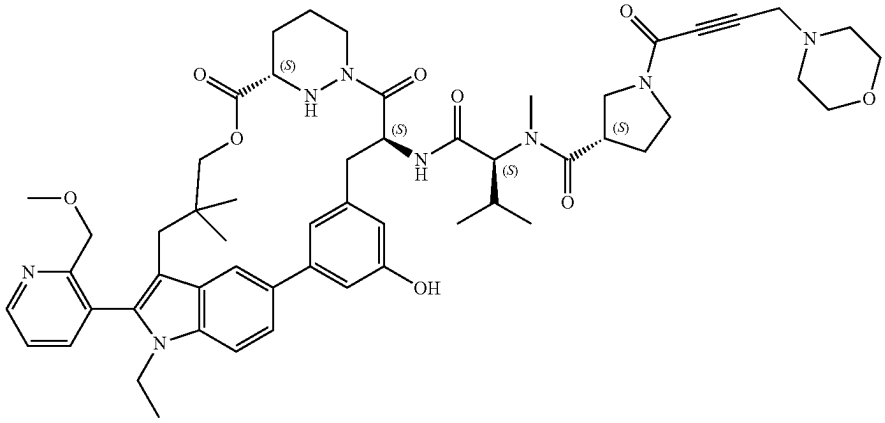
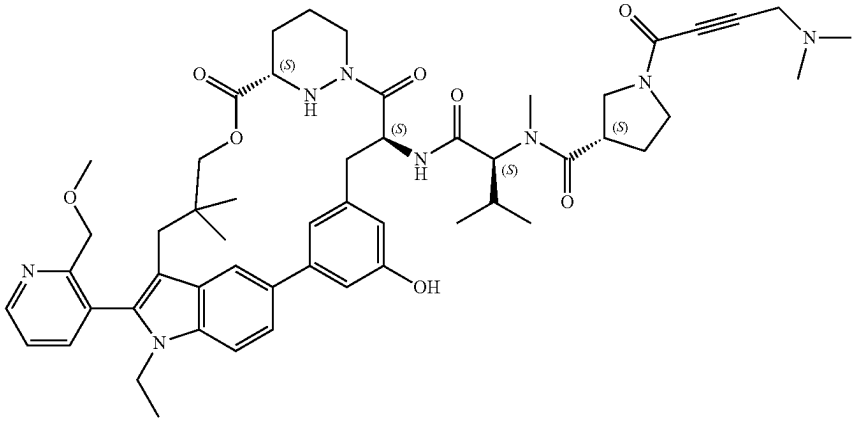
Certain Compounds of the Present Invention	
Ex#	Structure
A94	
A95	
A96	

TABLE 1-continued

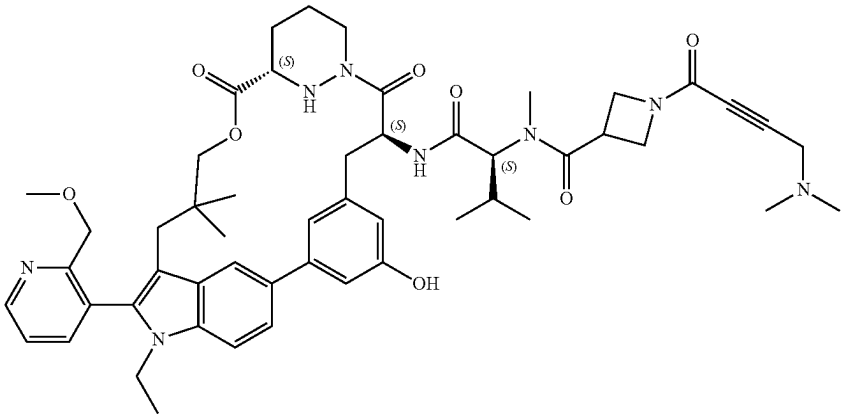
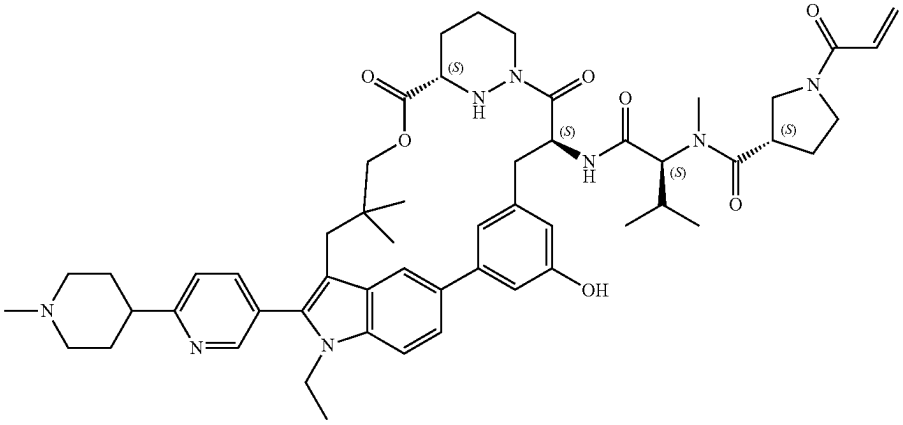
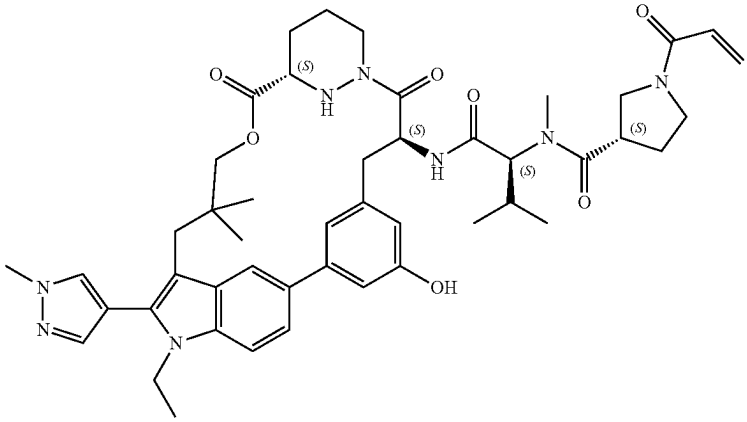
Certain Compounds of the Present Invention	
Ex#	Structure
A97	
A98	
A99	

TABLE 1-continued

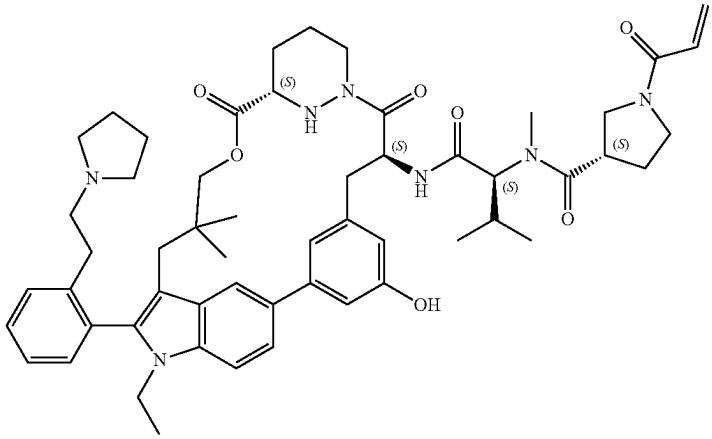
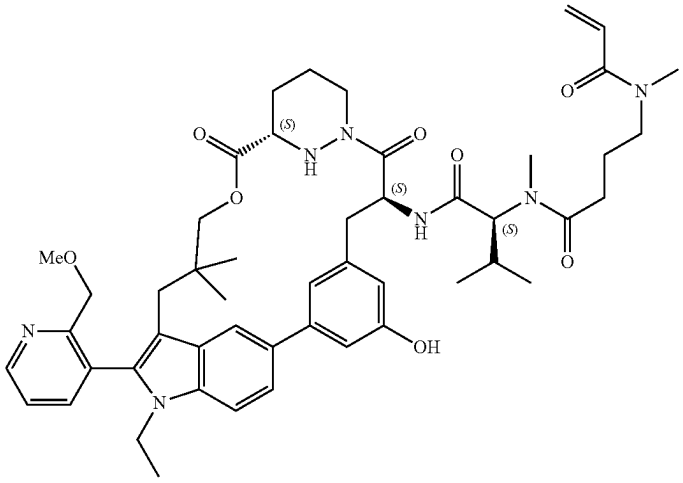
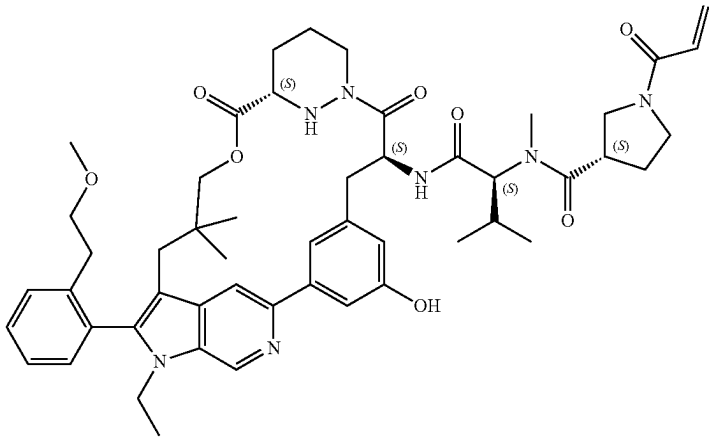
Certain Compounds of the Present Invention	
Ex#	Structure
A100	
A101	
A102	

TABLE 1-continued

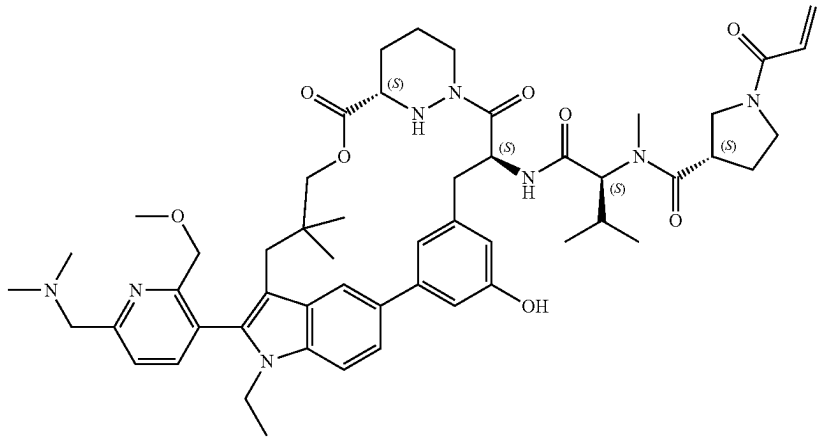
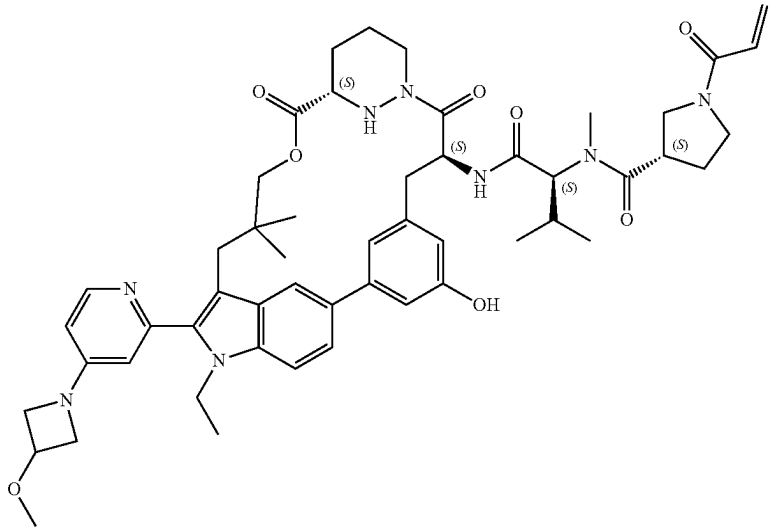
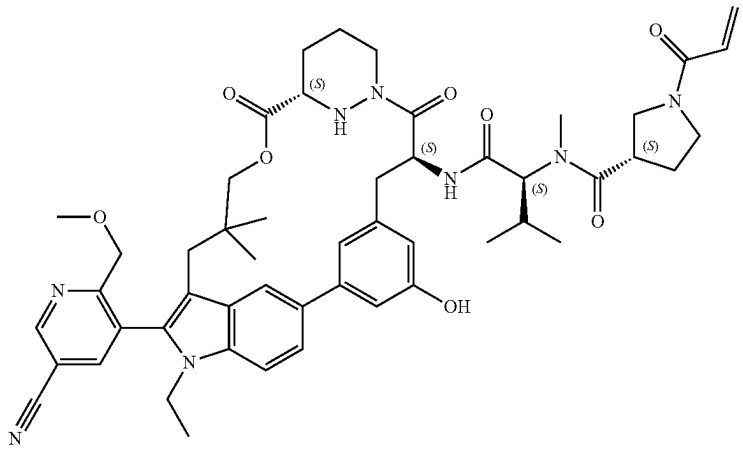
Certain Compounds of the Present Invention	
Ex#	Structure
A103	
A104	
A105	

TABLE 1-continued

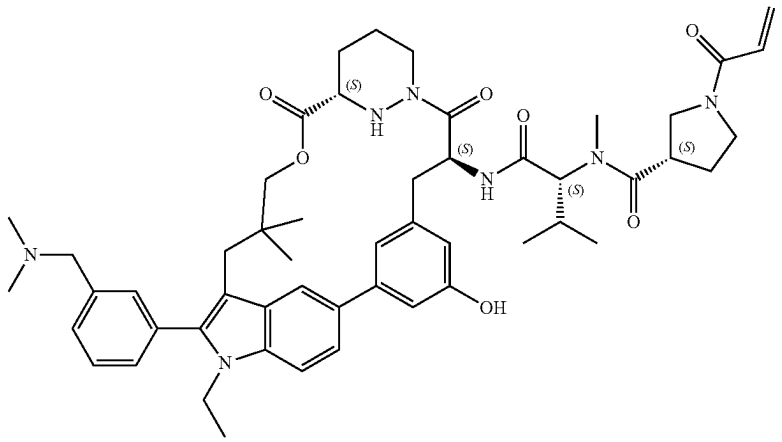
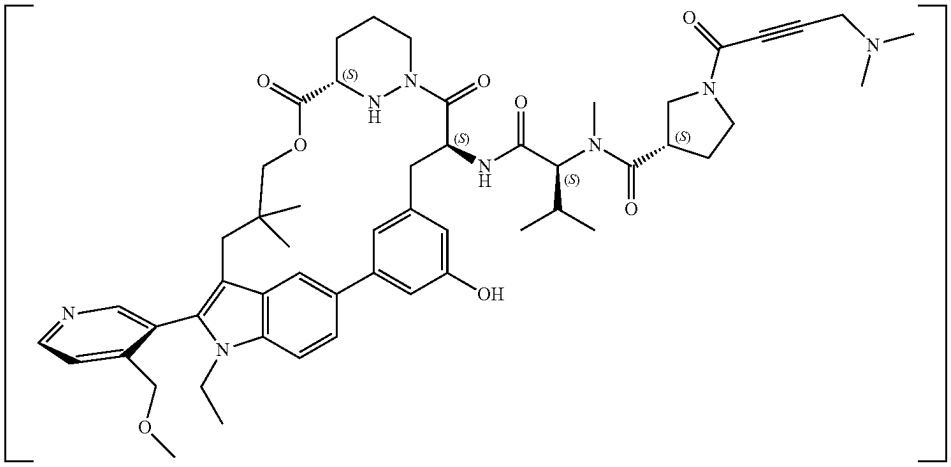
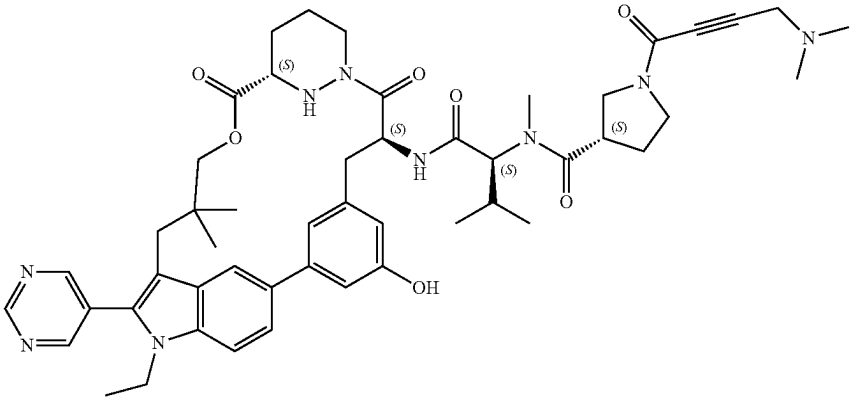
Certain Compounds of the Present Invention	
Ex#	Structure
A106	
A107	
A108	

TABLE 1-continued

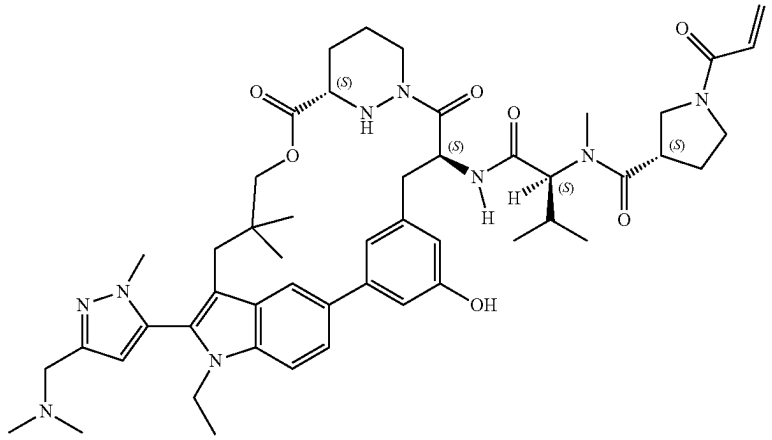
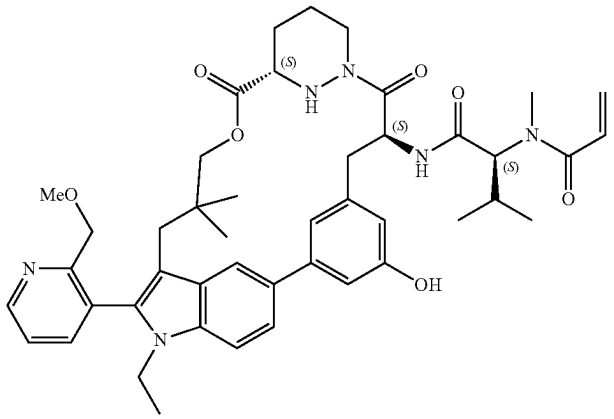
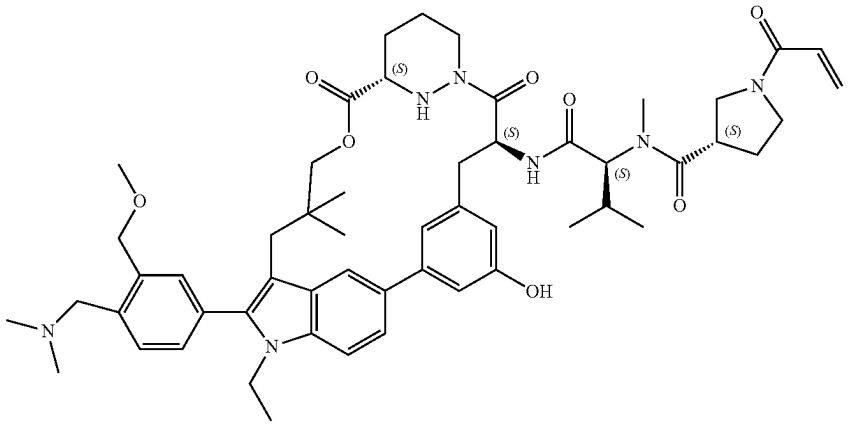
Certain Compounds of the Present Invention	
Ex#	Structure
A109	
A110	
A111	

TABLE 1-continued

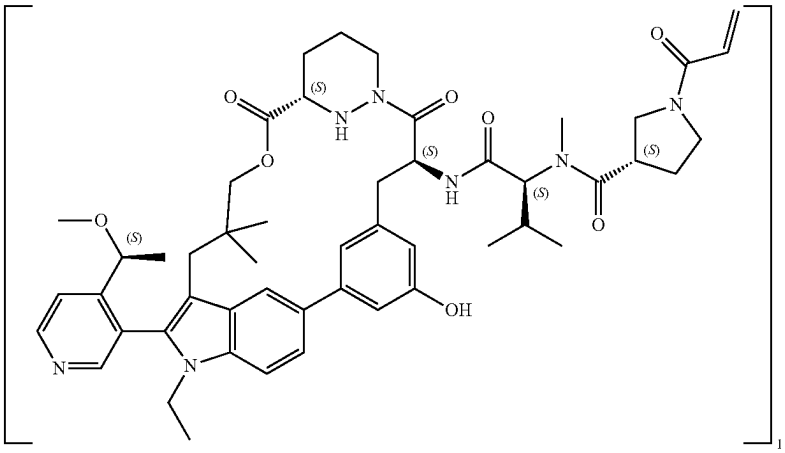
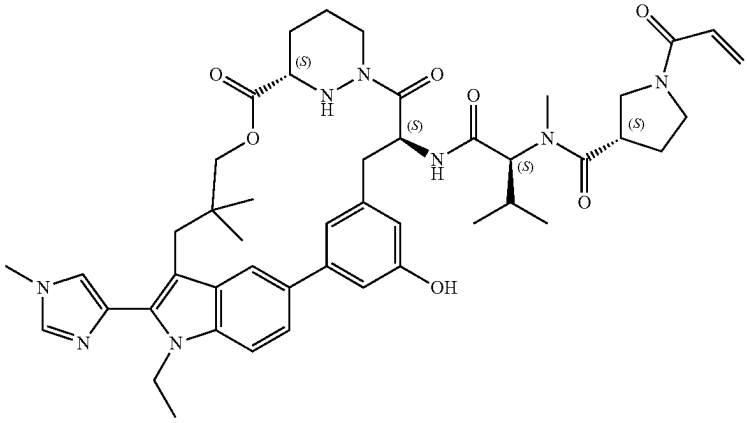
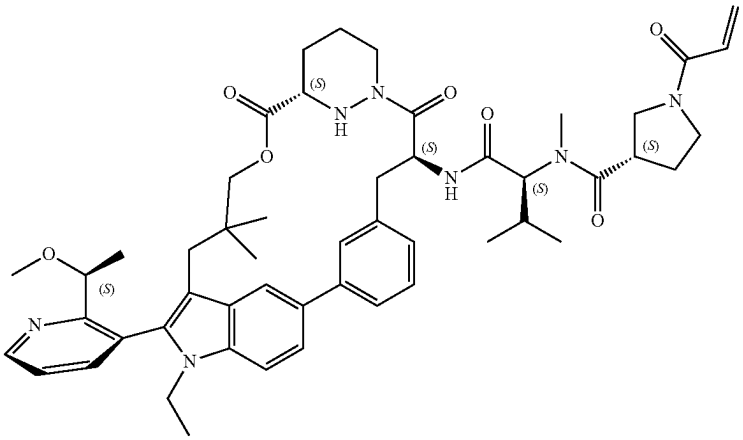
Certain Compounds of the Present Invention	
Ex#	Structure
A112	
A113	
A114	

TABLE 1-continued

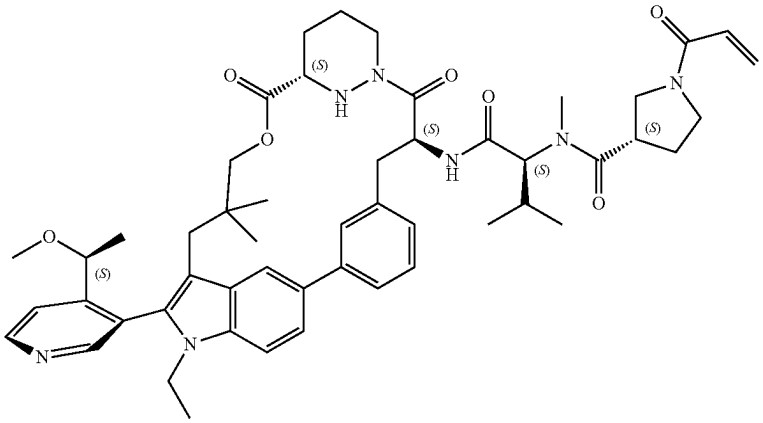
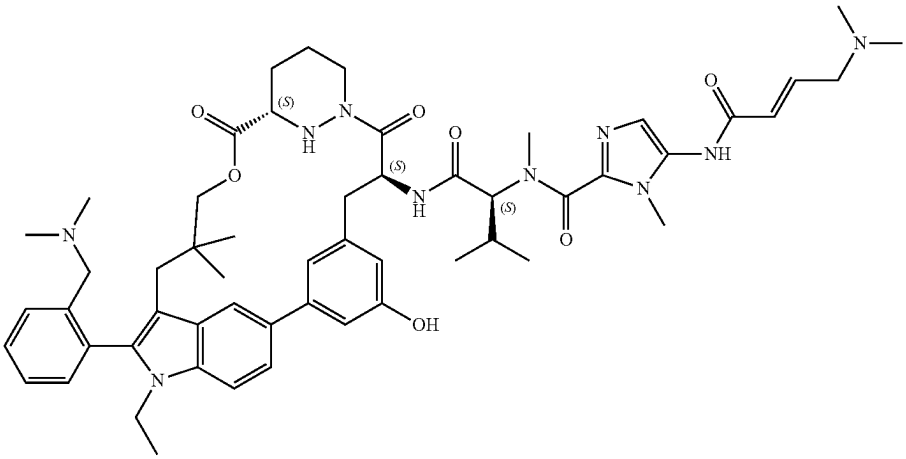
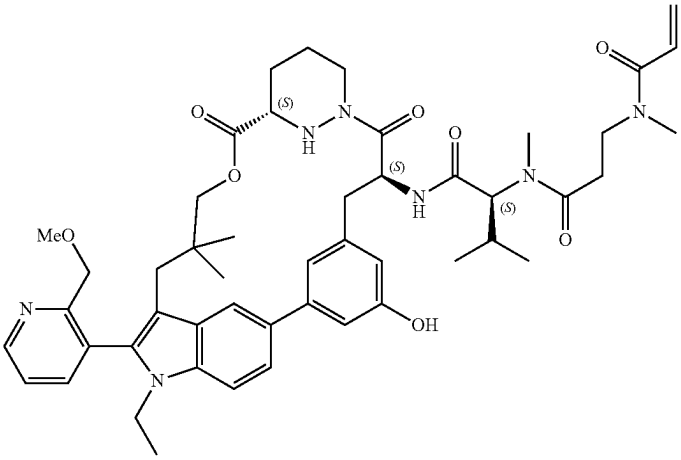
Certain Compounds of the Present Invention	
Ex#	Structure
A115	
A116	
A117	

TABLE 1-continued

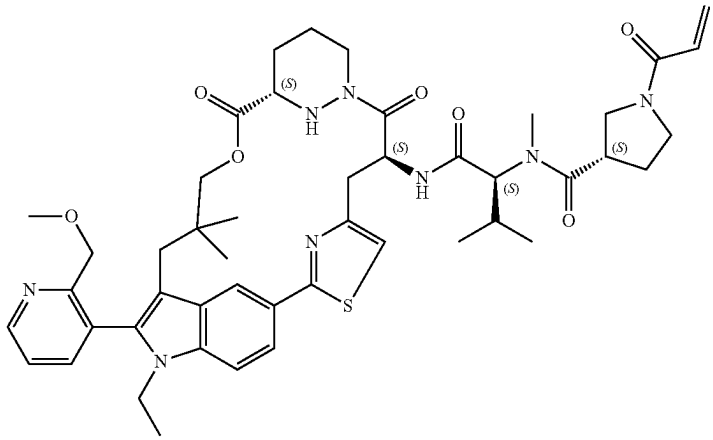
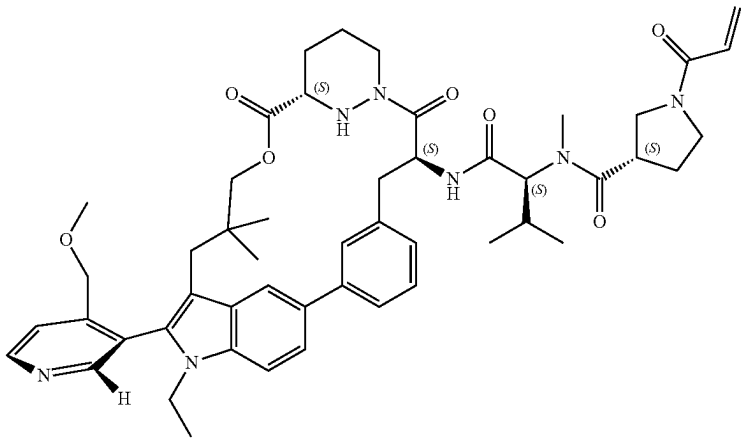
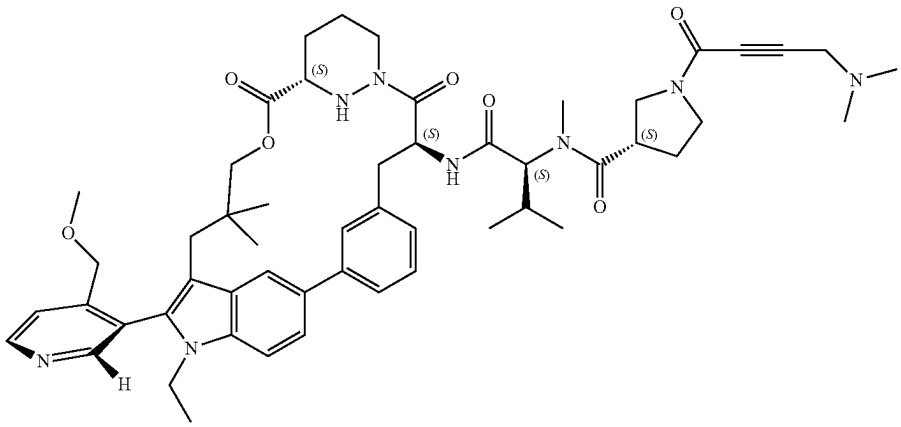
Certain Compounds of the Present Invention	
Ex#	Structure
A118	
A119	
A120	

TABLE 1-continued

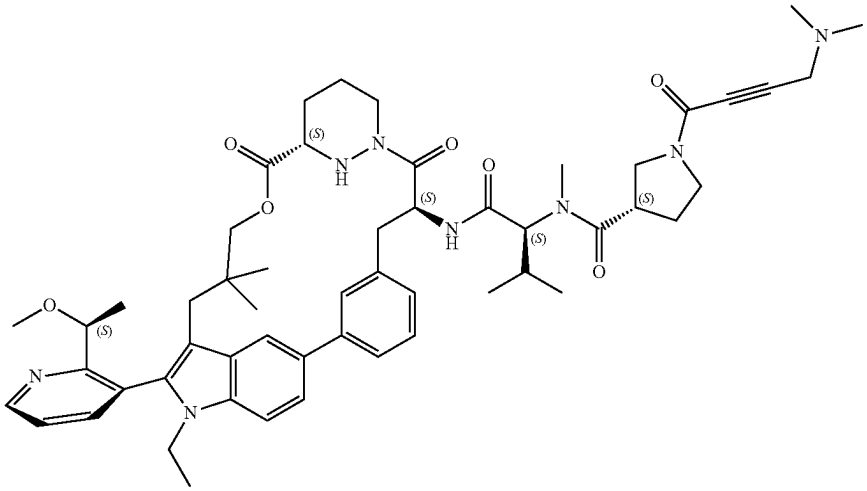
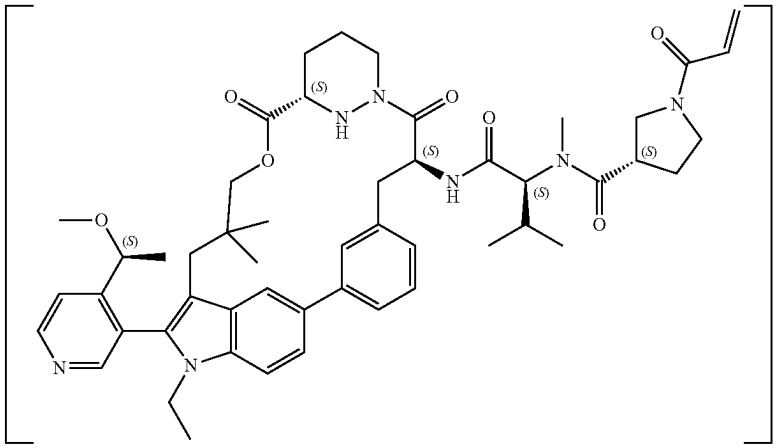
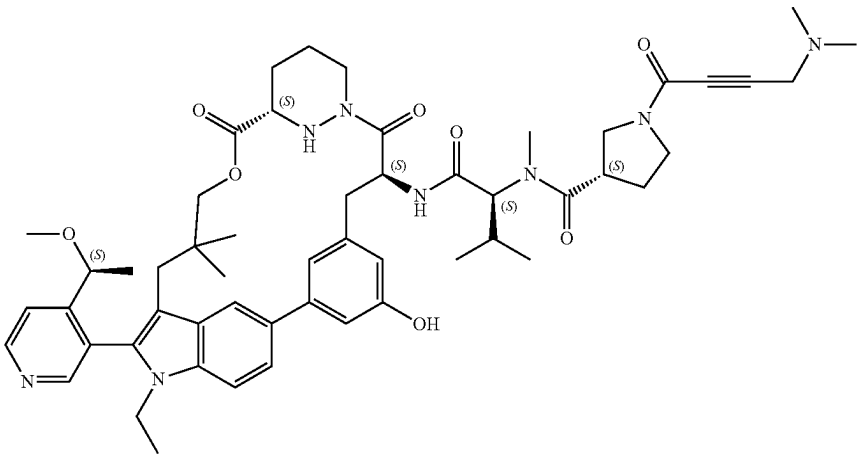
Certain Compounds of the Present Invention	
Ex#	Structure
A121	
A122	
A123	

TABLE 1-continued

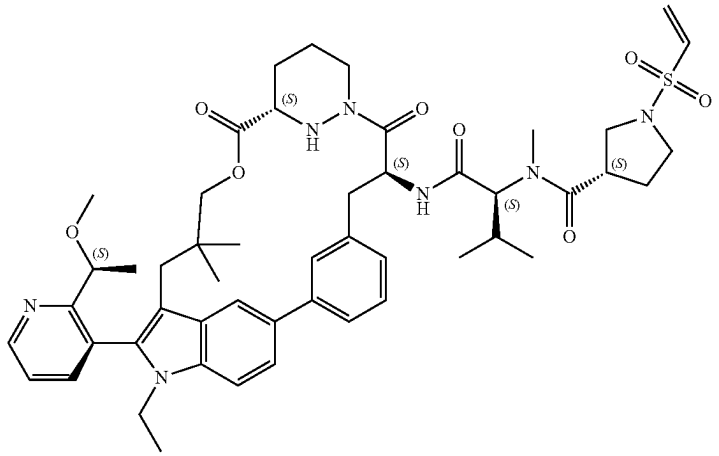
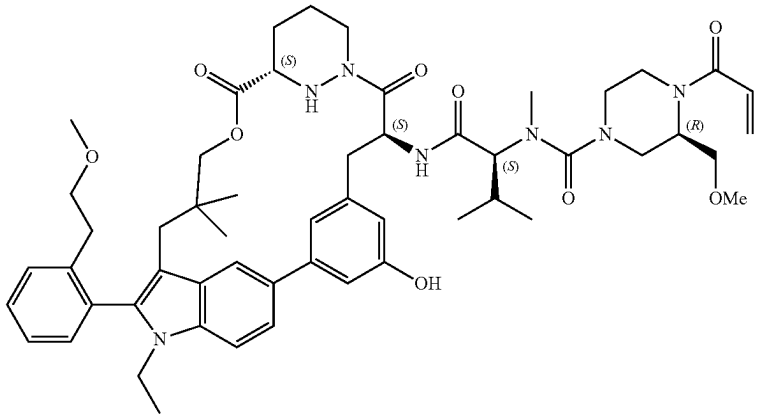
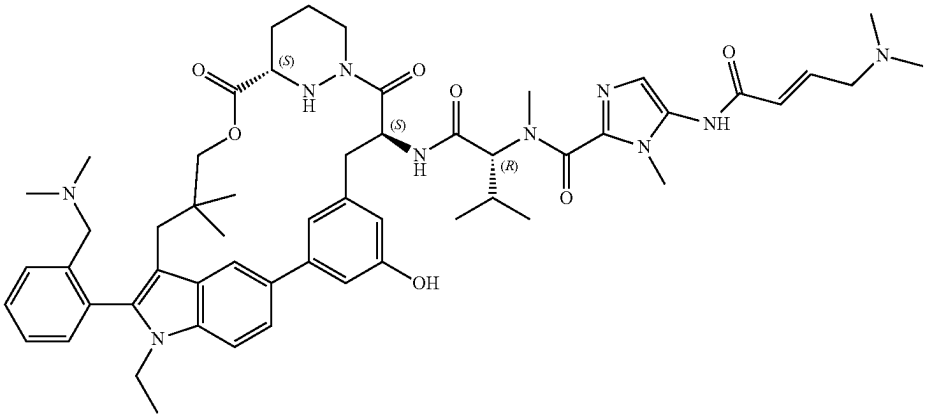
Certain Compounds of the Present Invention	
Ex#	Structure
A124	
A125	
A126	

TABLE 1-continued

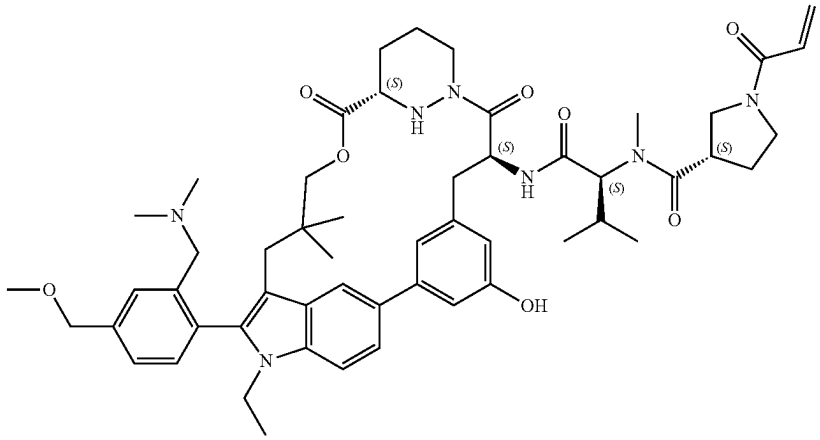
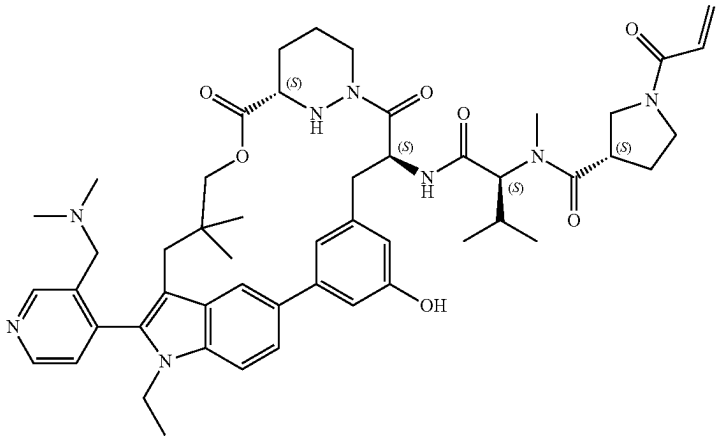
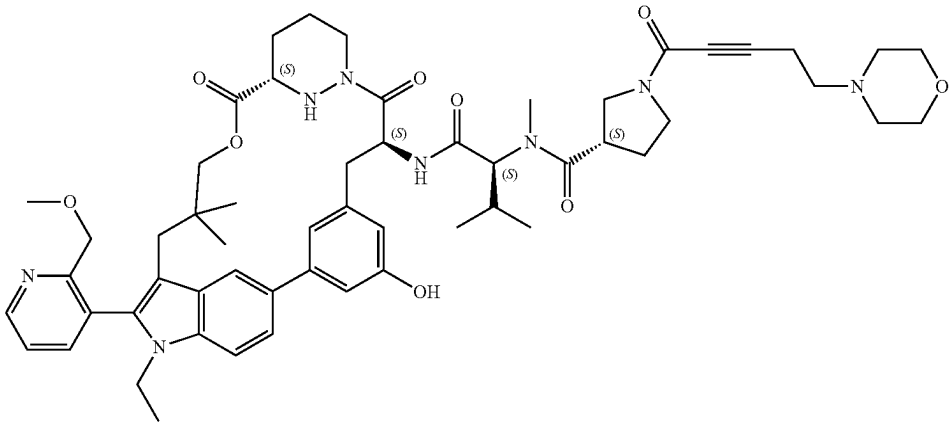
Certain Compounds of the Present Invention	
Ex#	Structure
A127	
A128	
A129	

TABLE 1-continued

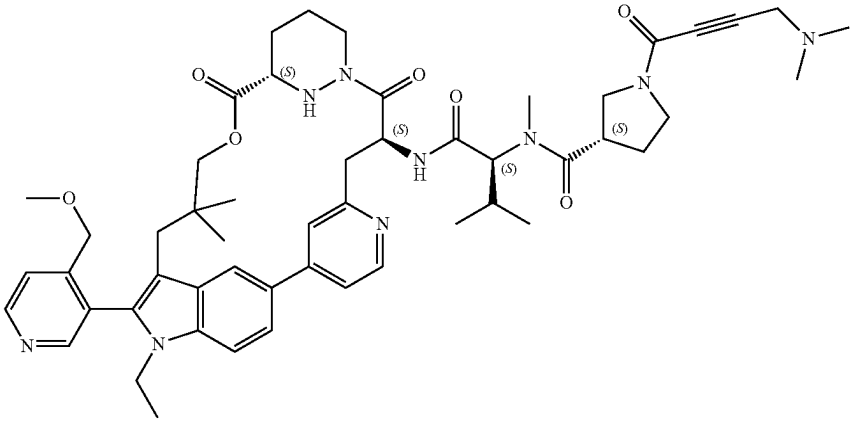
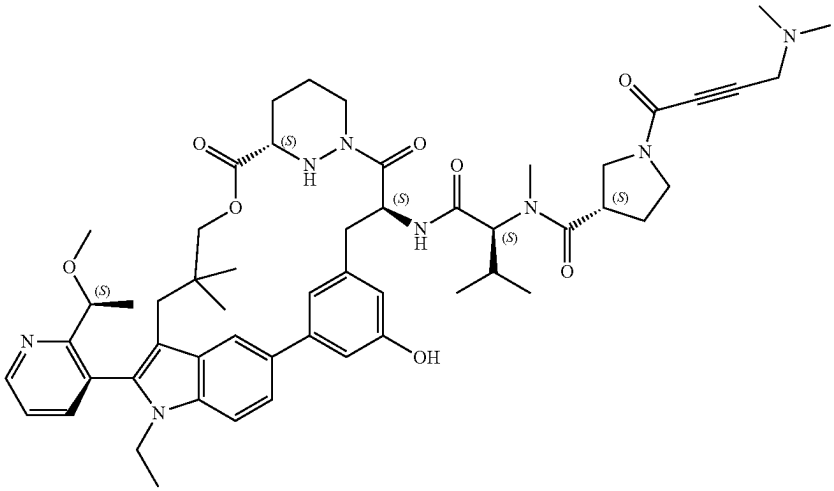
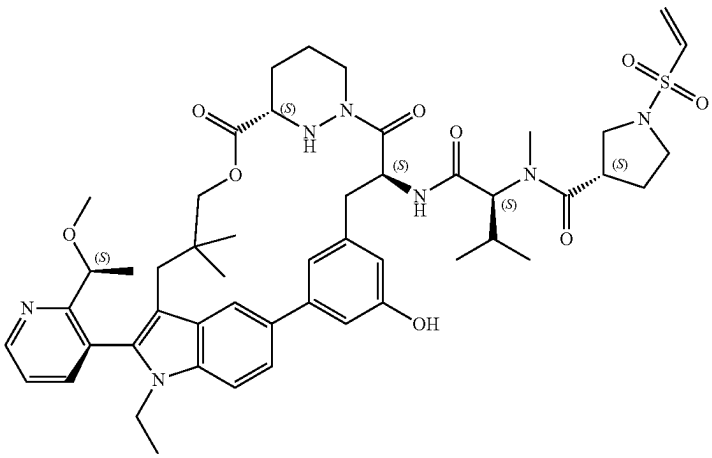
Certain Compounds of the Present Invention	
Ex#	Structure
A130	
A131	
A132	

TABLE 1-continued

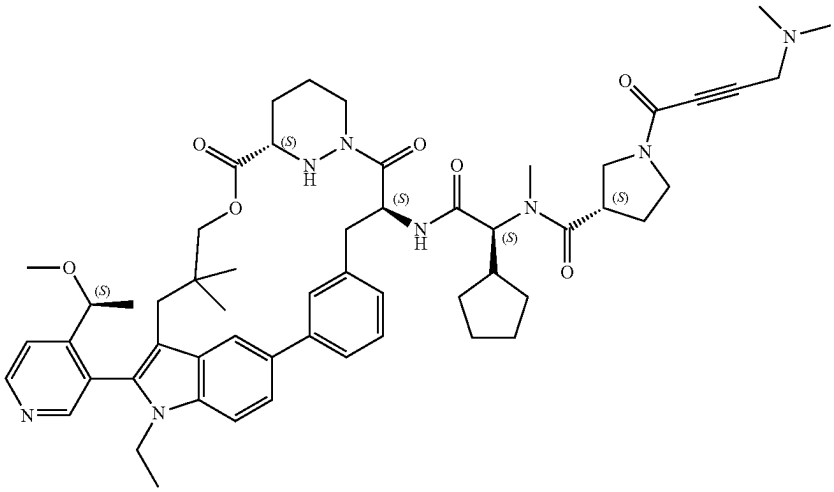
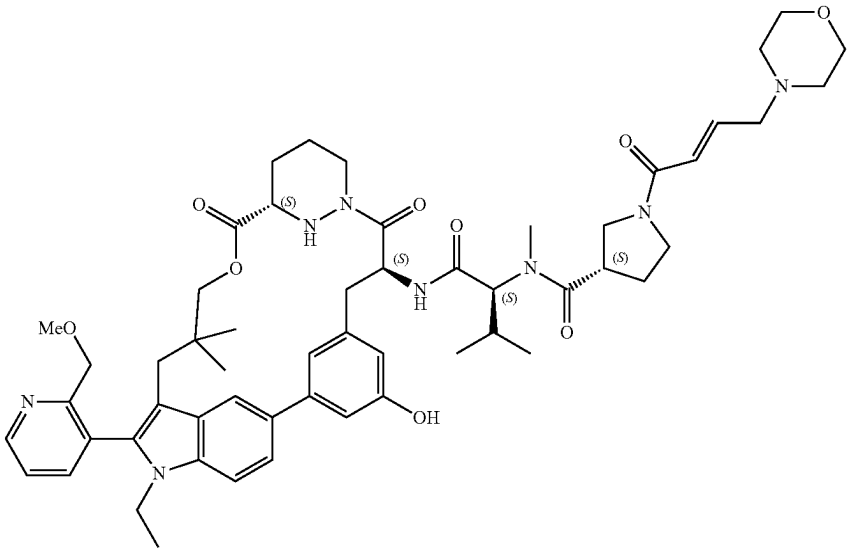
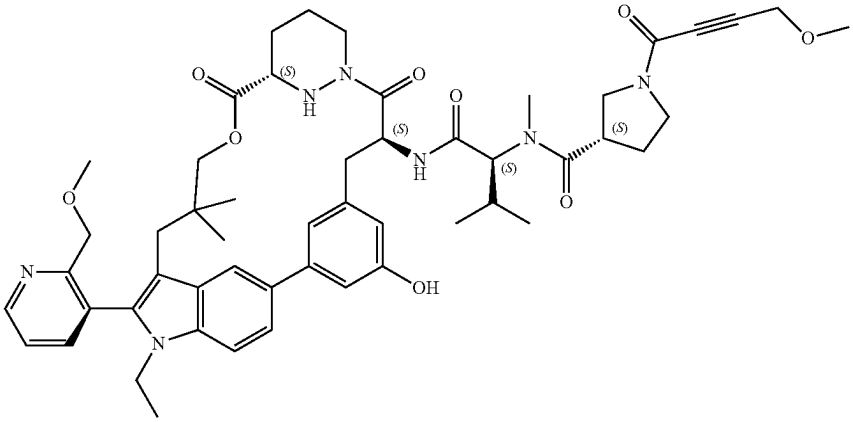
Certain Compounds of the Present Invention	
Ex#	Structure
A133	
A134	
A135	

TABLE 1-continued

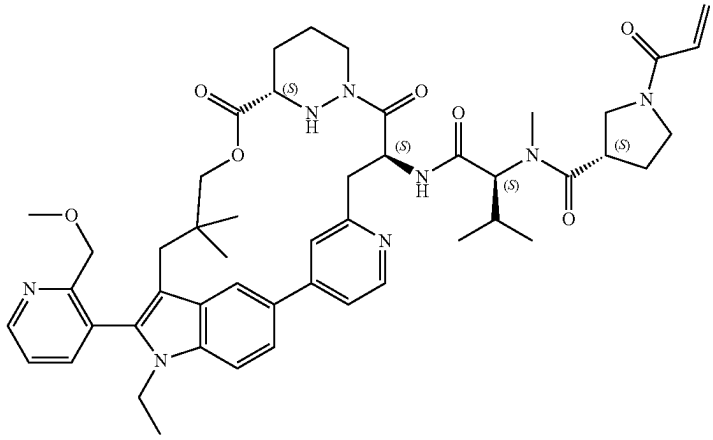
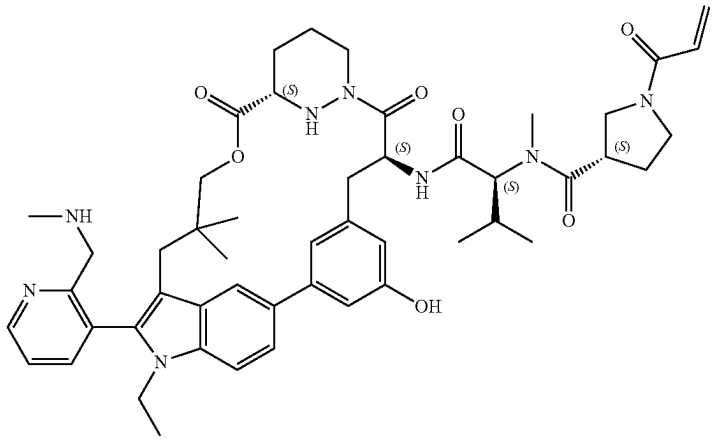
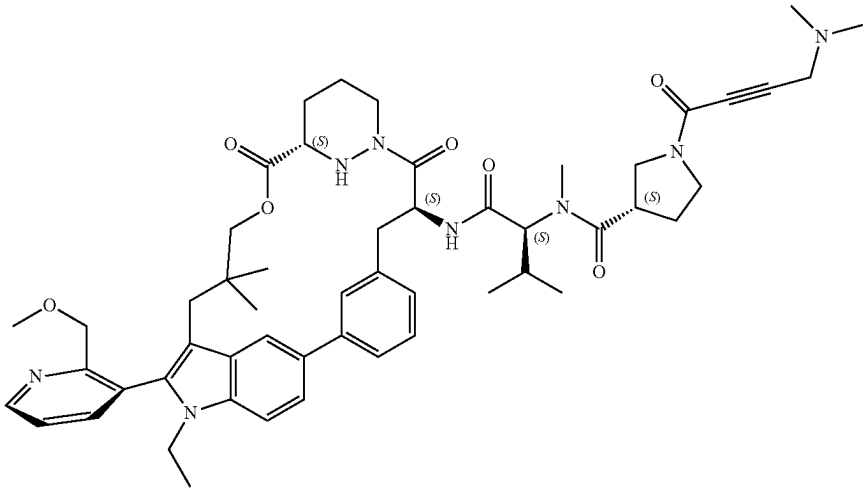
Certain Compounds of the Present Invention	
Ex#	Structure
A136	
A137	
A138	

TABLE 1-continued

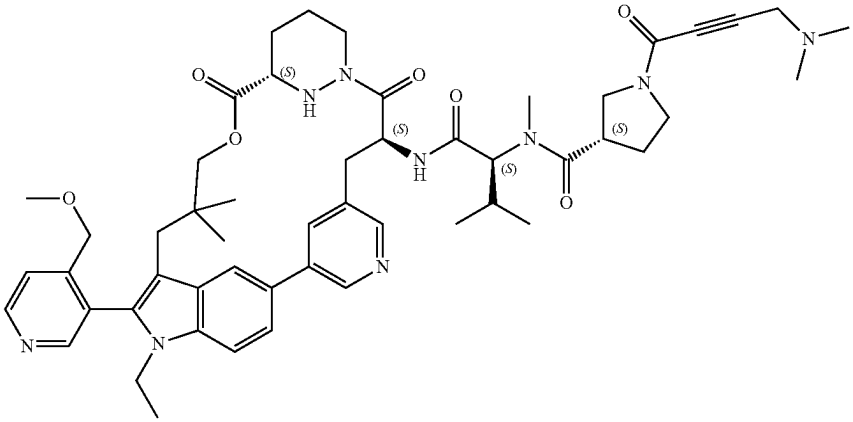
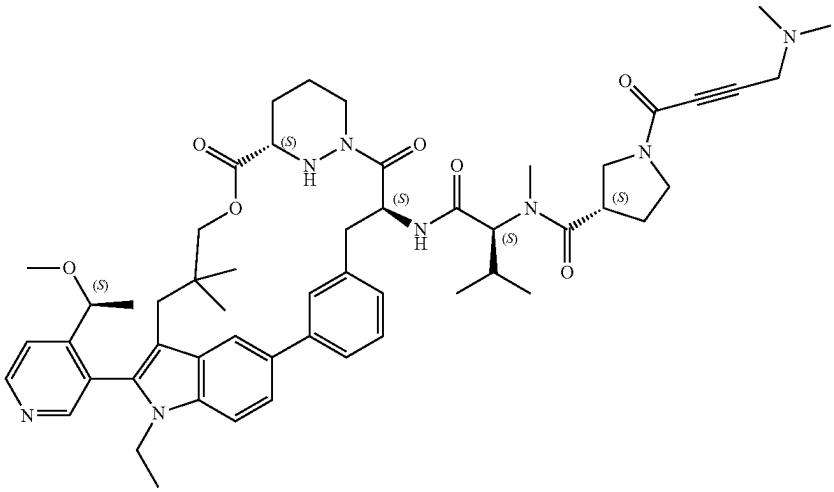
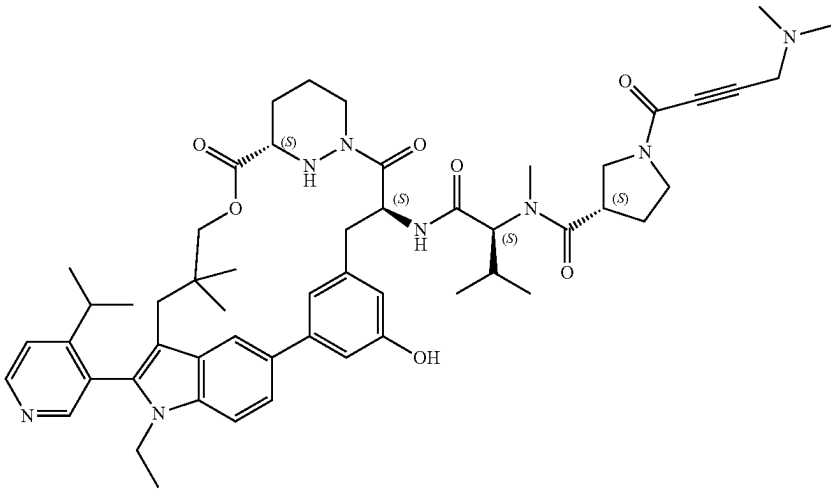
Certain Compounds of the Present Invention	
Ex#	Structure
A139	
A140	
A141	

TABLE 1-continued

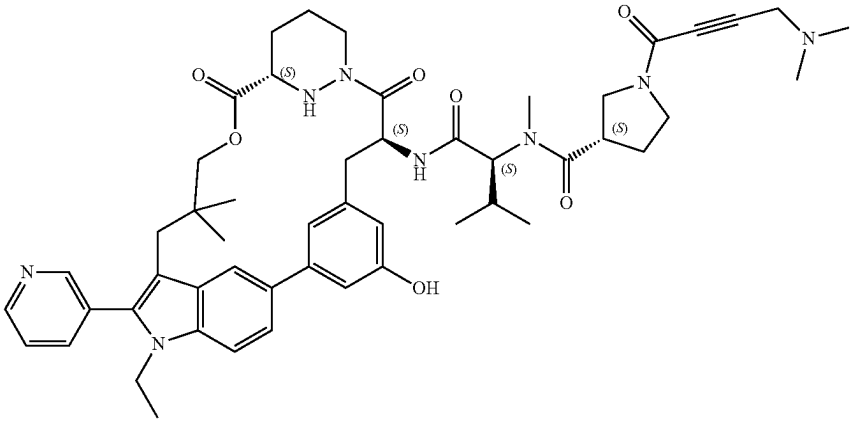
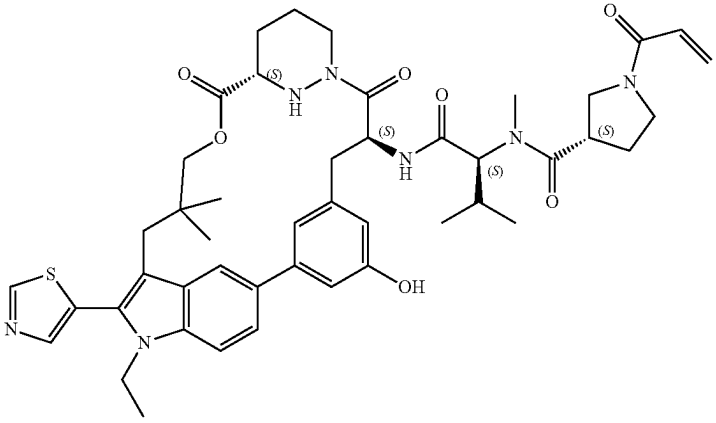
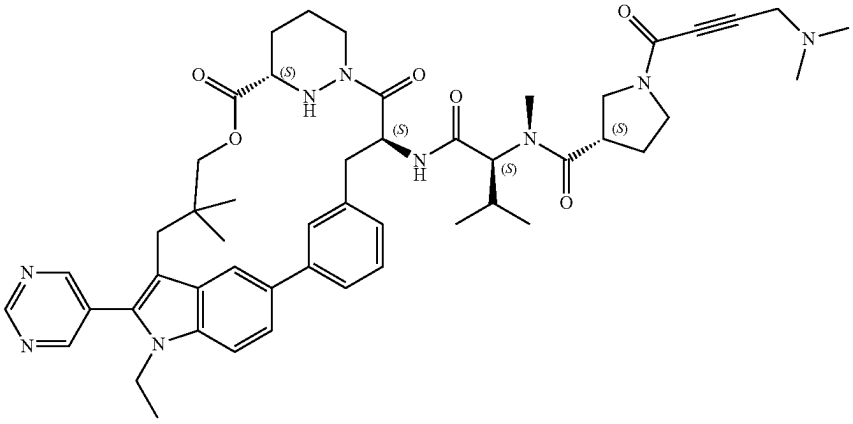
Certain Compounds of the Present Invention	
Ex#	Structure
A142	
A143	
A144	

TABLE 1-continued

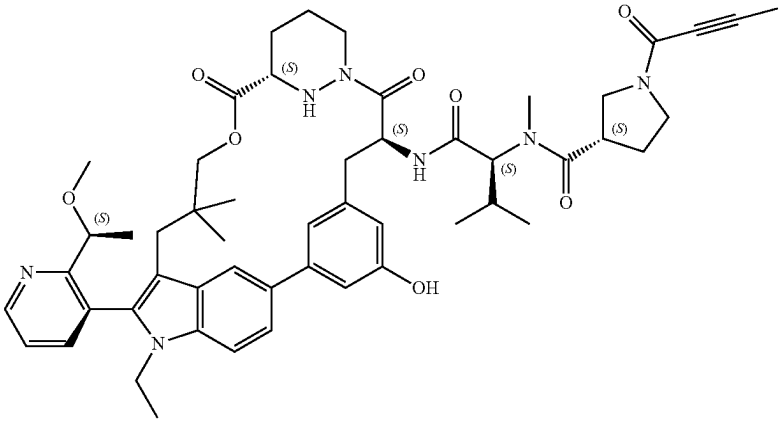
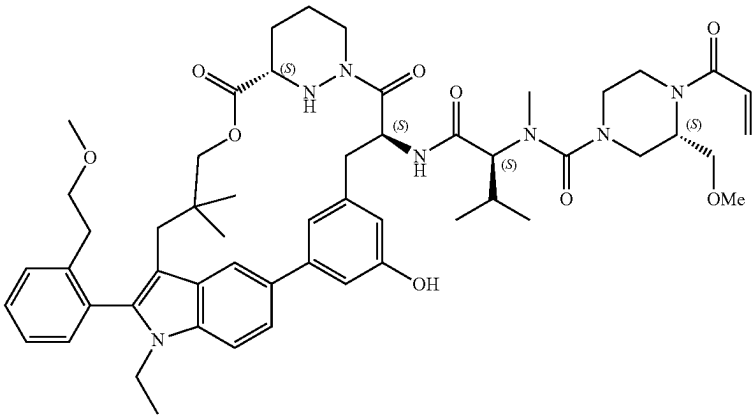
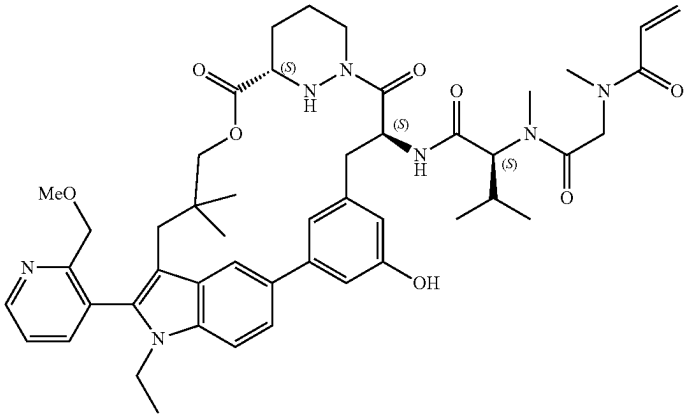
Certain Compounds of the Present Invention	
Ex#	Structure
A145	
A146	
A147	

TABLE 1-continued

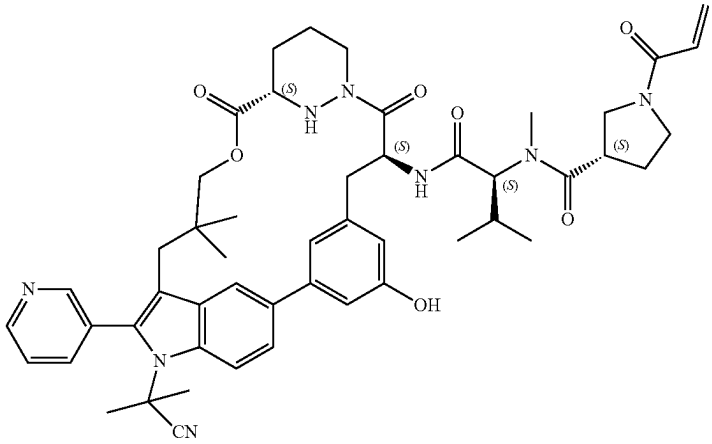
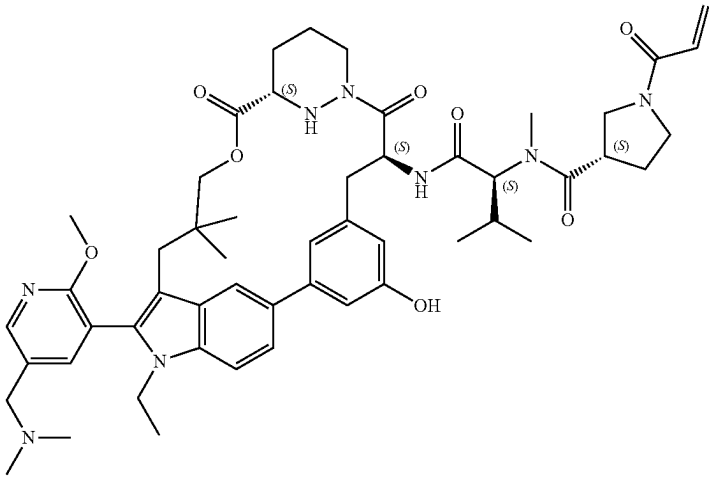
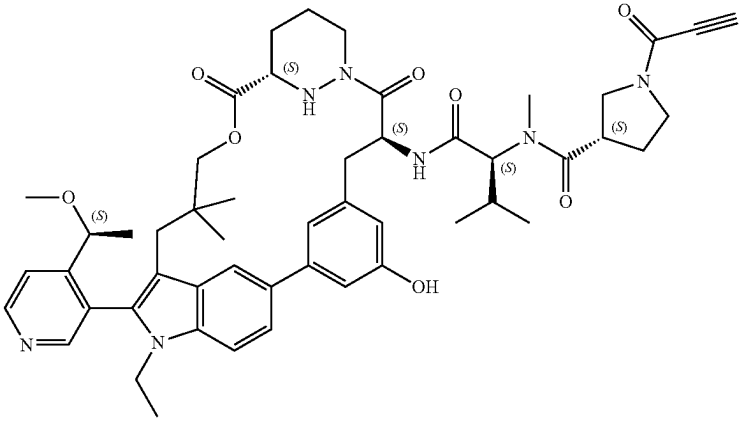
Certain Compounds of the Present Invention	
Ex#	Structure
A148	
A149	
A150	

TABLE 1-continued

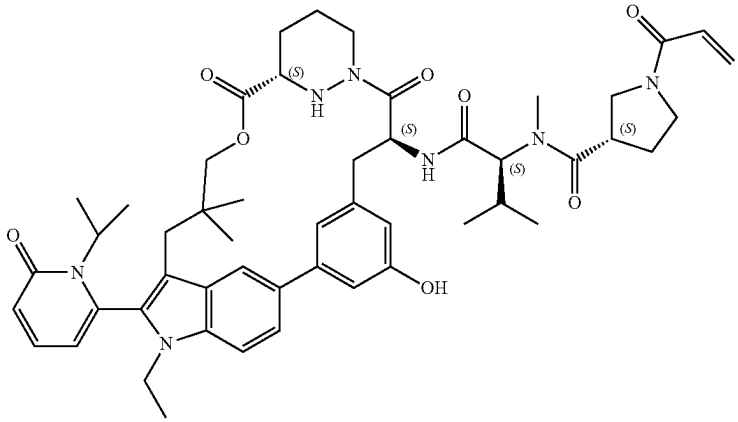
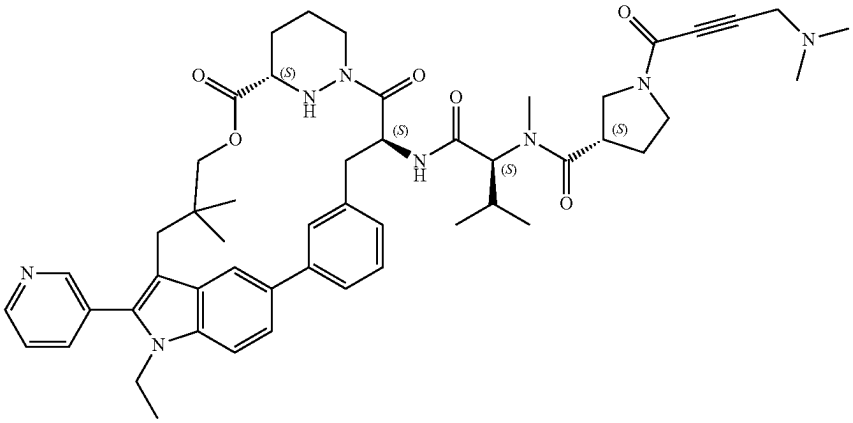
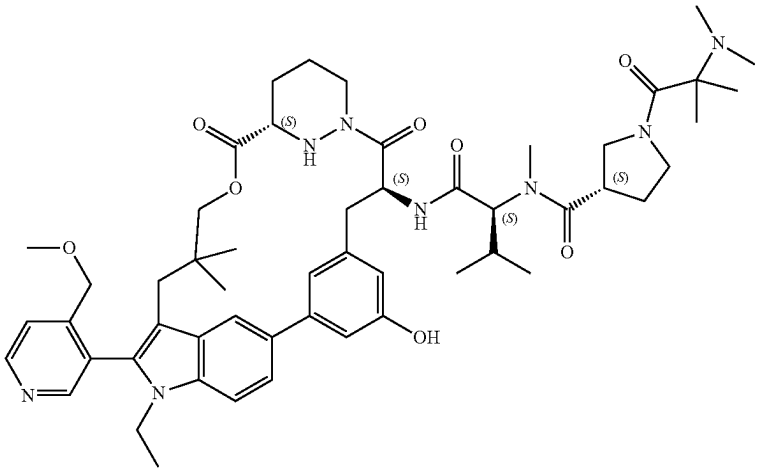
Certain Compounds of the Present Invention	
Ex#	Structure
A151	
A152	
A153	

TABLE 1-continued

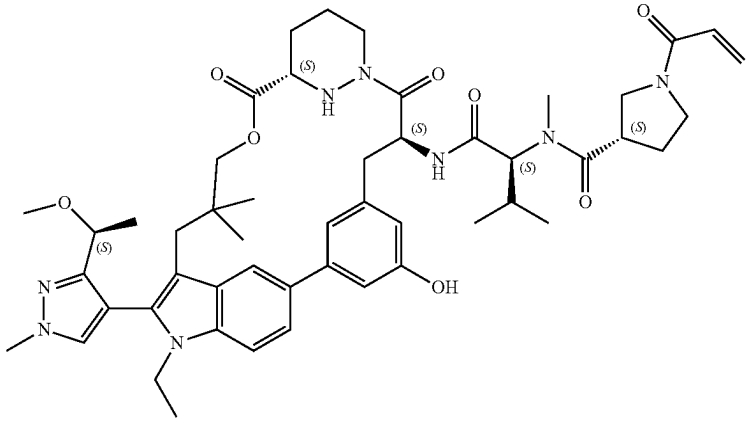
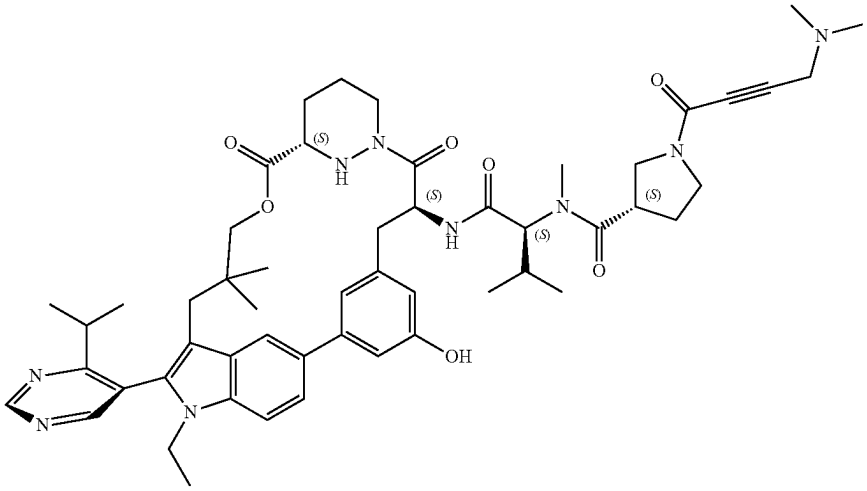
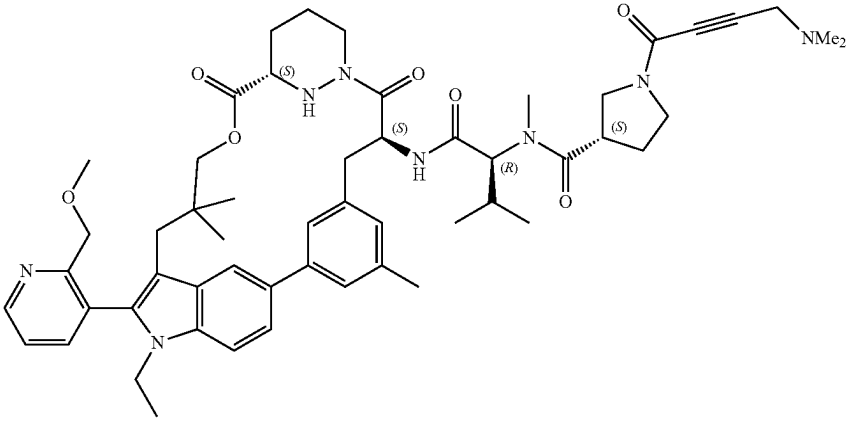
Certain Compounds of the Present Invention	
Ex#	Structure
A154	
A155	
A156	

TABLE 1-continued

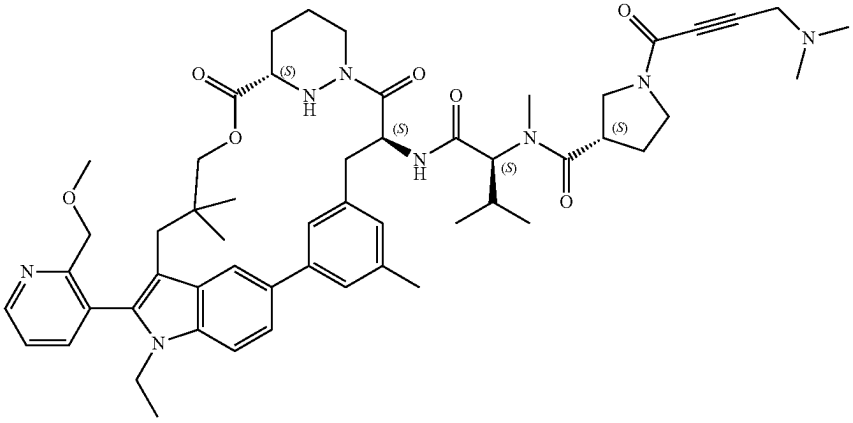
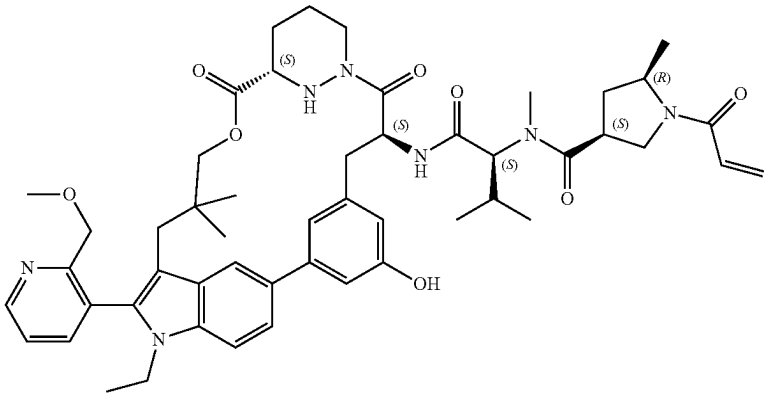
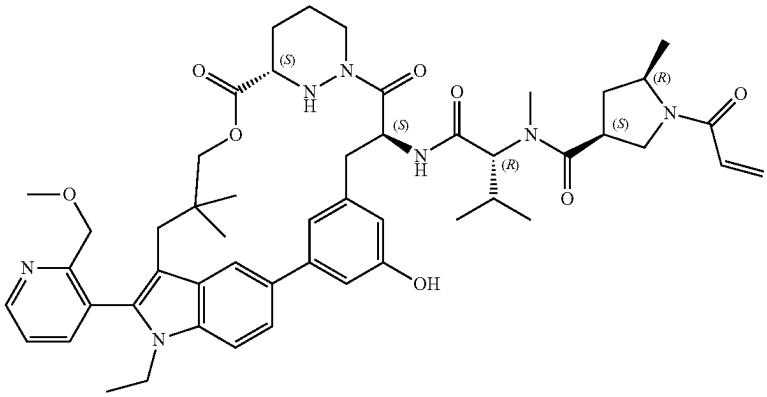
Certain Compounds of the Present Invention	
Ex#	Structure
A157	
A158	
A159	

TABLE 1-continued

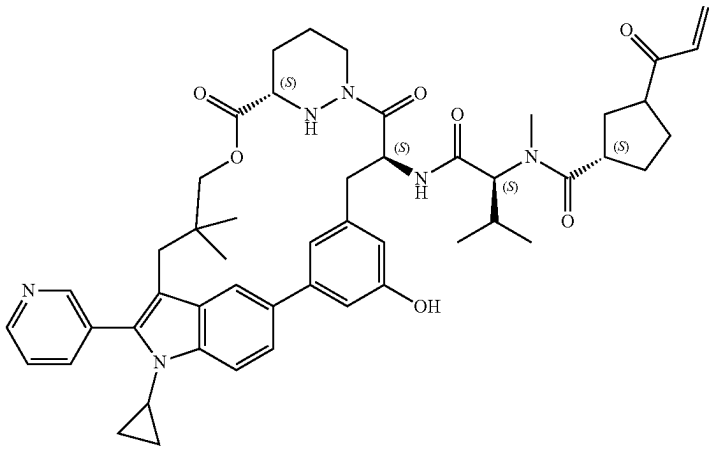
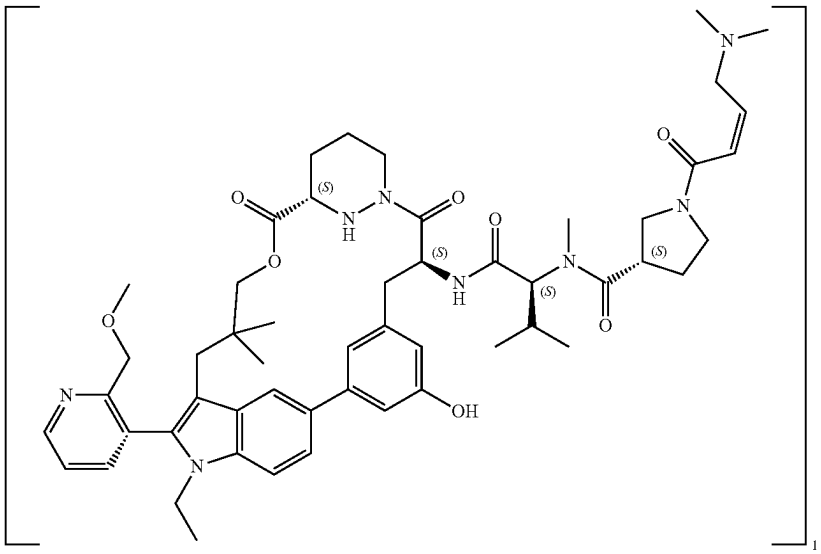
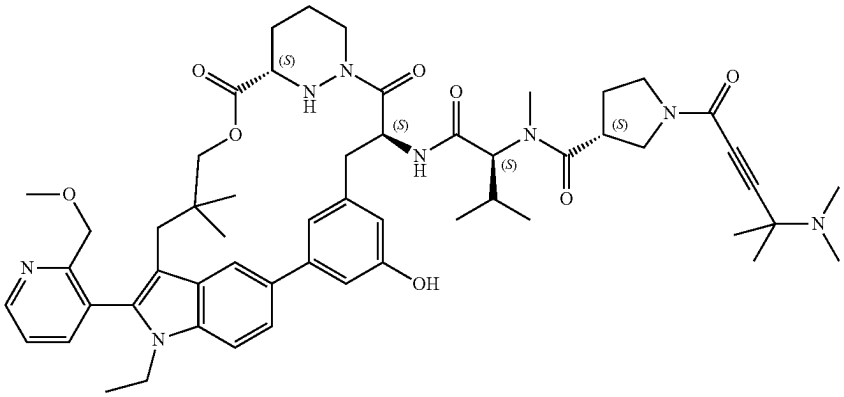
Certain Compounds of the Present Invention	
Ex#	Structure
A160	
A161	
A162	

TABLE 1-continued

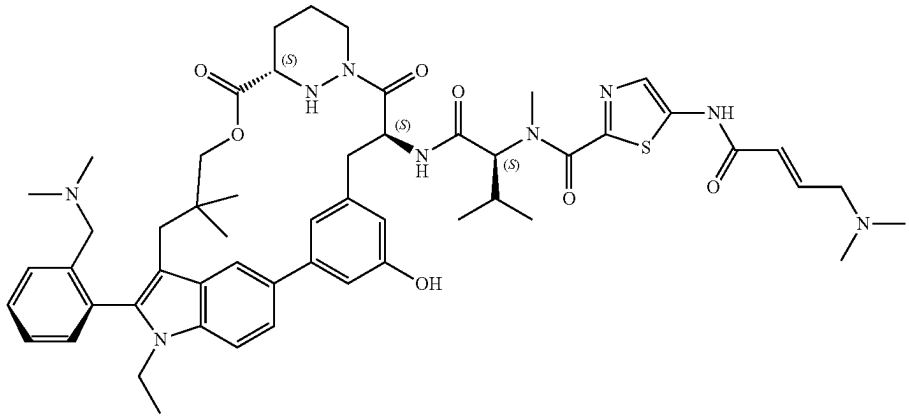
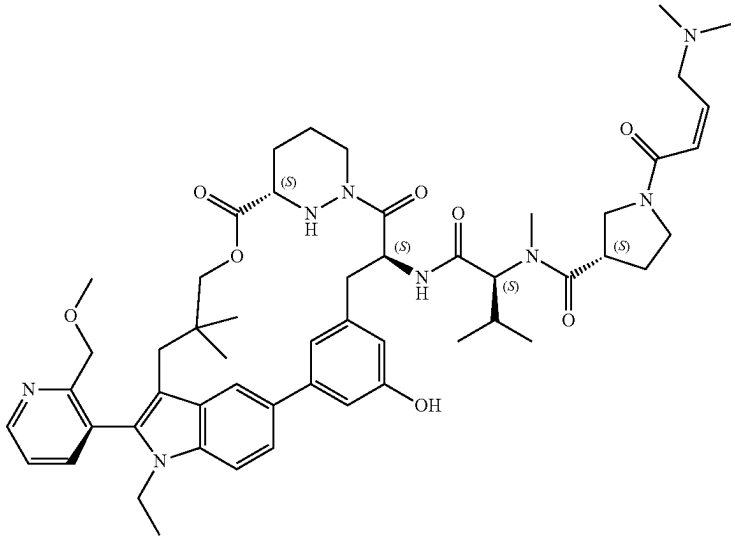
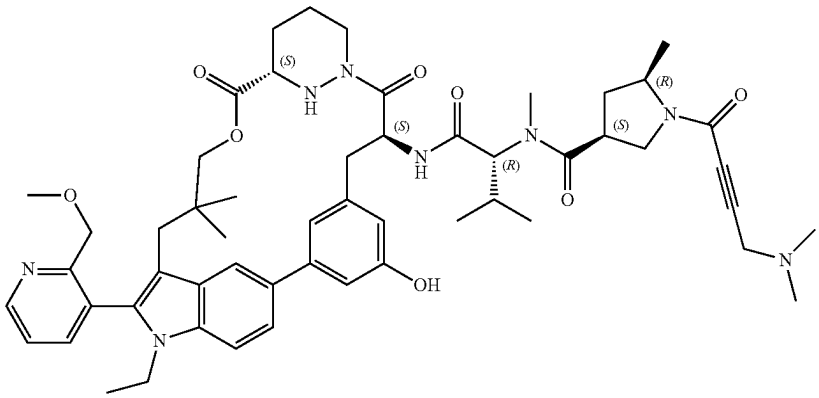
Certain Compounds of the Present Invention	
Ex#	Structure
A163	
A164	
A165	

TABLE 1-continued

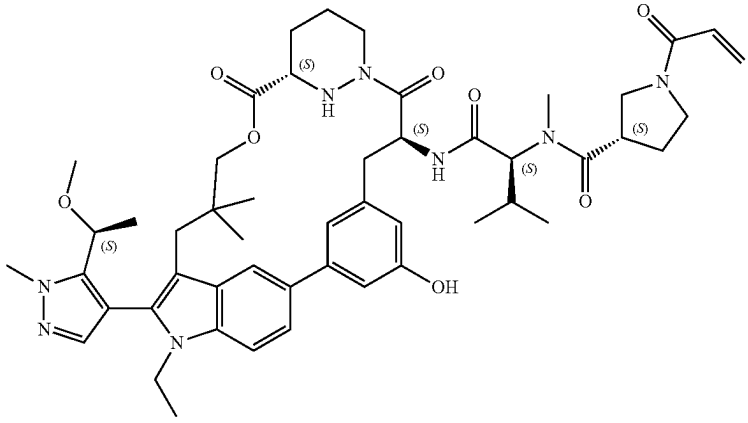
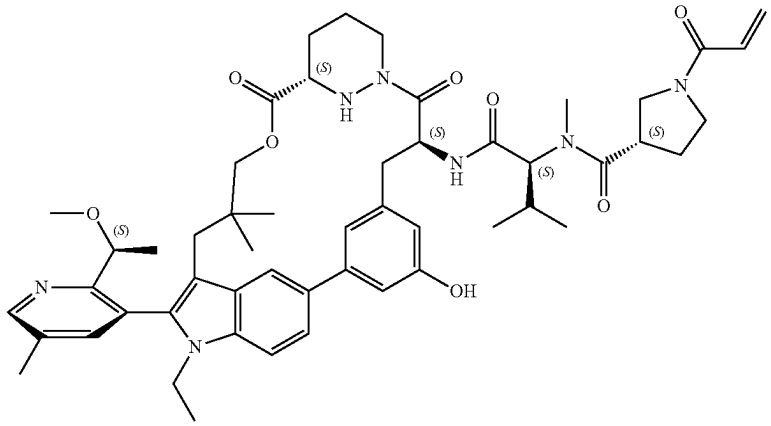
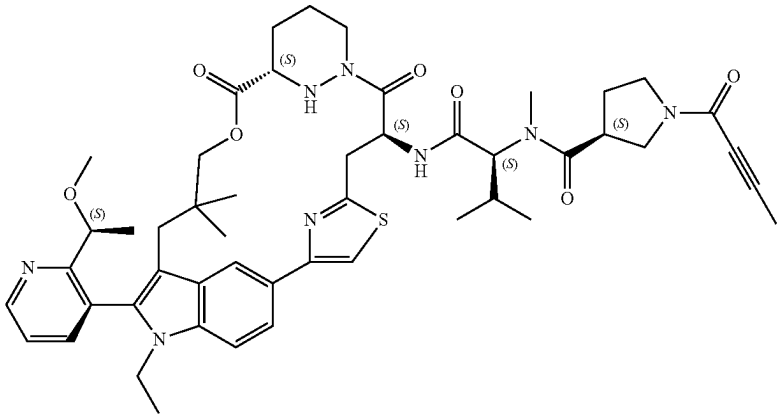
Certain Compounds of the Present Invention	
Ex#	Structure
A166	
A167	
A168	

TABLE 1-continued

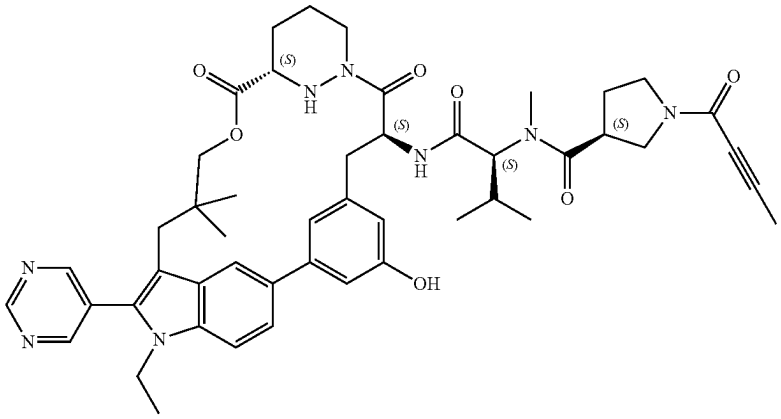
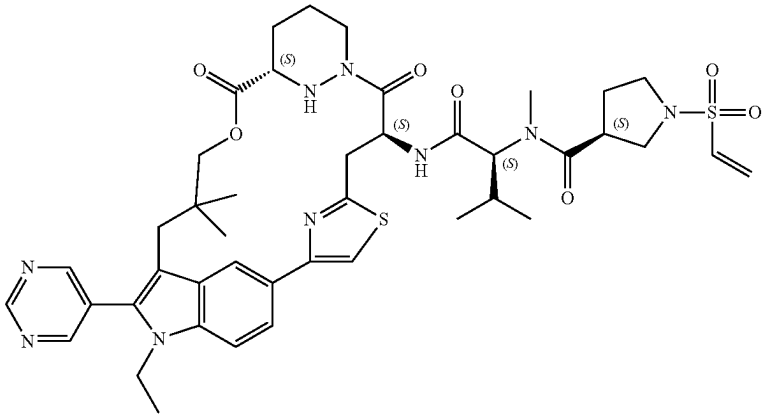
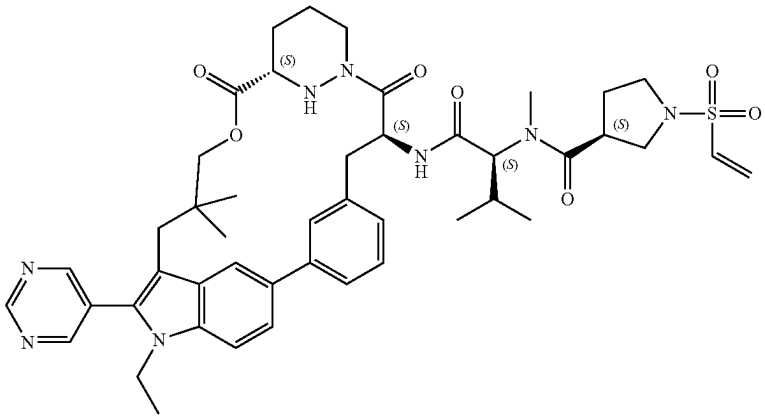
Certain Compounds of the Present Invention	
Ex#	Structure
A169	
A170	
A171	

TABLE 1-continued

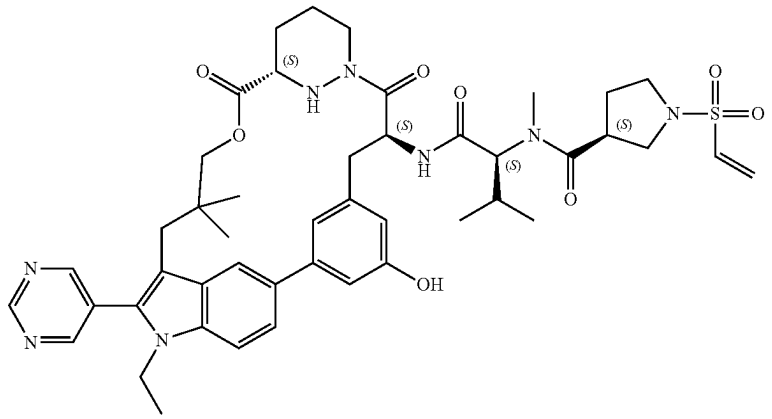
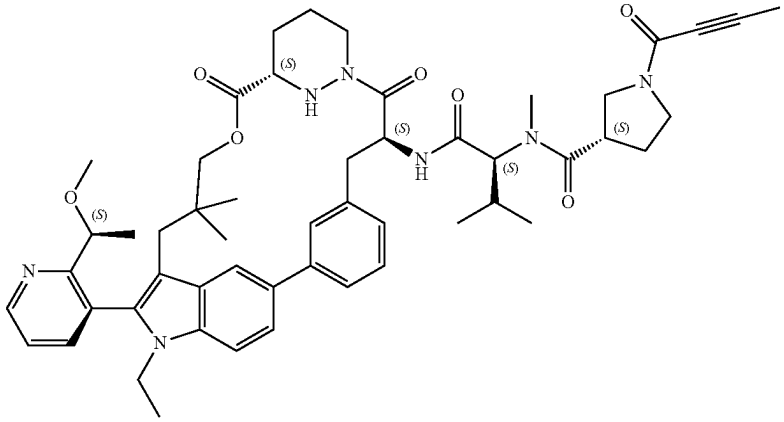
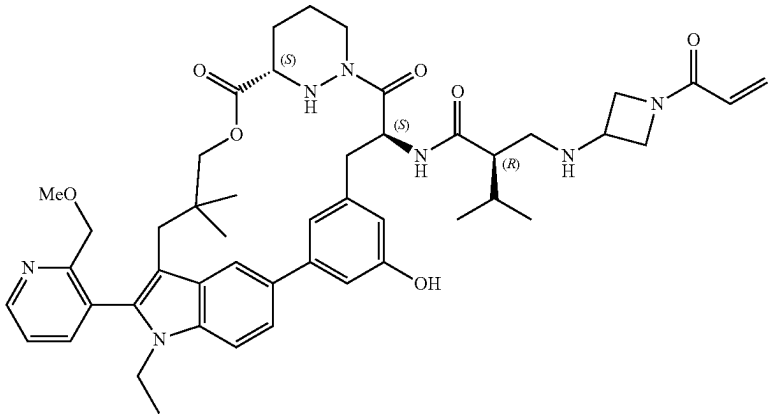
Certain Compounds of the Present Invention	
Ex#	Structure
A172	
A173	
A174	

TABLE 1-continued

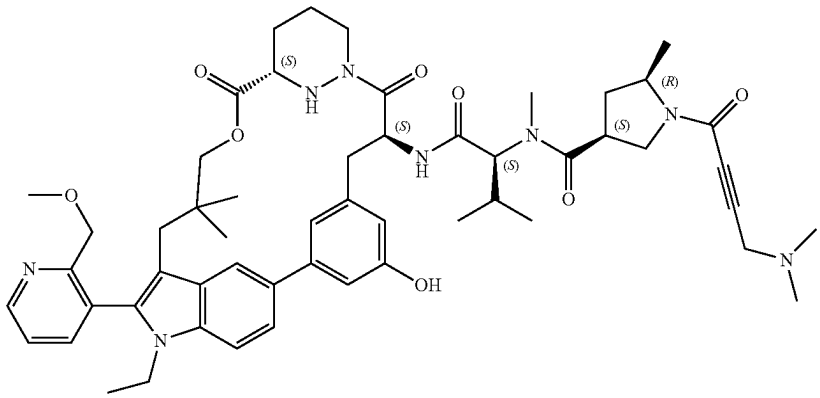
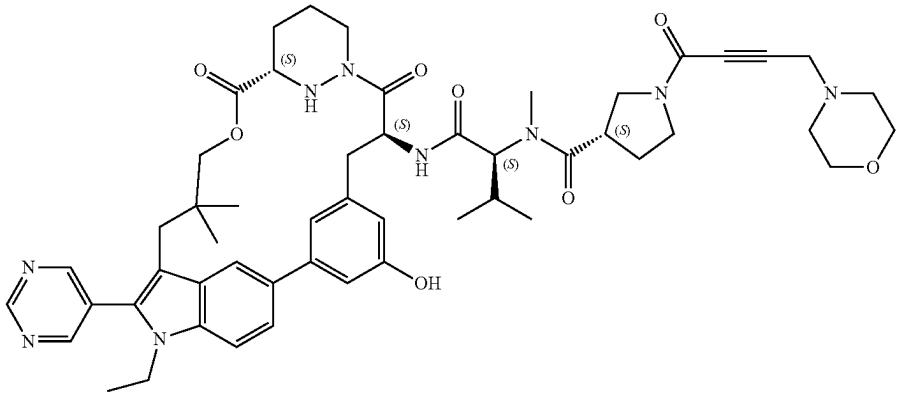
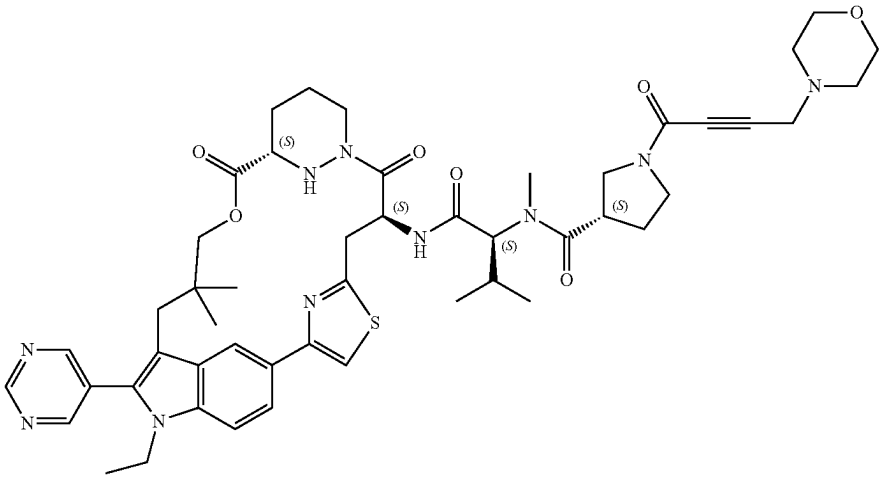
Certain Compounds of the Present Invention	
Ex#	Structure
A175	
A176	
A177	

TABLE 1-continued

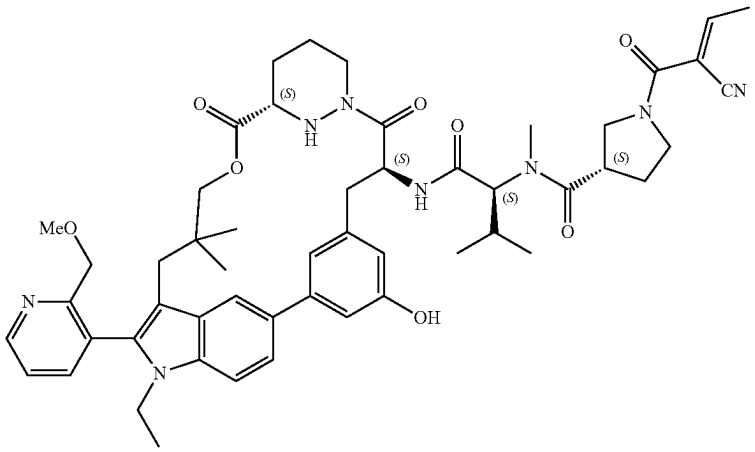
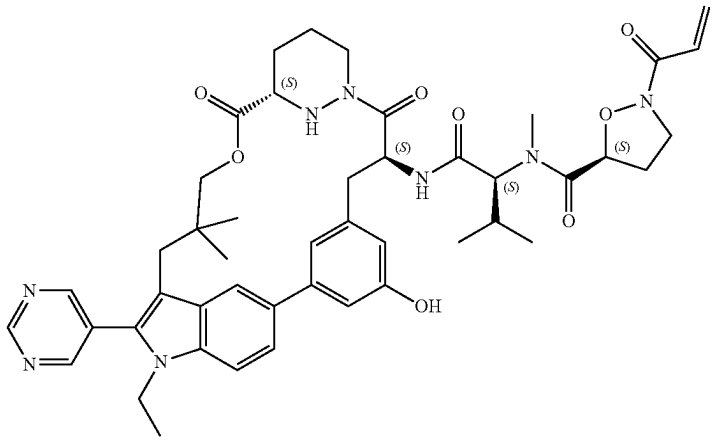
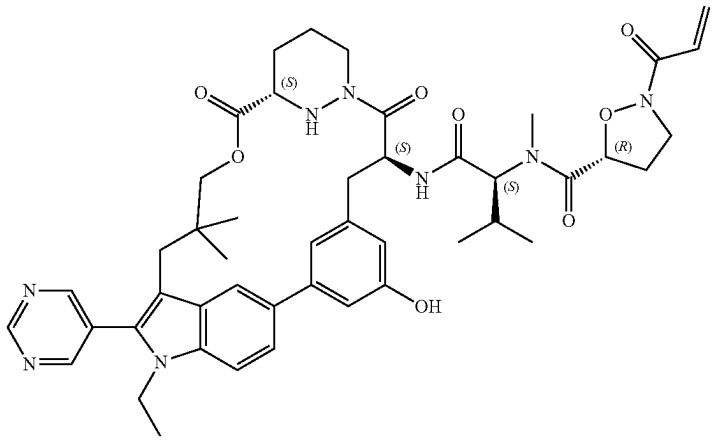
Certain Compounds of the Present Invention	
Ex#	Structure
A178	
A179	
A180	

TABLE 1-continued

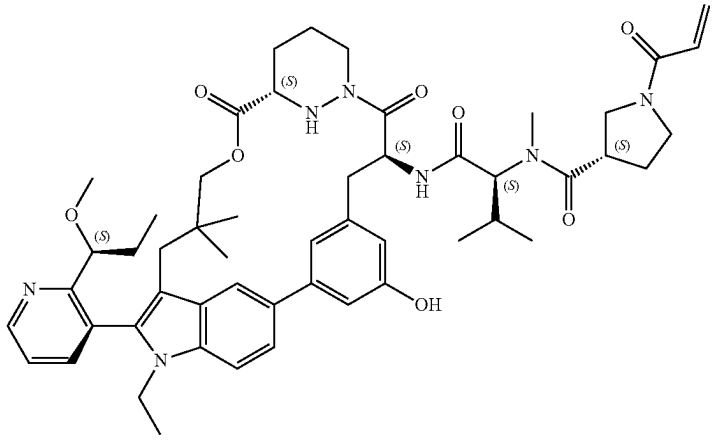
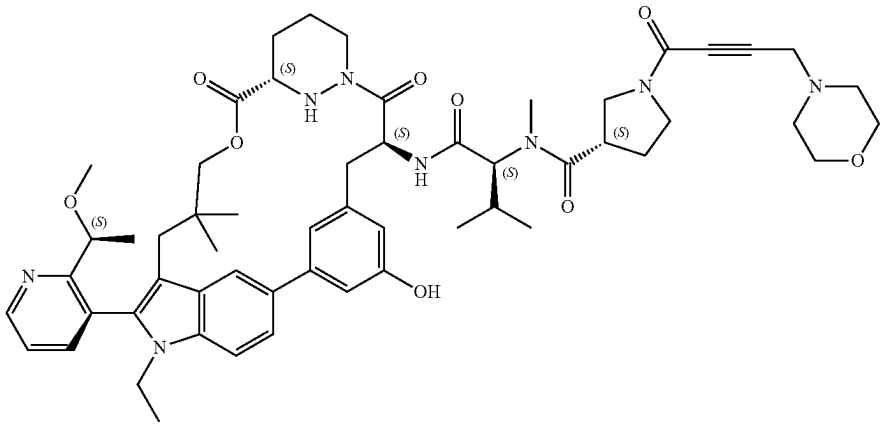
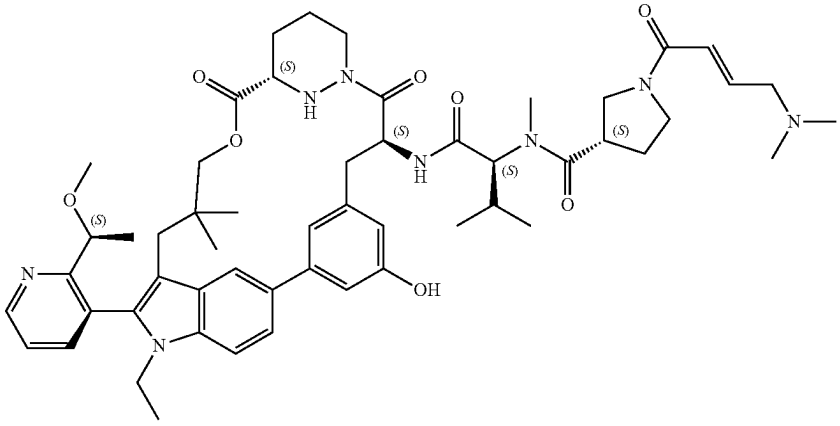
Certain Compounds of the Present Invention	
Ex#	Structure
A181	
A182	
A183	

TABLE 1-continued

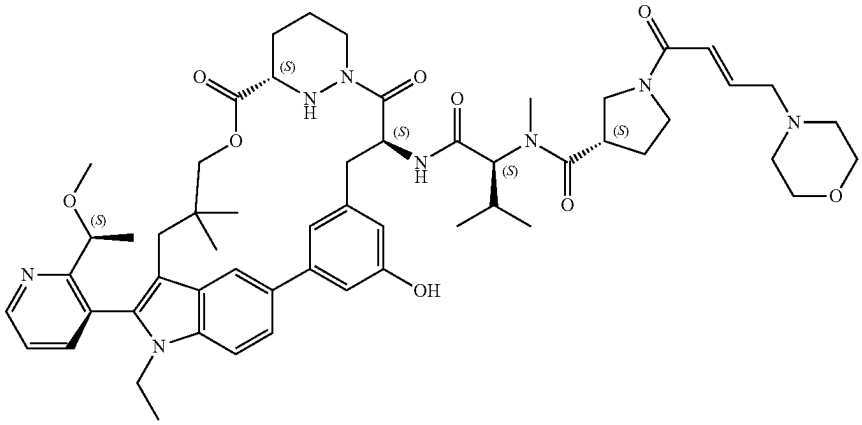
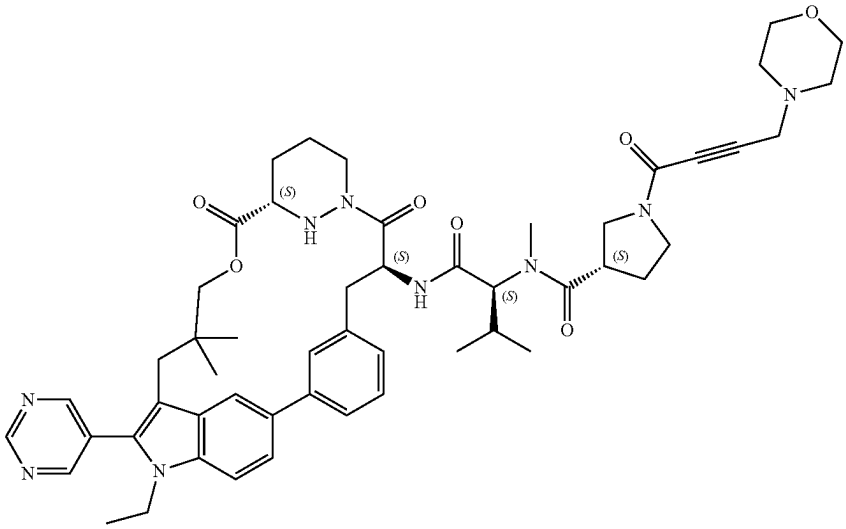
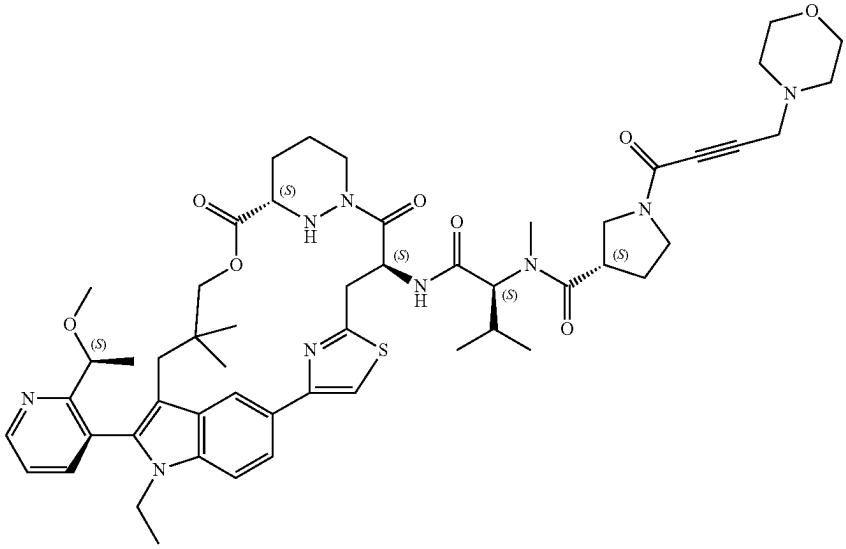
Certain Compounds of the Present Invention	
Ex#	Structure
A184	
A185	
A186	

TABLE 1-continued

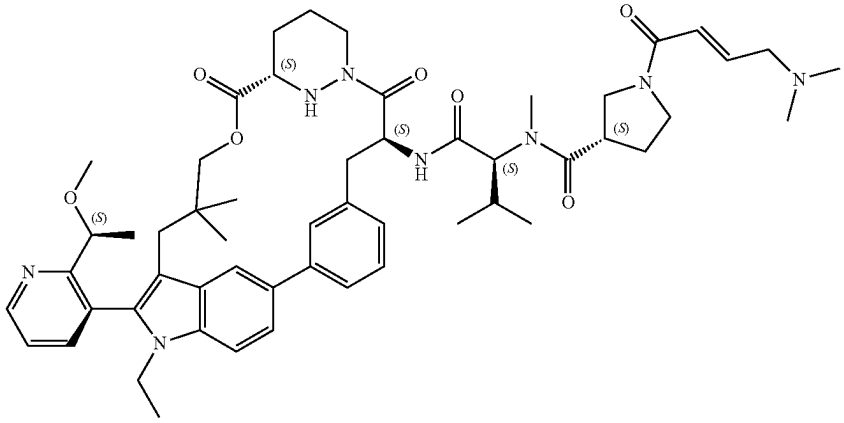
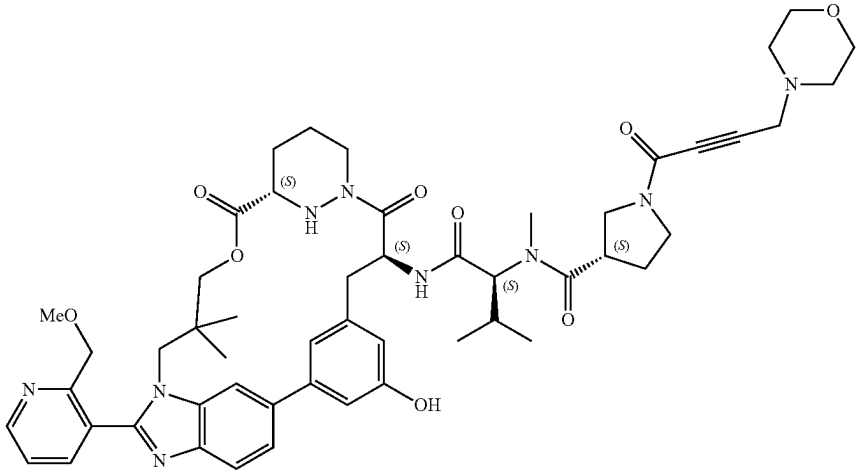
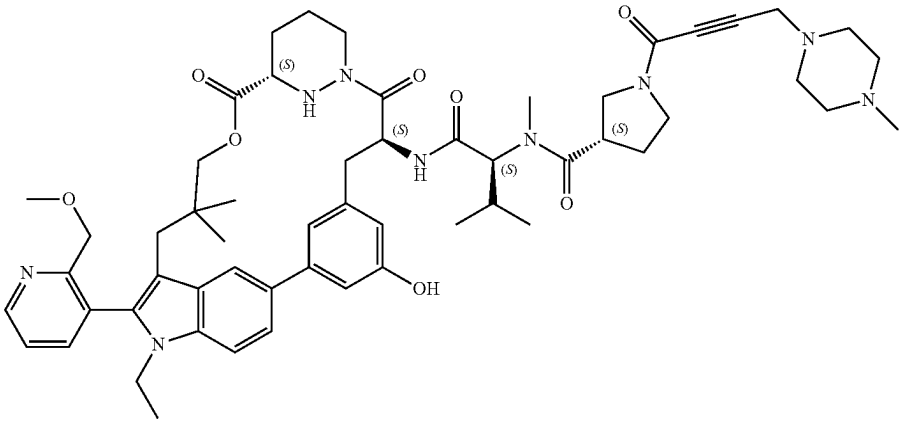
Certain Compounds of the Present Invention	
Ex#	Structure
A187	
A188	
A189	

TABLE 1-continued

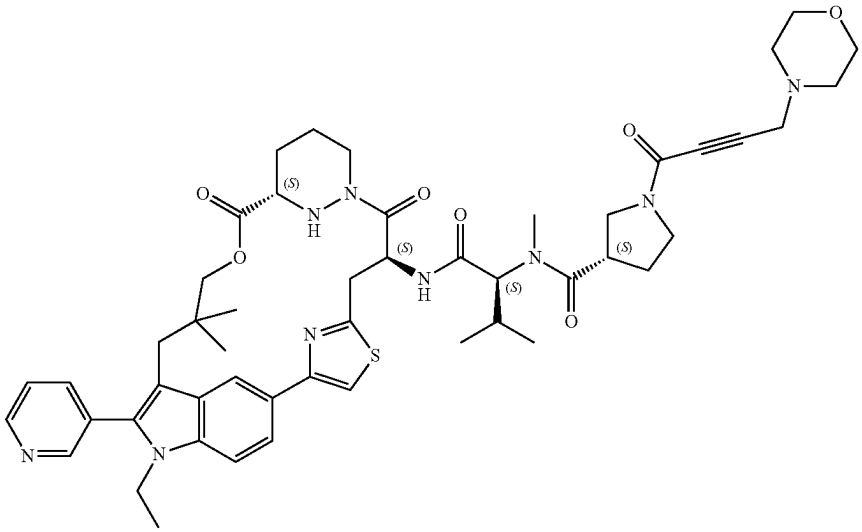
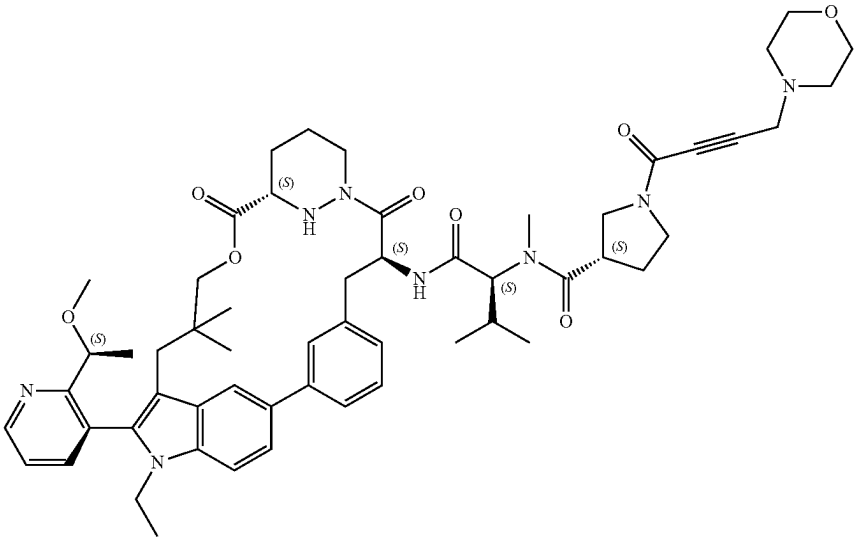
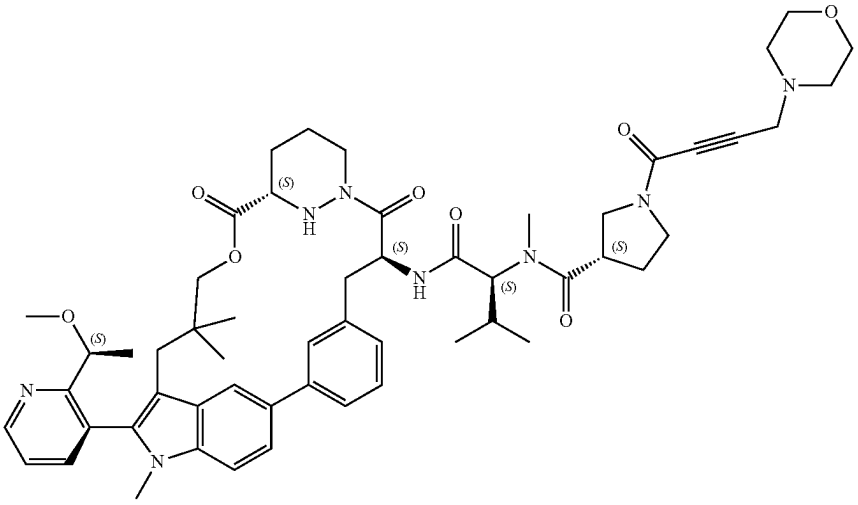
Certain Compounds of the Present Invention	
Ex#	Structure
A190	
A191	
A192	

TABLE 1-continued

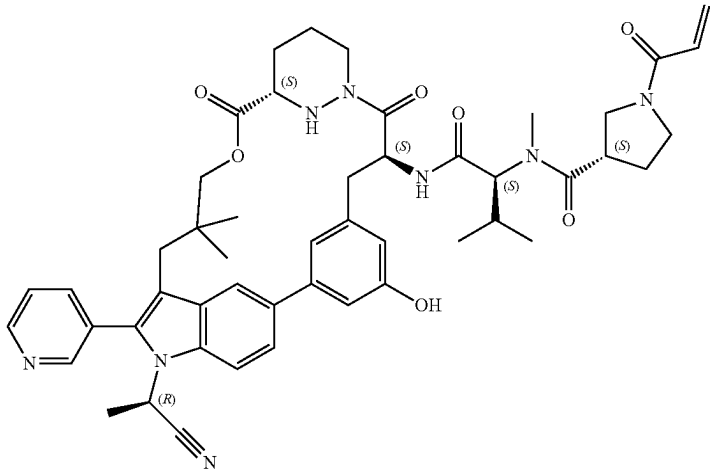
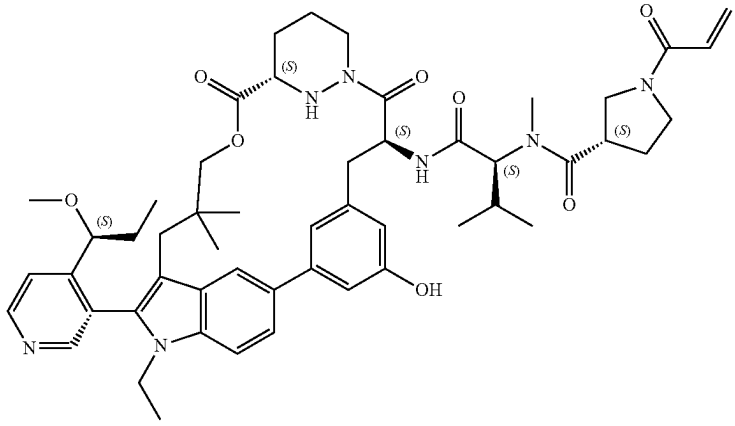
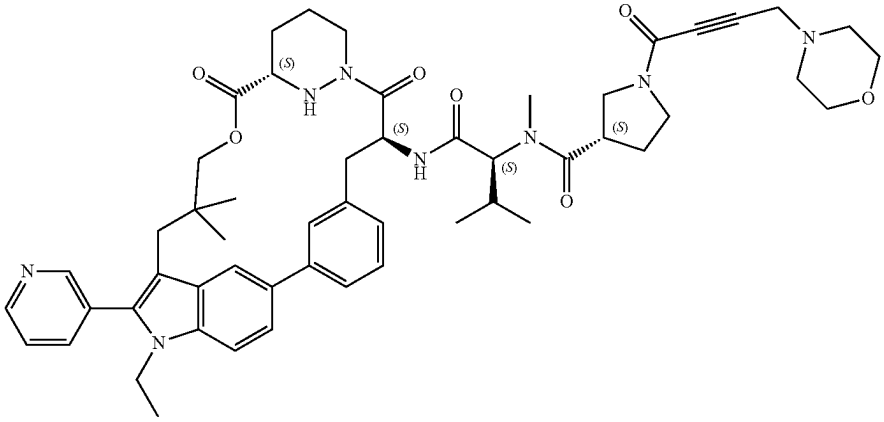
Certain Compounds of the Present Invention	
Ex#	Structure
A193	
A194	
A195	

TABLE 1-continued

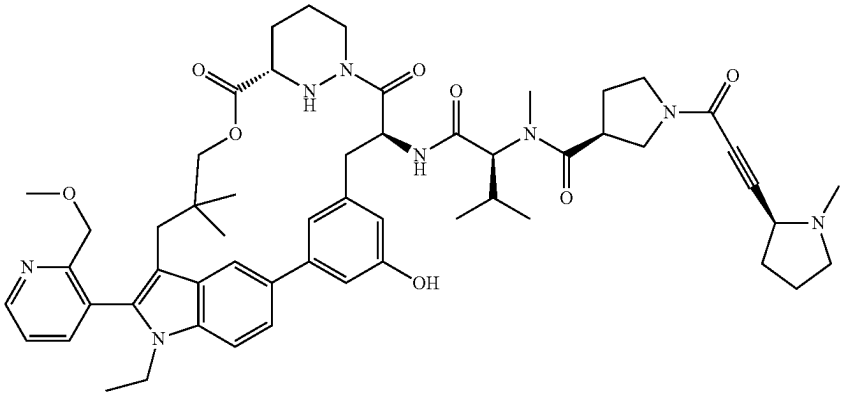
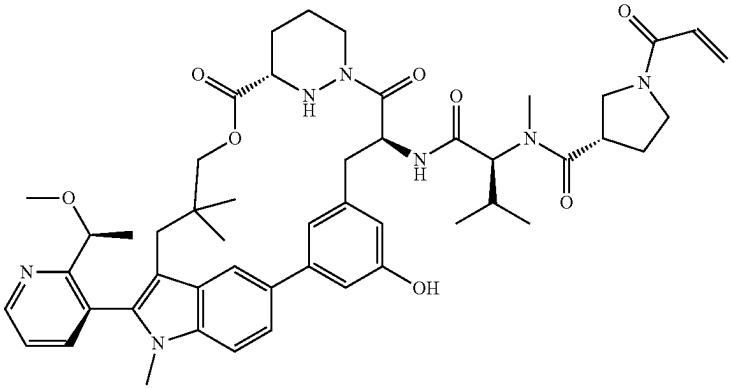
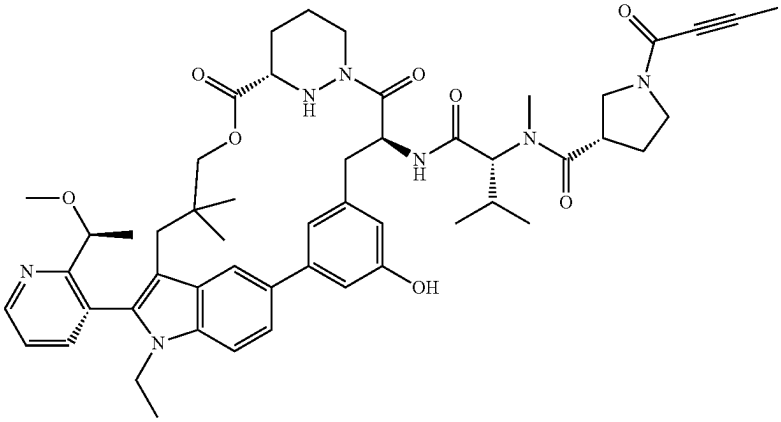
Certain Compounds of the Present Invention	
Ex#	Structure
A196	
A197	
A198	

TABLE 1-continued

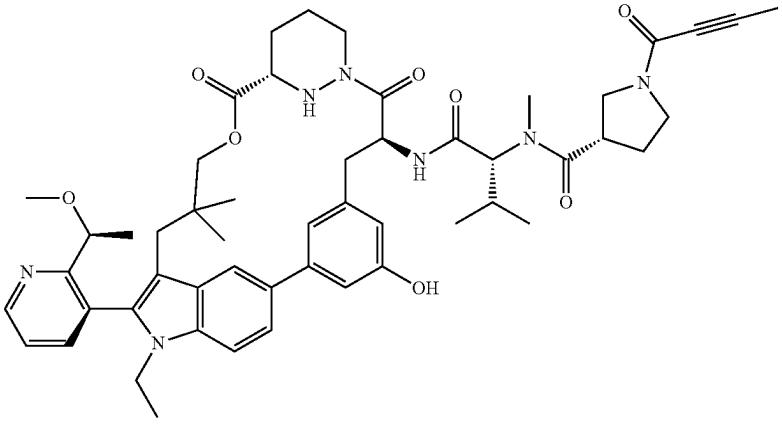
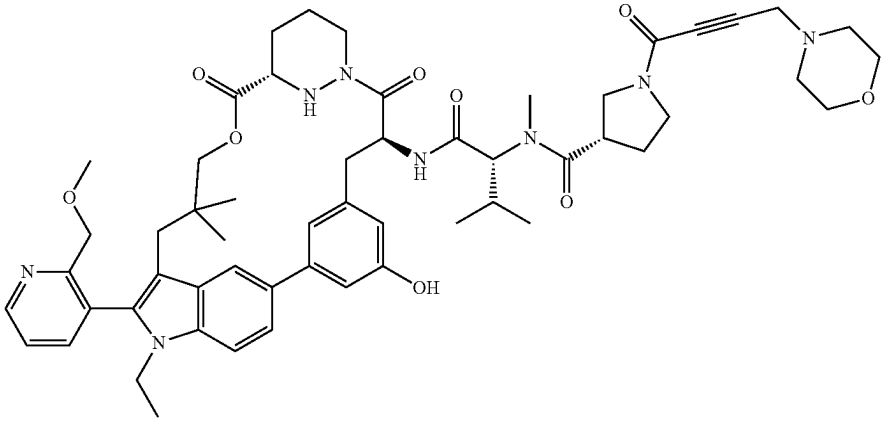
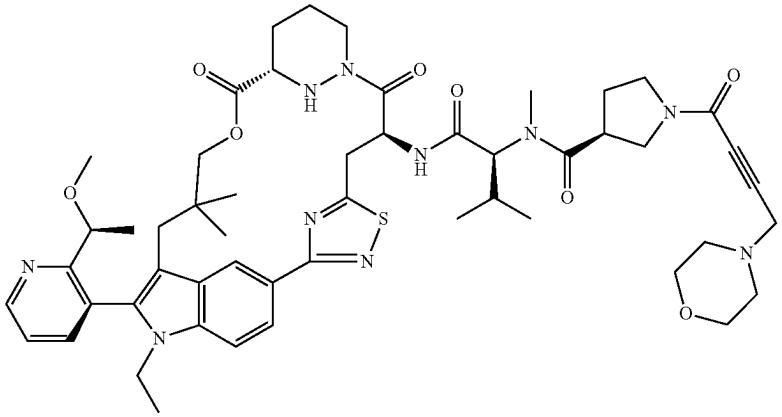
Certain Compounds of the Present Invention	
Ex#	Structure
A199	
A200	
A201	

TABLE 1-continued

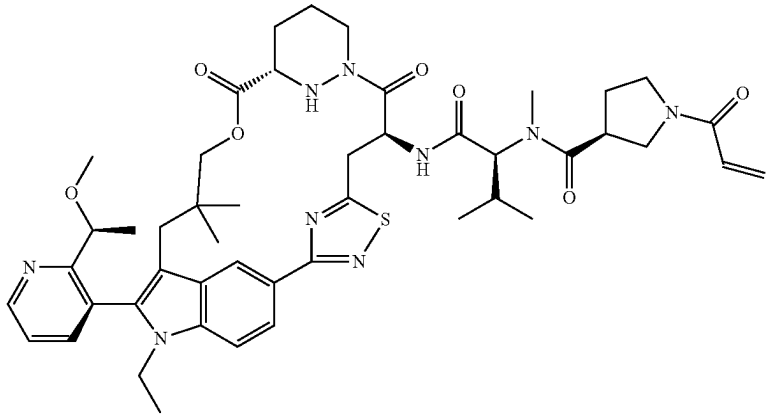
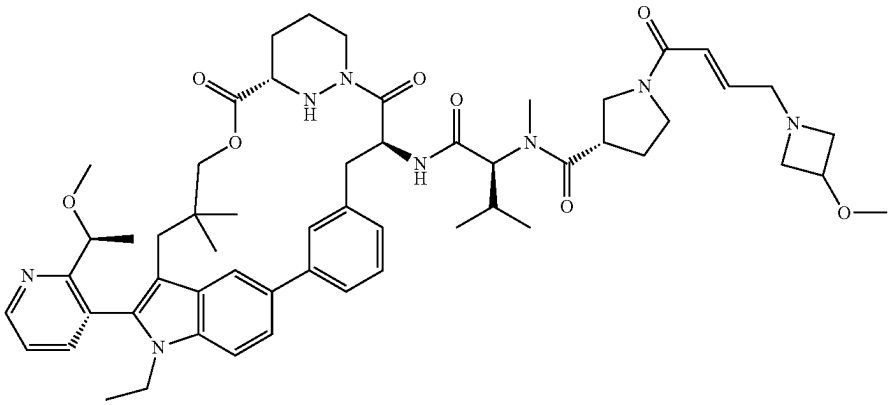
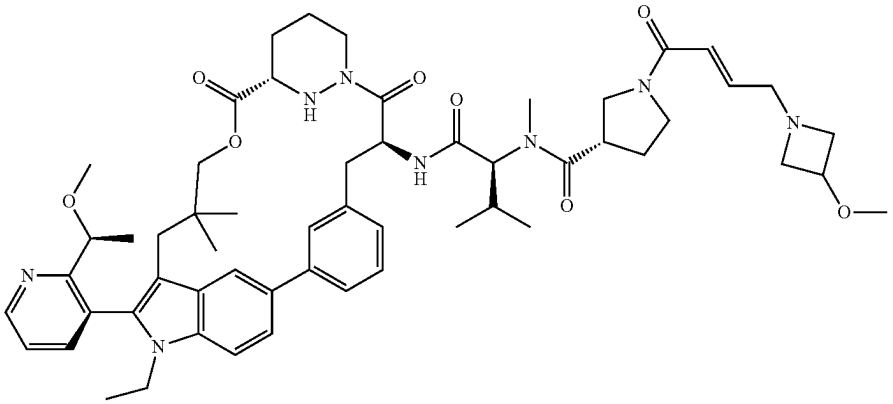
Certain Compounds of the Present Invention	
Ex#	Structure
A202	
A203	
A204	

TABLE 1-continued

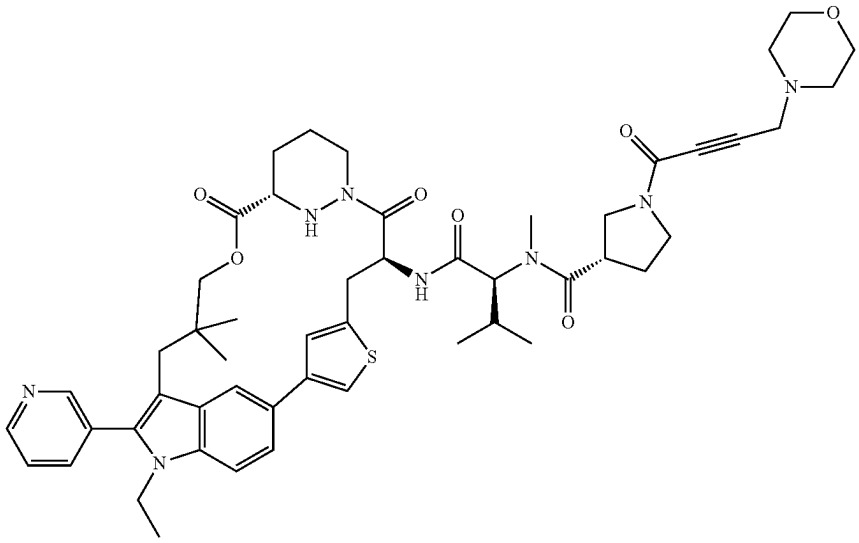
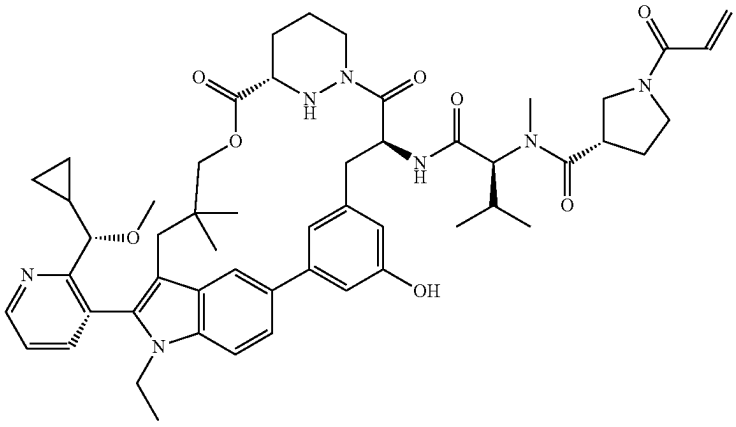
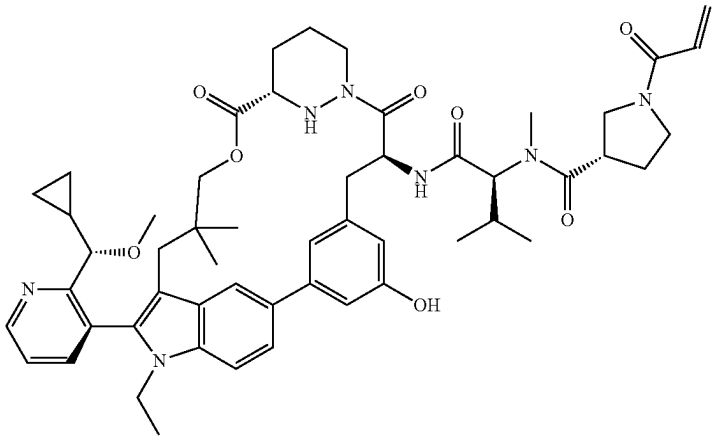
Certain Compounds of the Present Invention	
Ex#	Structure
A205	
A206	
A207	

TABLE 1-continued

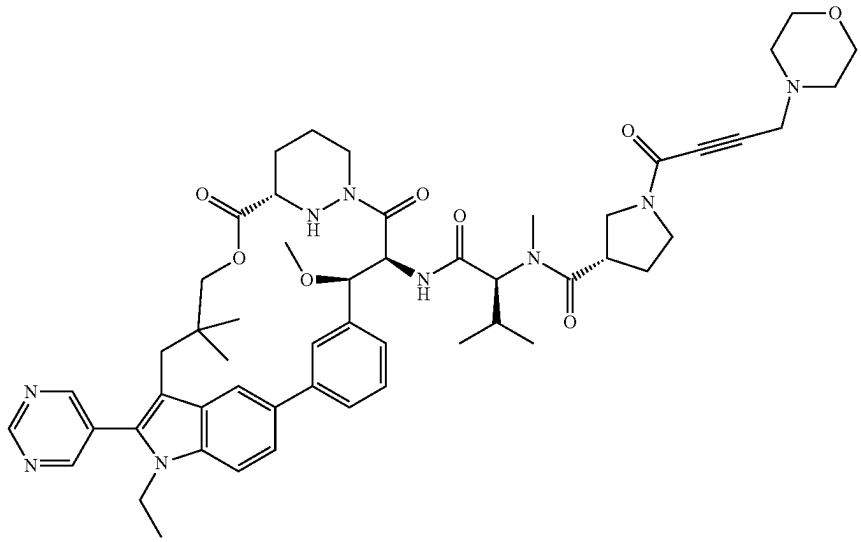
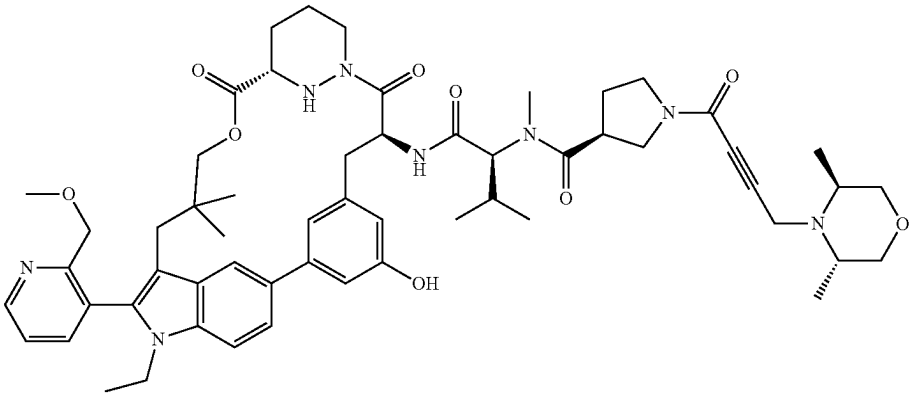
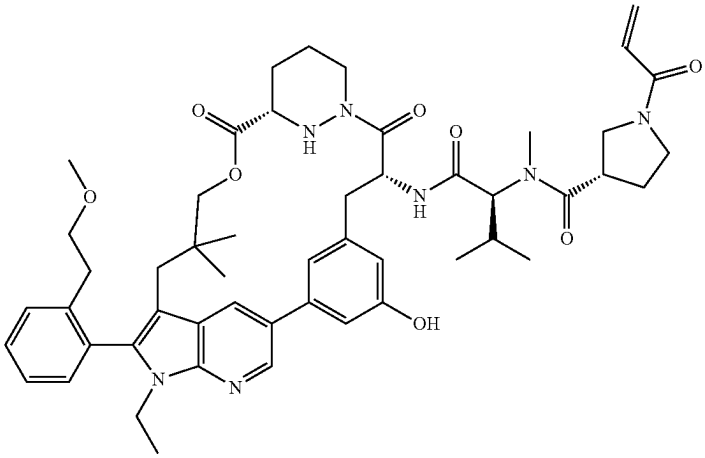
Certain Compounds of the Present Invention	
Ex#	Structure
A208	
A209	
A210	

TABLE 1-continued

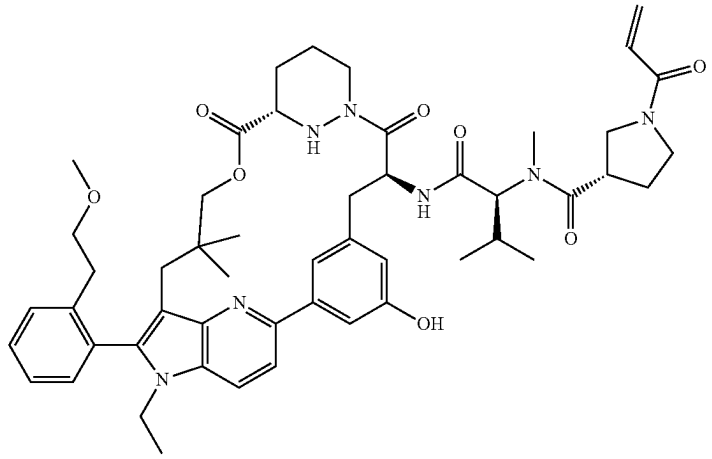
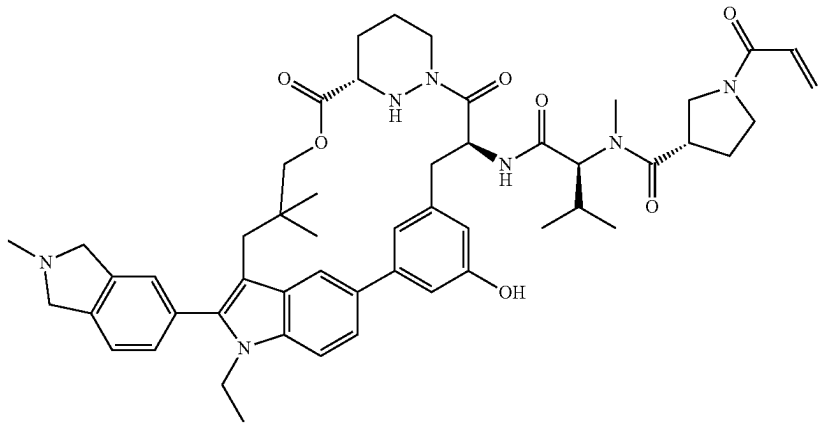
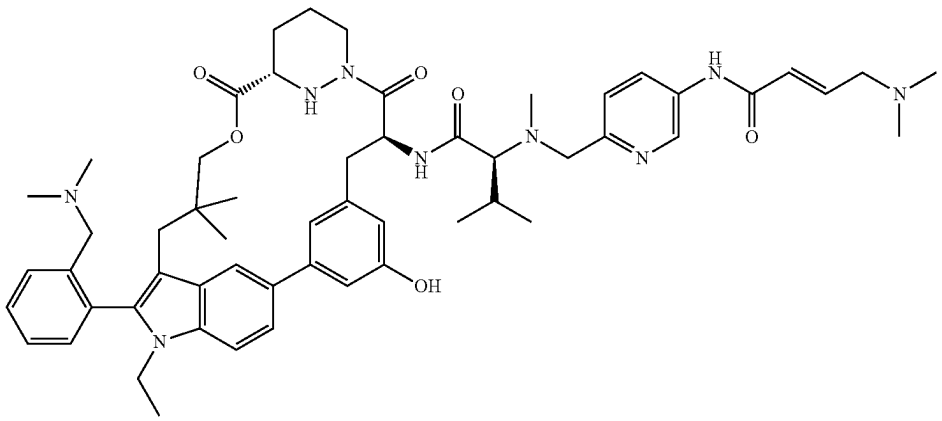
Certain Compounds of the Present Invention	
Ex#	Structure
A211	
A212	
A213	

TABLE 1-continued

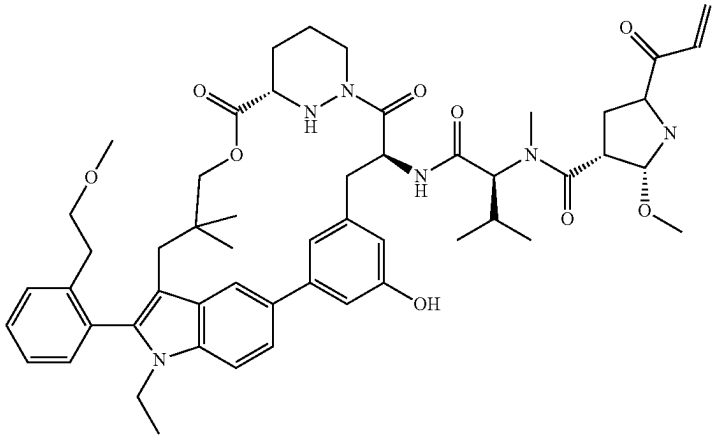
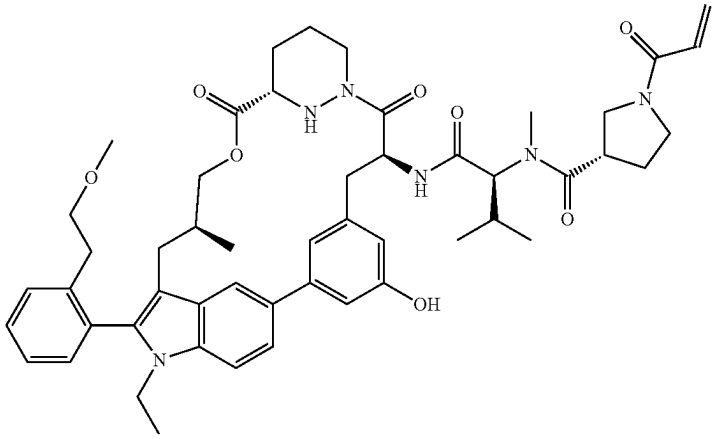
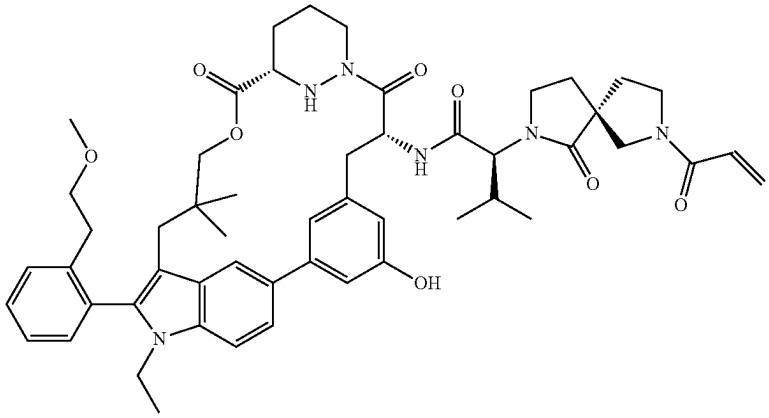
Certain Compounds of the Present Invention	
Ex#	Structure
A214	
A215	
A216	

TABLE 1-continued

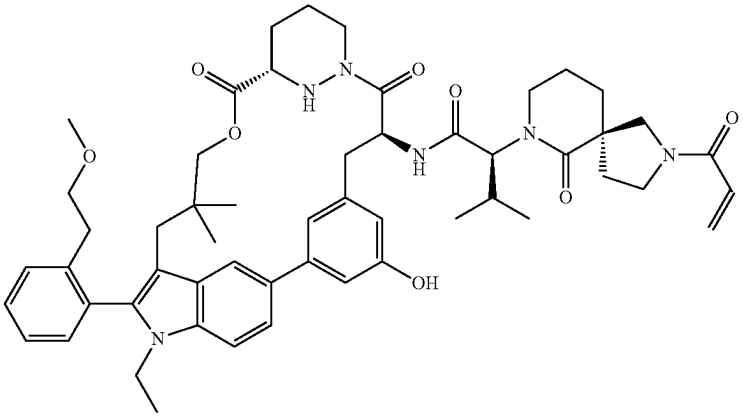
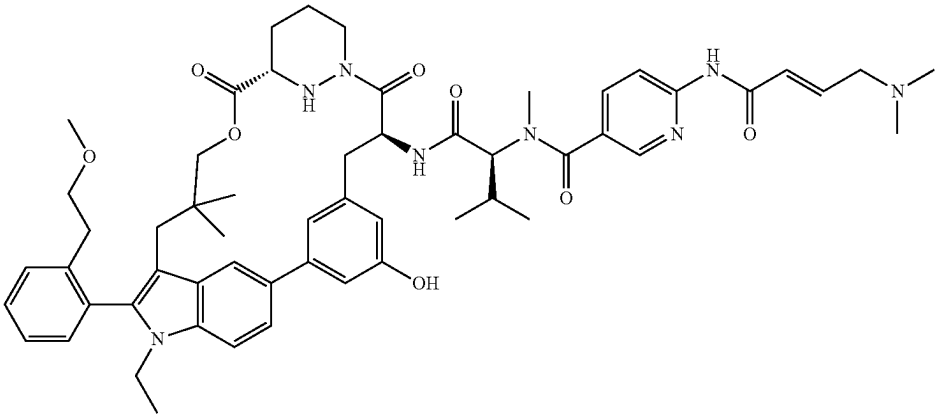
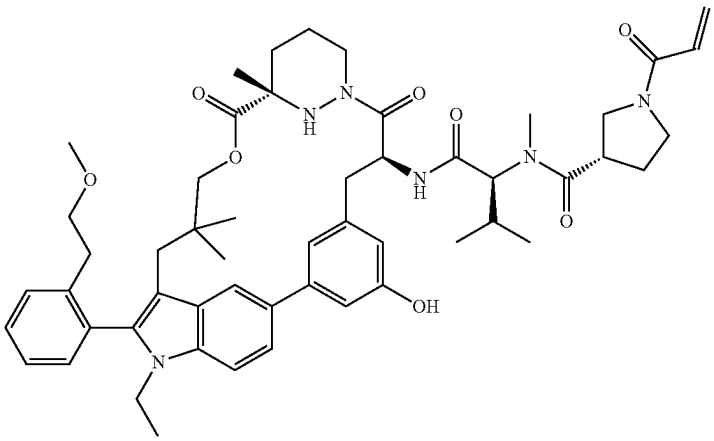
Certain Compounds of the Present Invention	
Ex#	Structure
A217	
A218	
A219	

TABLE 1-continued

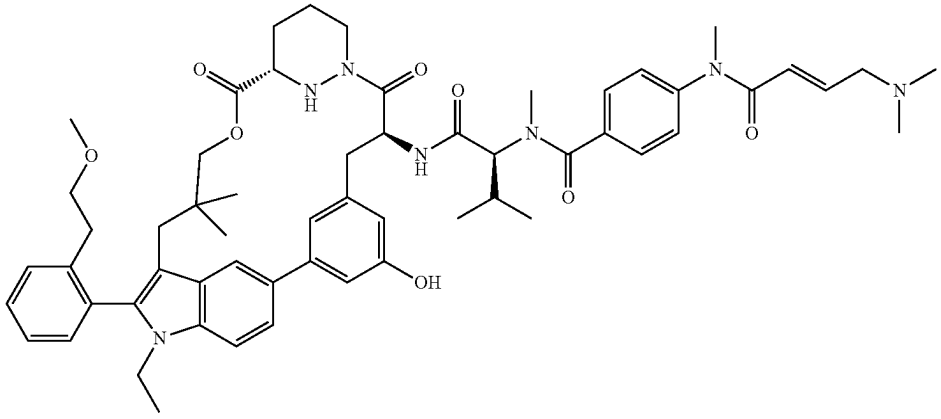
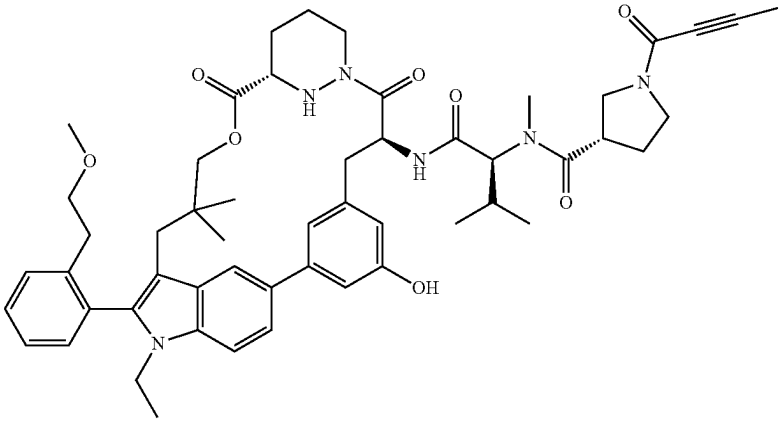
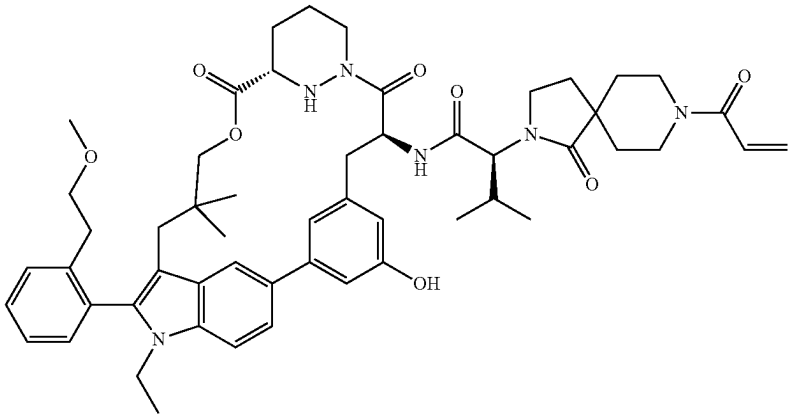
Certain Compounds of the Present Invention	
Ex#	Structure
A220	
A221	
A222	

TABLE 1-continued

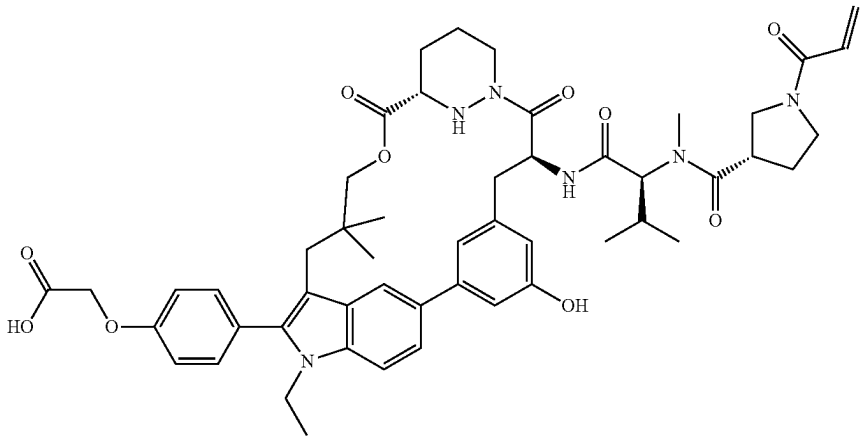
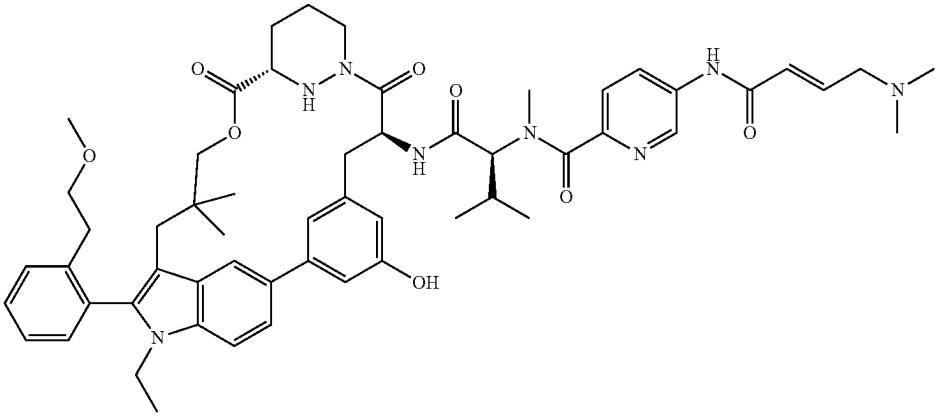
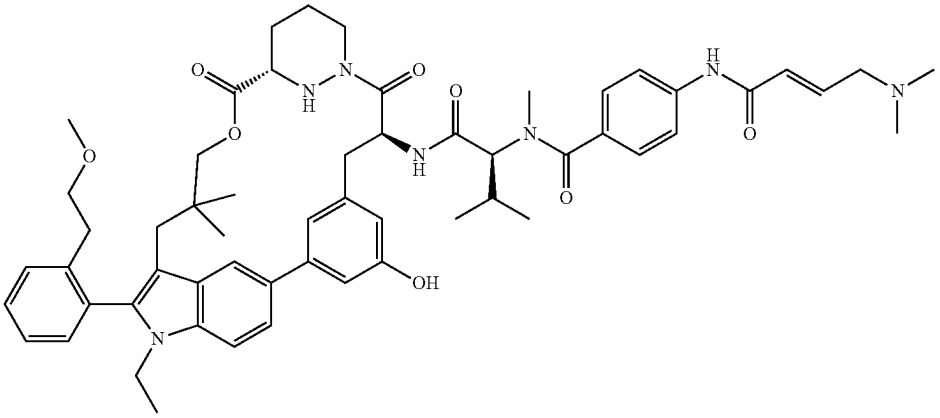
Certain Compounds of the Present Invention	
Ex#	Structure
A223	
A224	
A225	

TABLE 1-continued

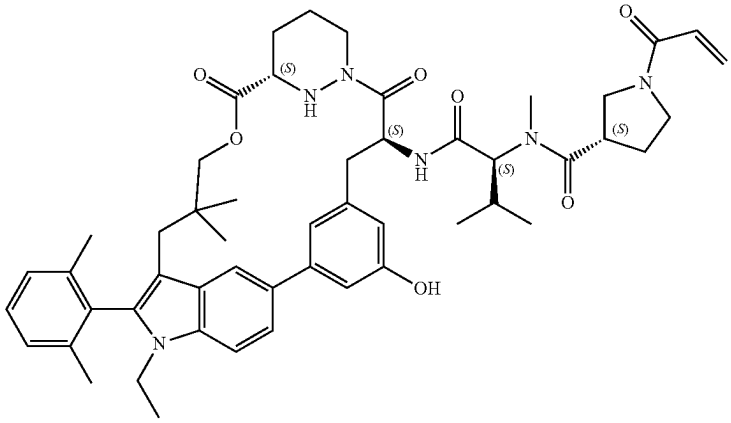
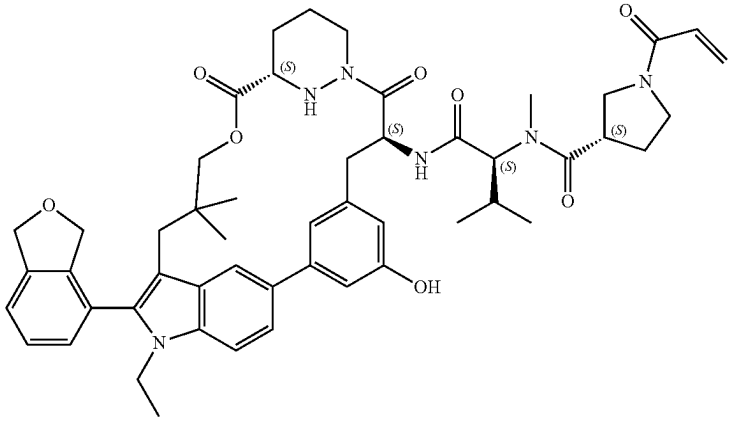
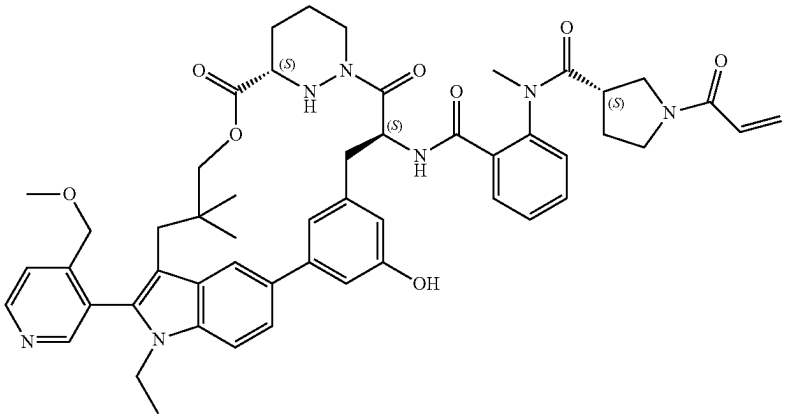
Certain Compounds of the Present Invention	
Ex#	Structure
A226	
A227	
A228	

TABLE 1-continued

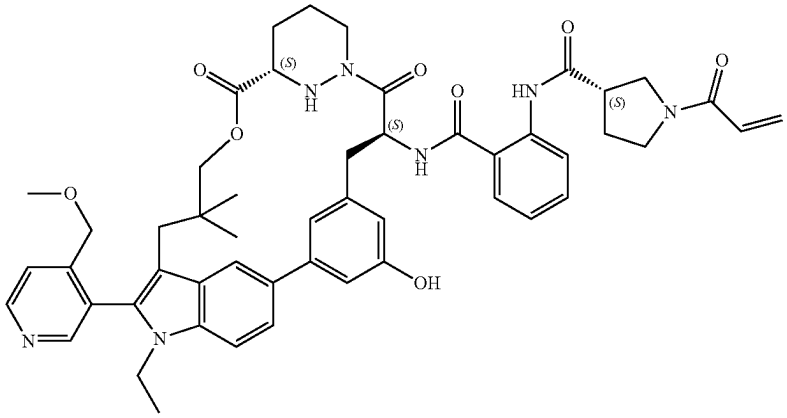
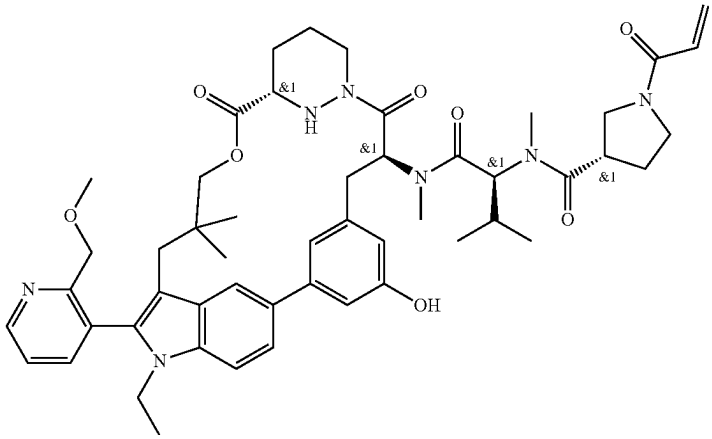
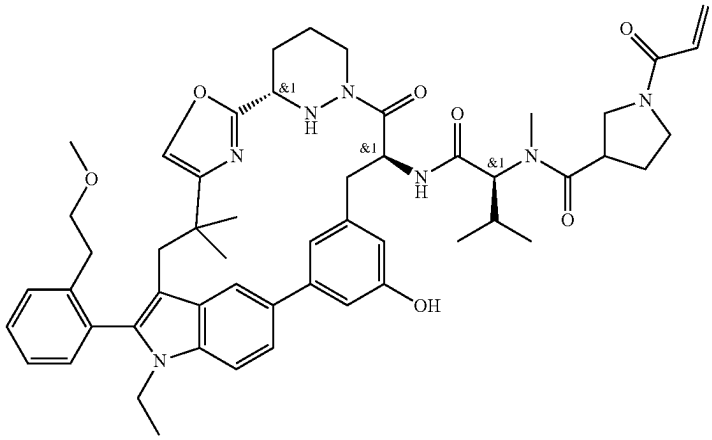
Certain Compounds of the Present Invention	
Ex#	Structure
A229	
A230	
A231	

TABLE 1-continued

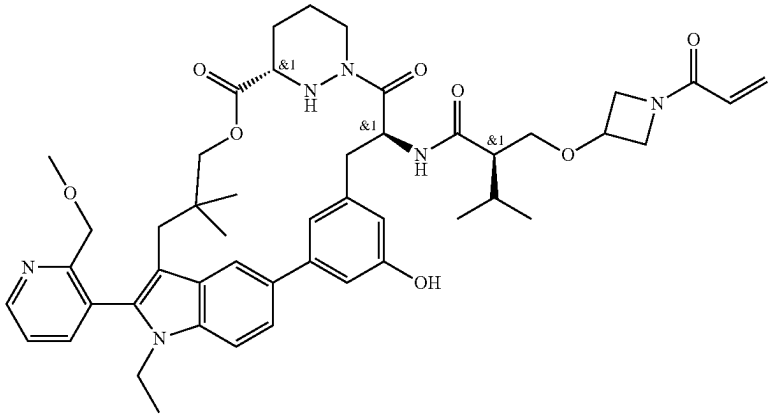
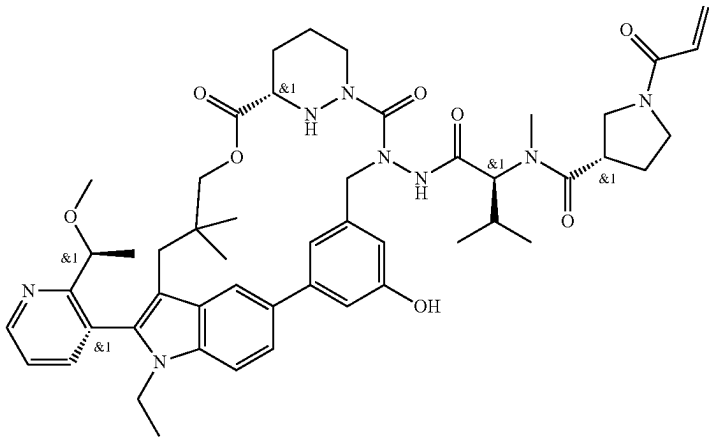
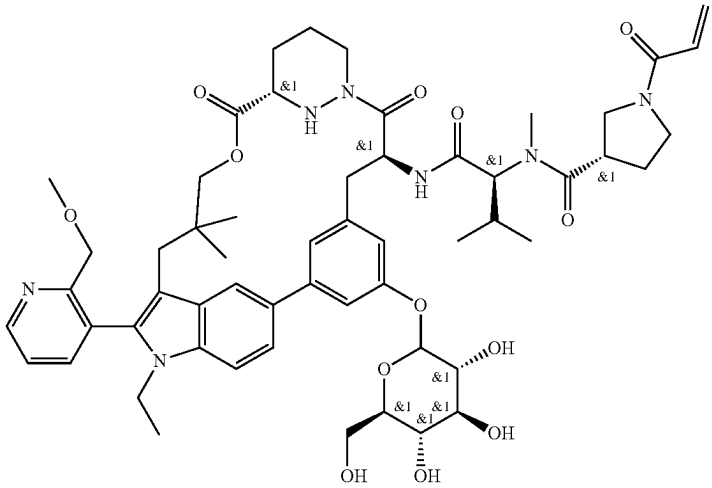
Certain Compounds of the Present Invention	
Ex#	Structure
A232	
A233	
A234	

TABLE 1-continued

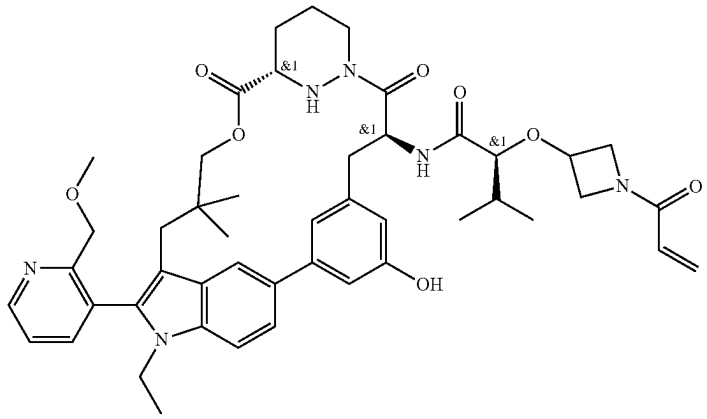
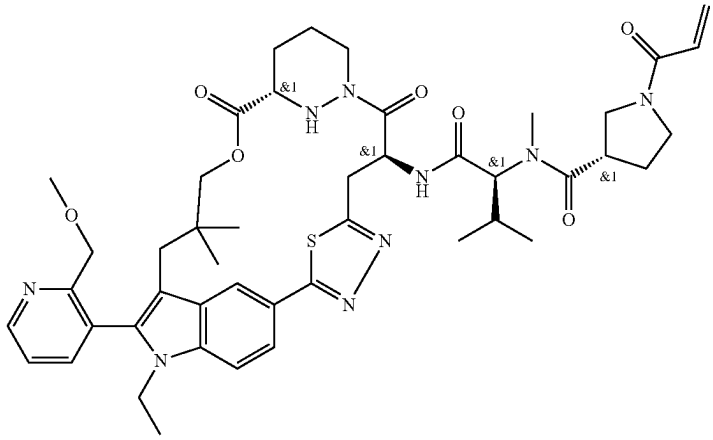
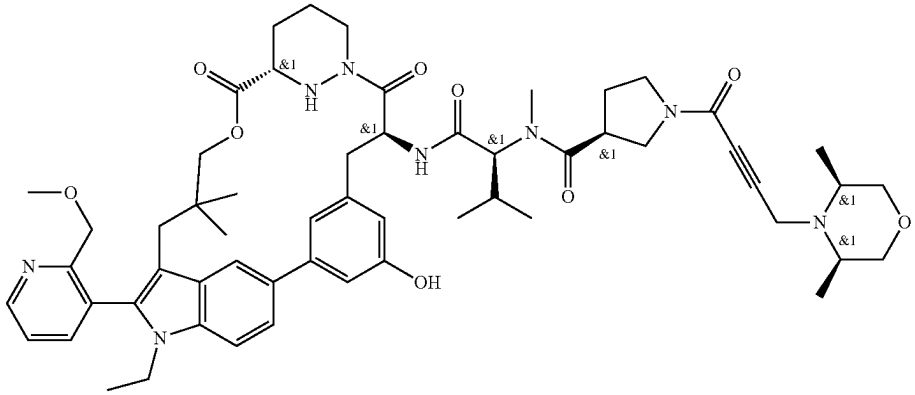
Certain Compounds of the Present Invention	
Ex#	Structure
A235	
A236	
A237	

TABLE 1-continued

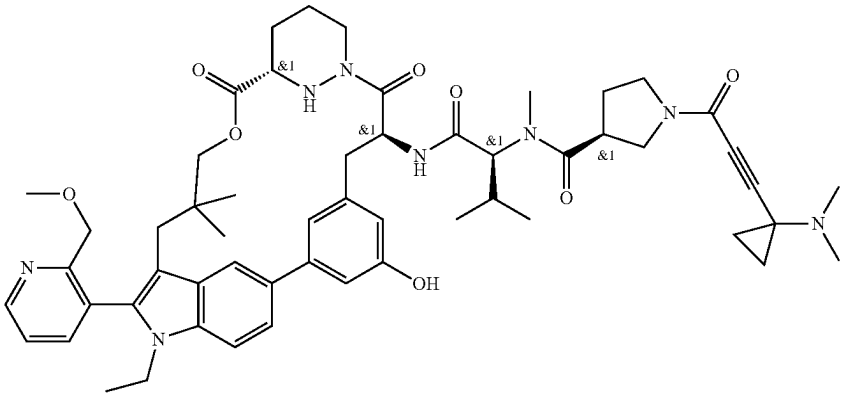
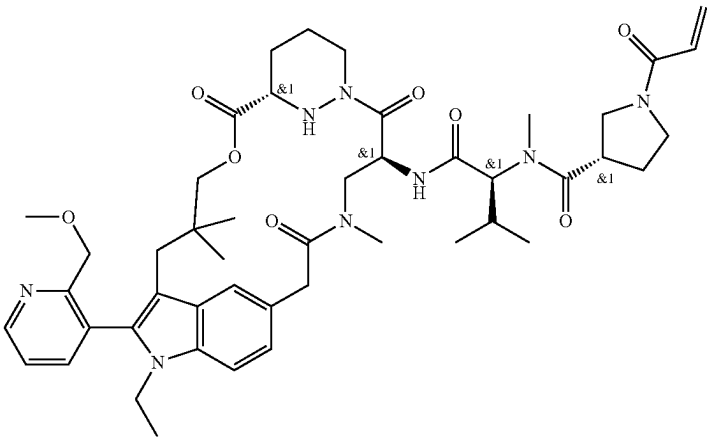
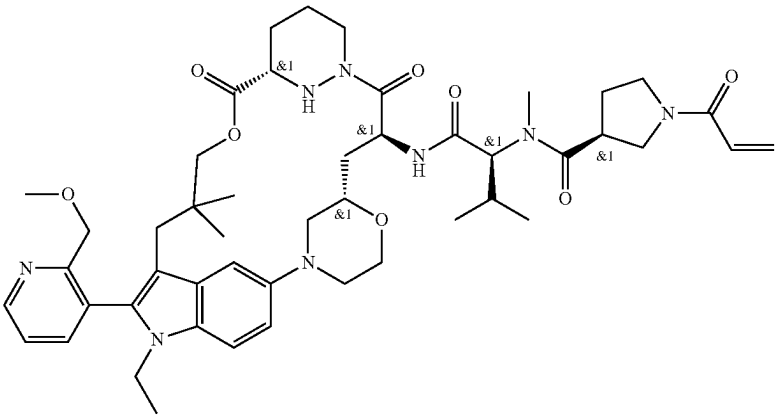
Certain Compounds of the Present Invention	
Ex#	Structure
A238	
A239	
A240	

TABLE 1-continued

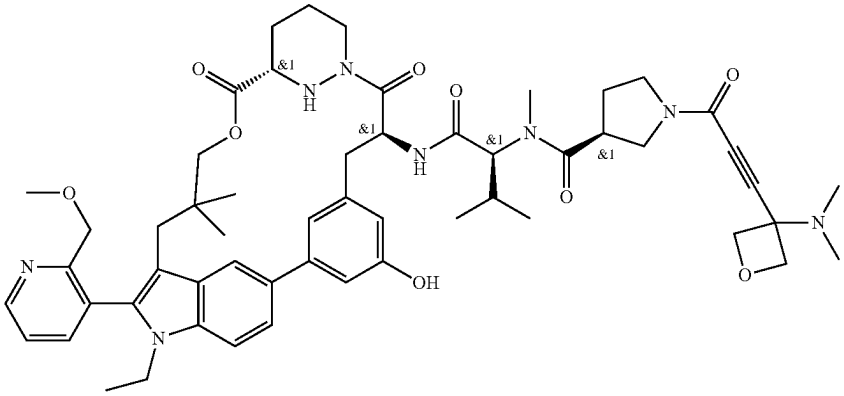
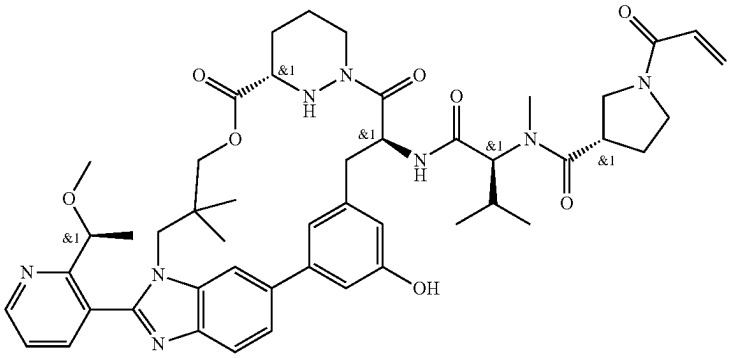
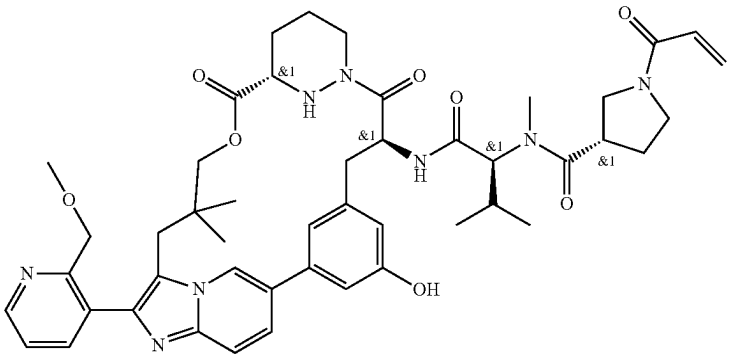
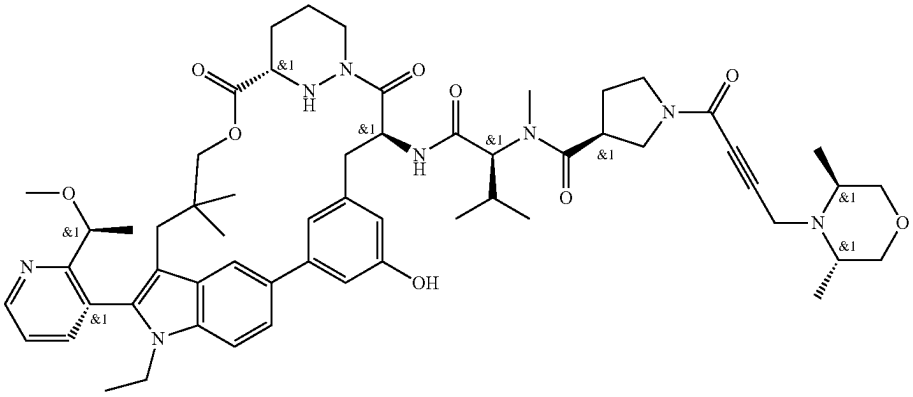
Certain Compounds of the Present Invention	
Ex#	Structure
A241	
A242	
A243	
A244	

TABLE 1-continued

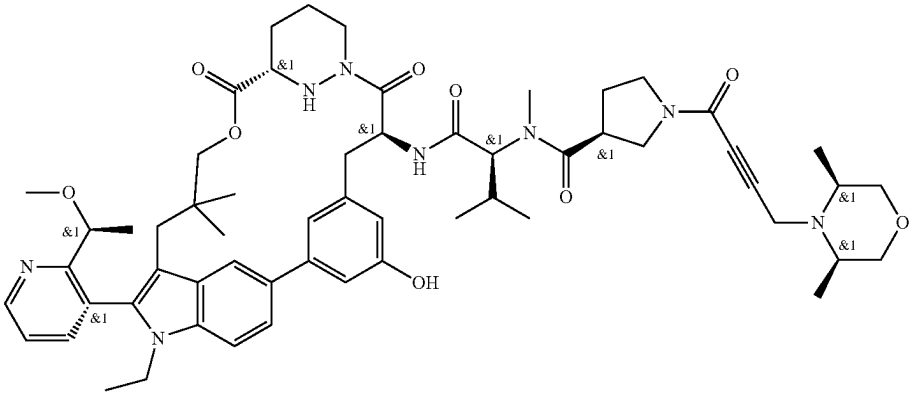
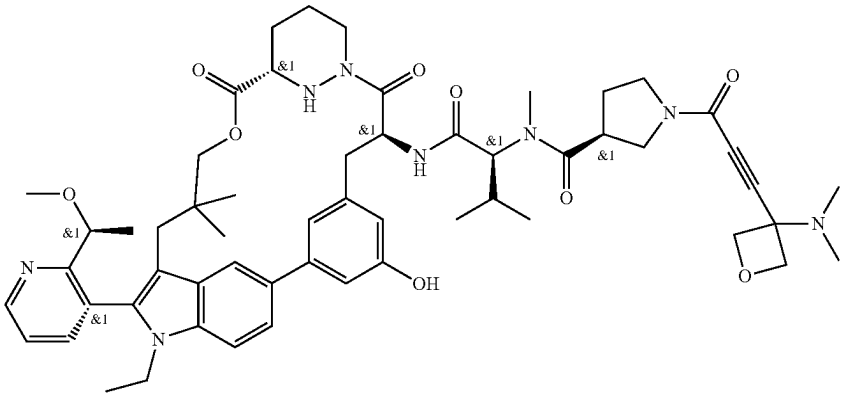
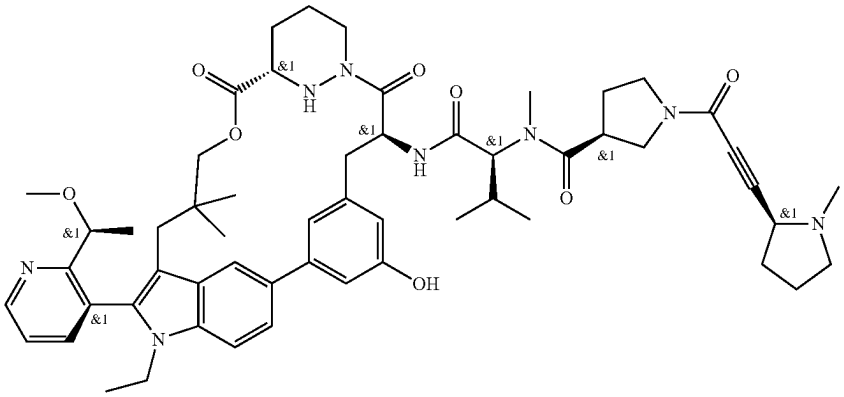
Certain Compounds of the Present Invention	
Ex#	Structure
A245	
A246	
A247	

TABLE 1-continued

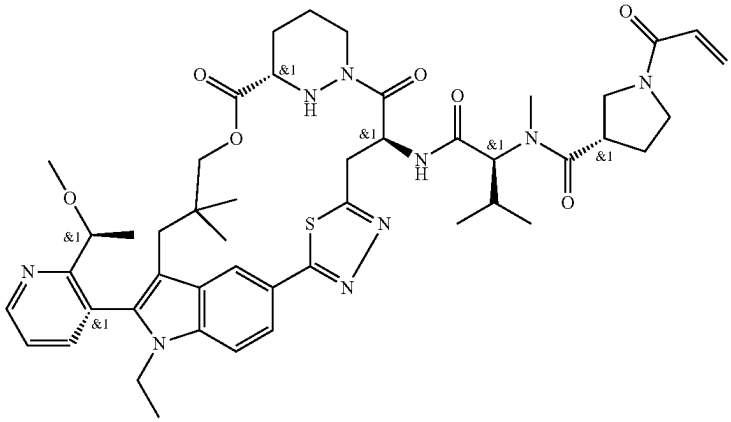
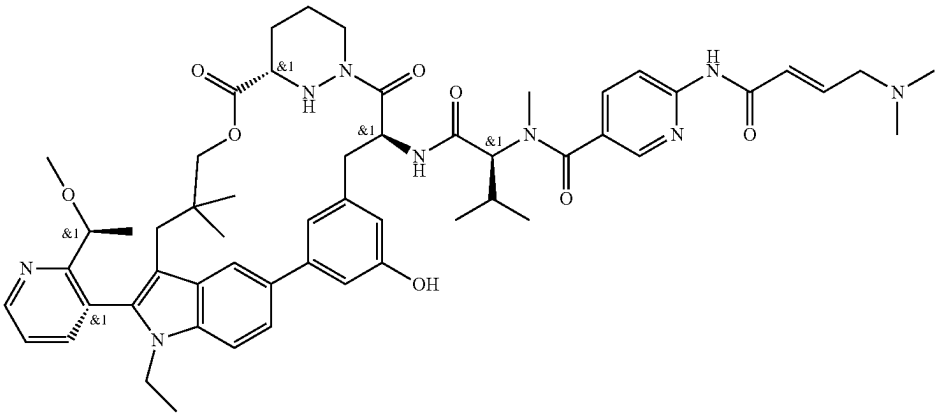
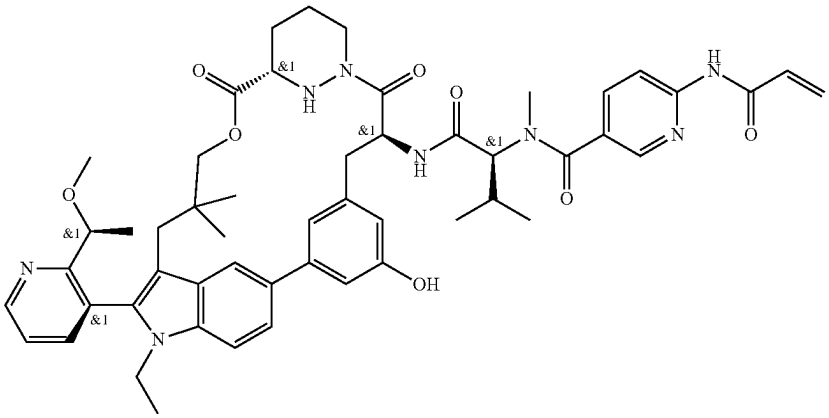
Certain Compounds of the Present Invention	
Ex#	Structure
A248	
A249	
A250	

TABLE 1-continued

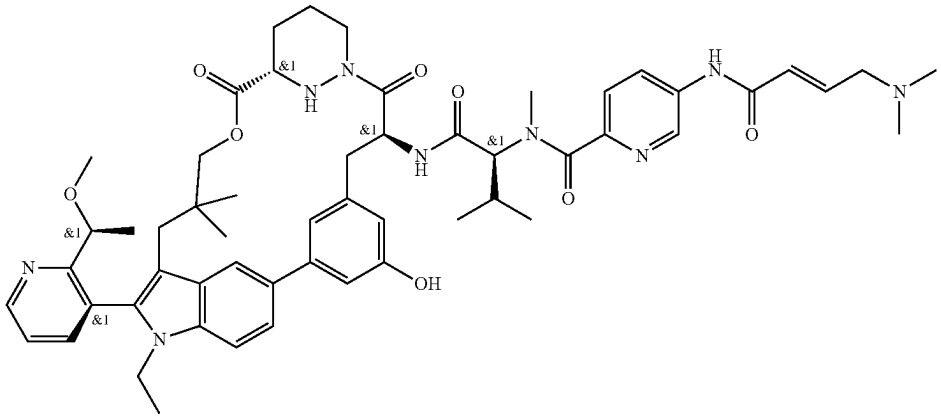
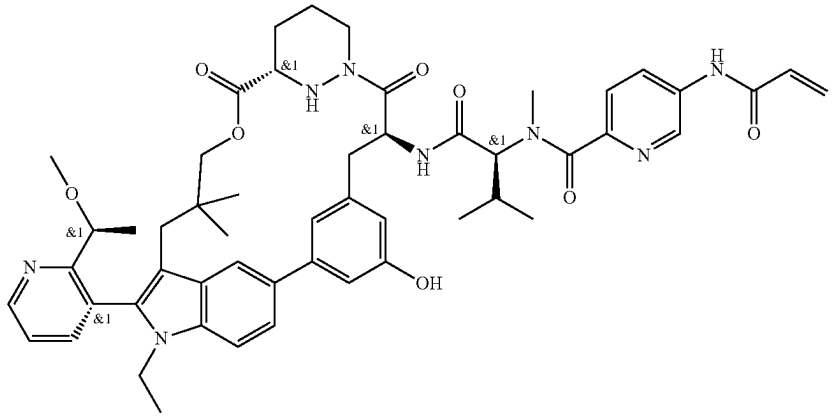
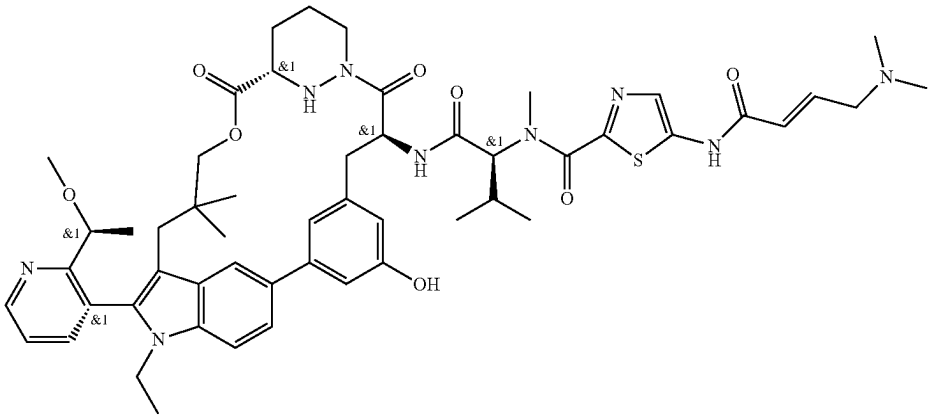
Certain Compounds of the Present Invention	
Ex#	Structure
A251	
A252	
A253	

TABLE 1-continued

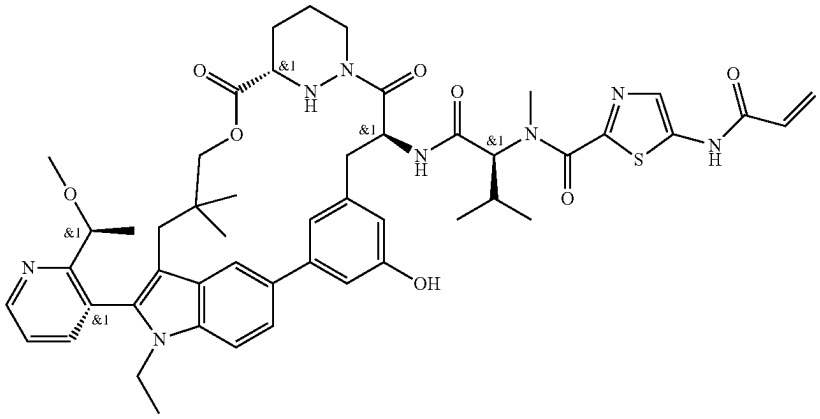
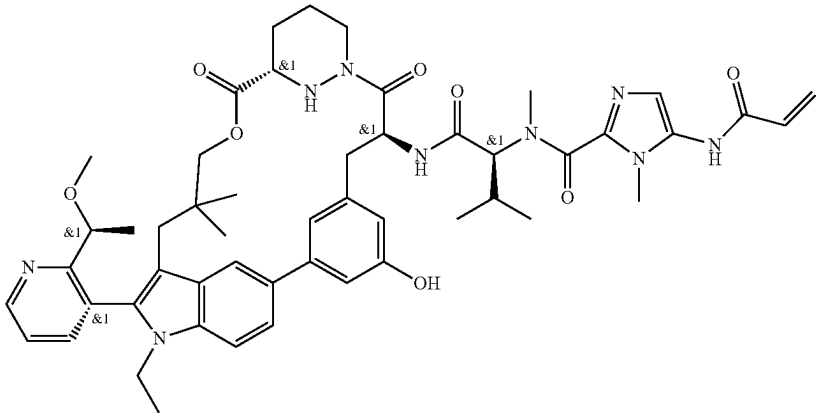
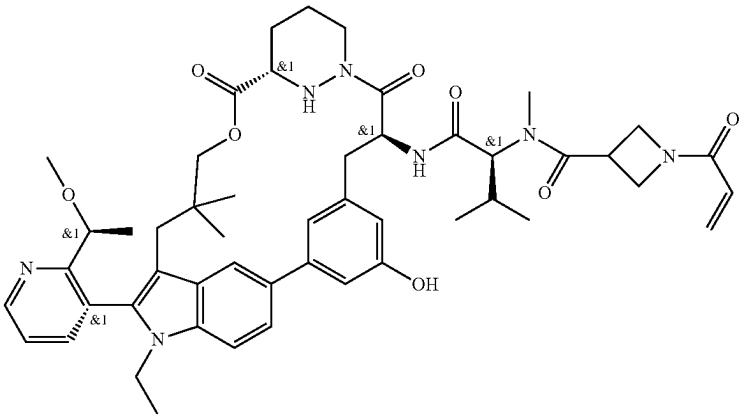
Certain Compounds of the Present Invention	
Ex#	Structure
A254	
A255	
A256	

TABLE 1-continued

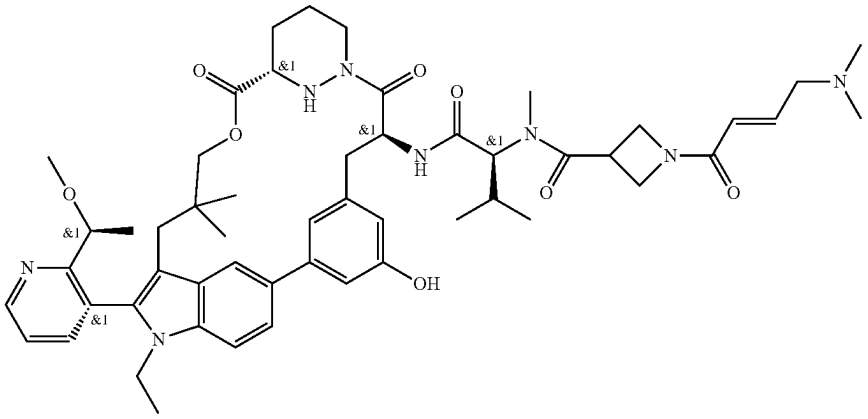
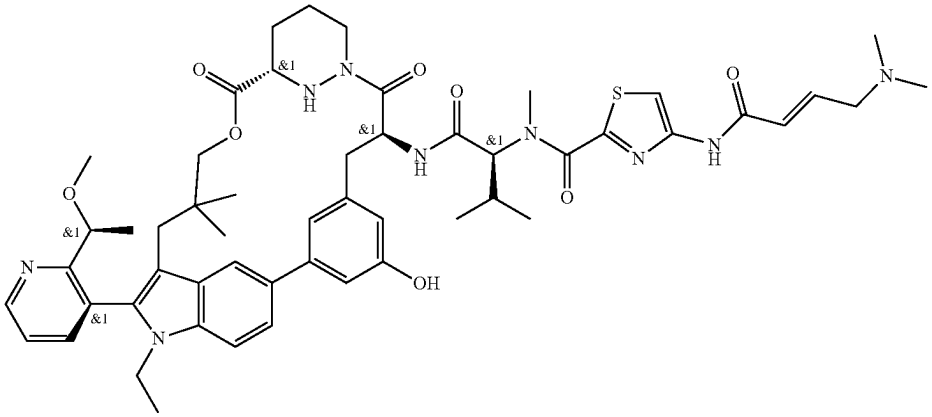
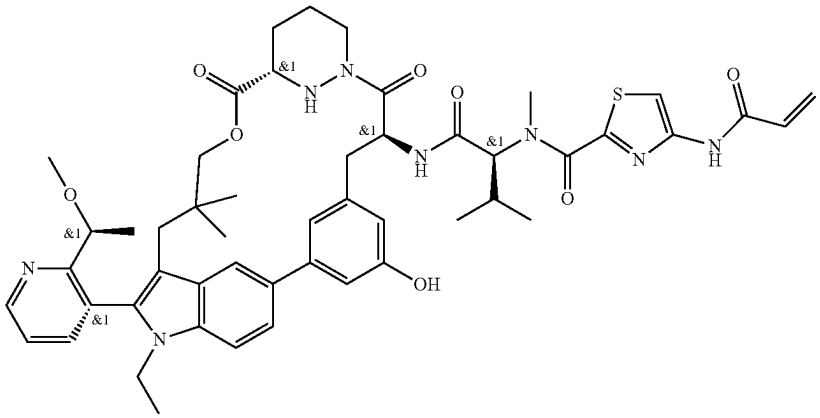
Certain Compounds of the Present Invention	
Ex#	Structure
A257	
A258	
A259	

TABLE 1-continued

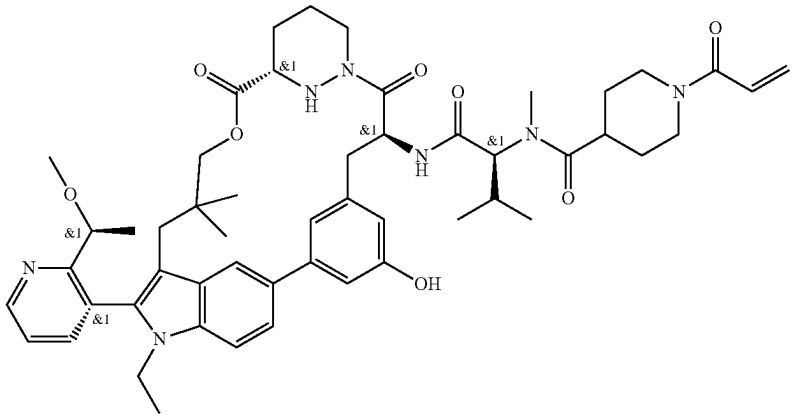
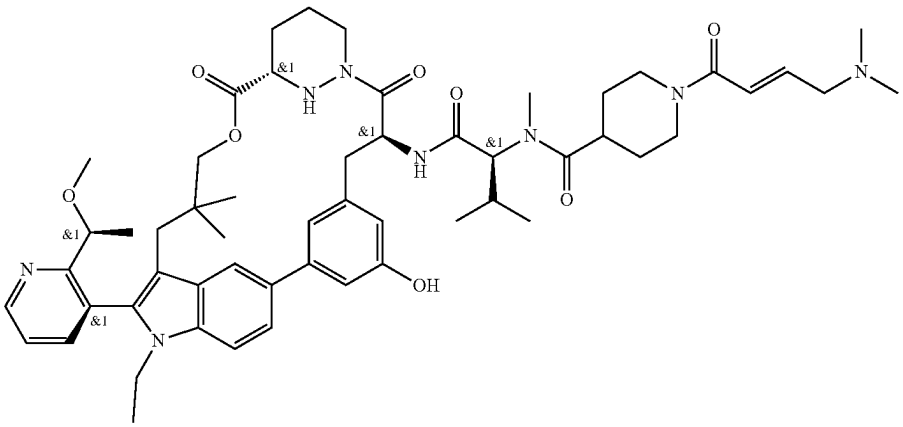
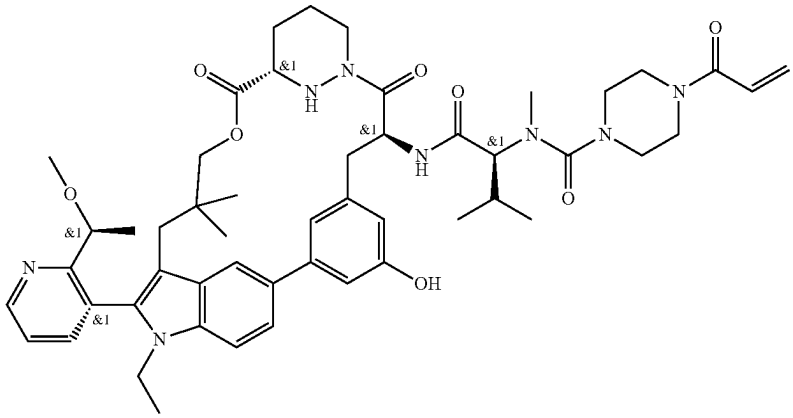
Certain Compounds of the Present Invention	
Ex#	Structure
A260	
A261	
A262	

TABLE 1-continued

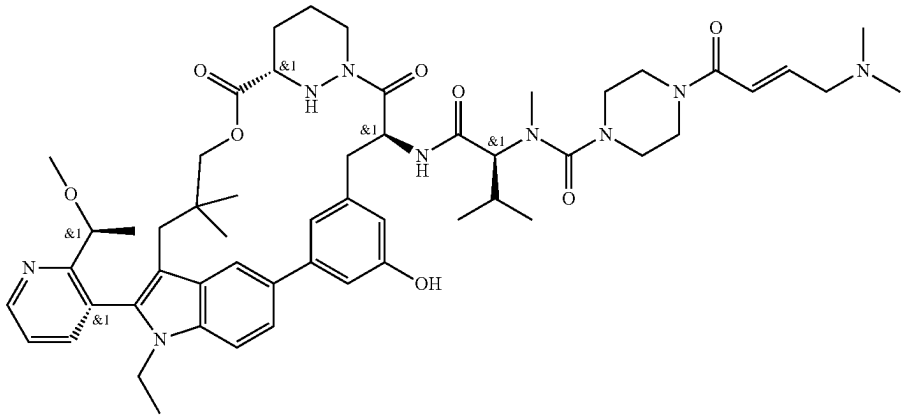
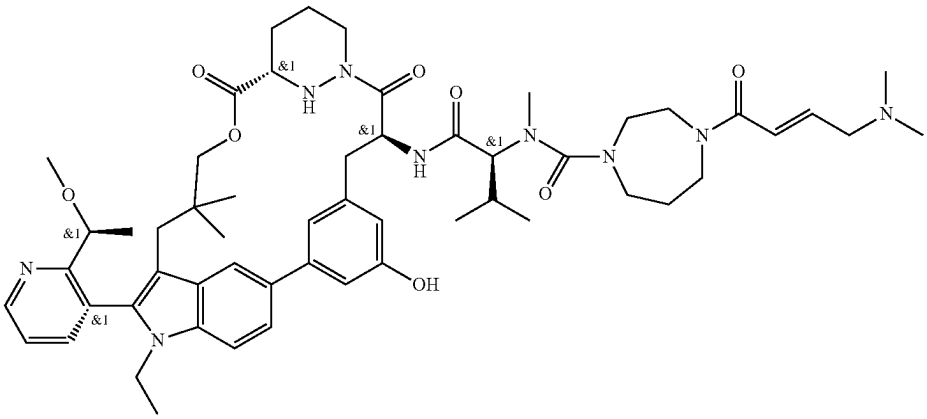
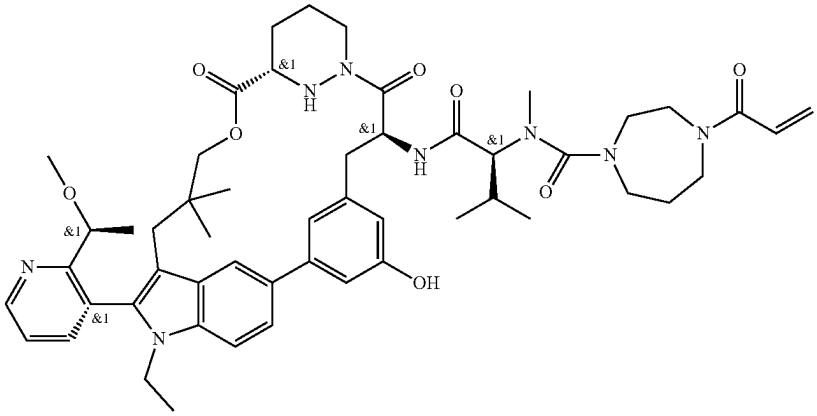
Certain Compounds of the Present Invention	
Ex#	Structure
A263	
A264	
A265	

TABLE 1-continued

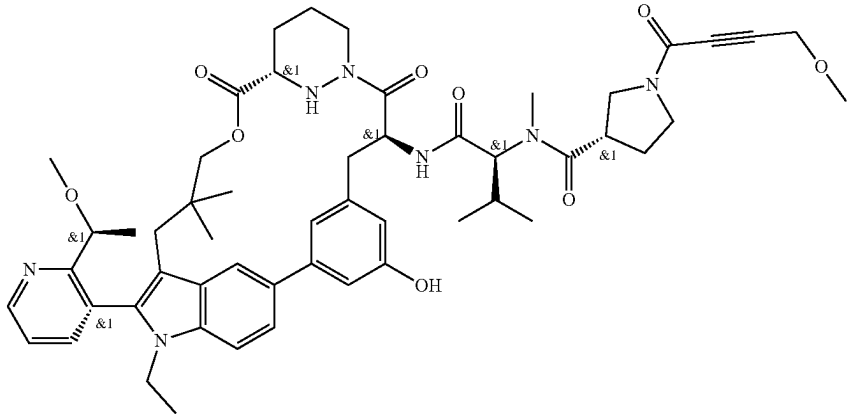
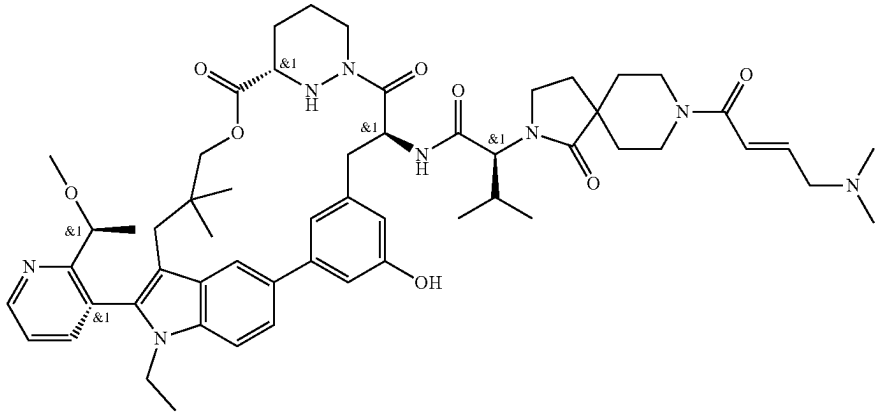
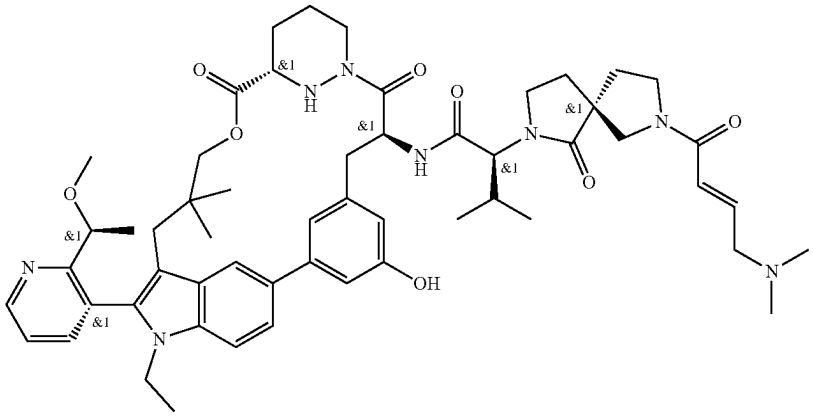
Certain Compounds of the Present Invention	
Ex#	Structure
A266	
A267	
A268	

TABLE 1-continued

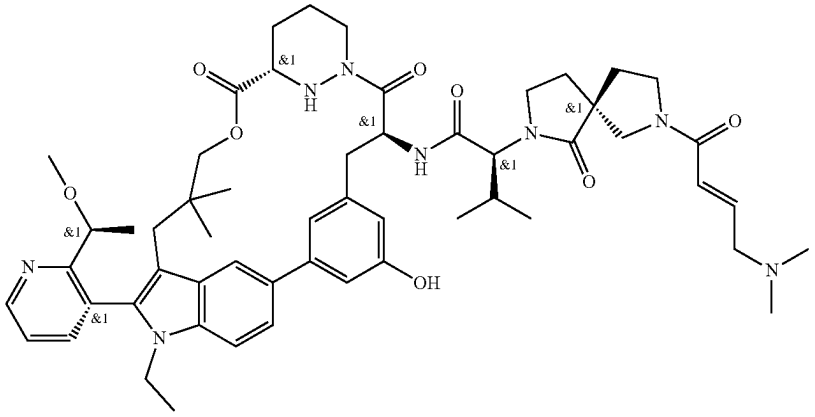
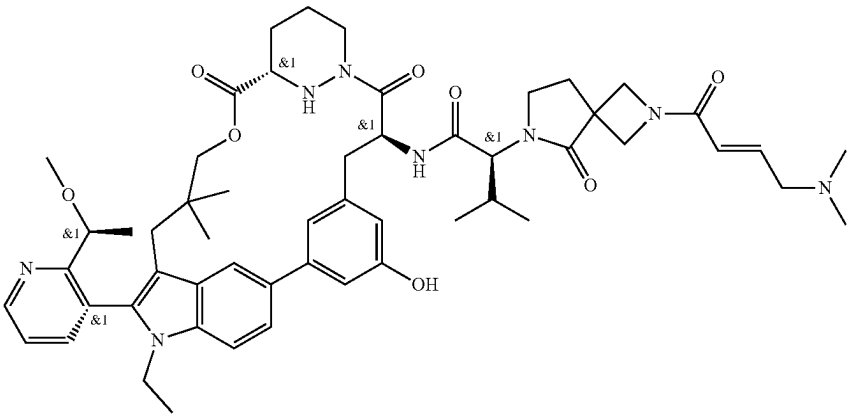
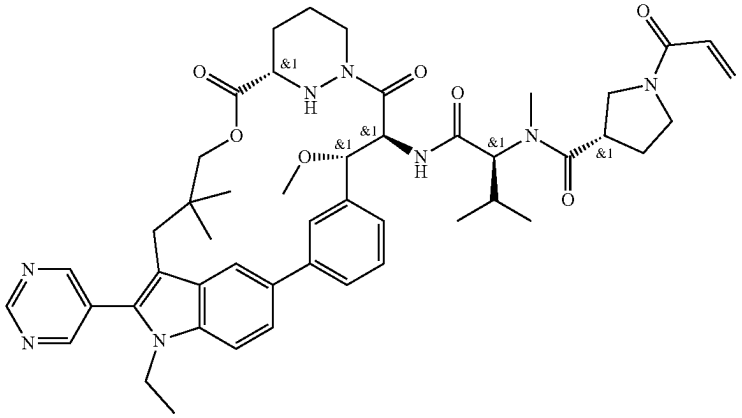
Certain Compounds of the Present Invention	
Ex#	Structure
A269	
A270	
A271	

TABLE 1-continued

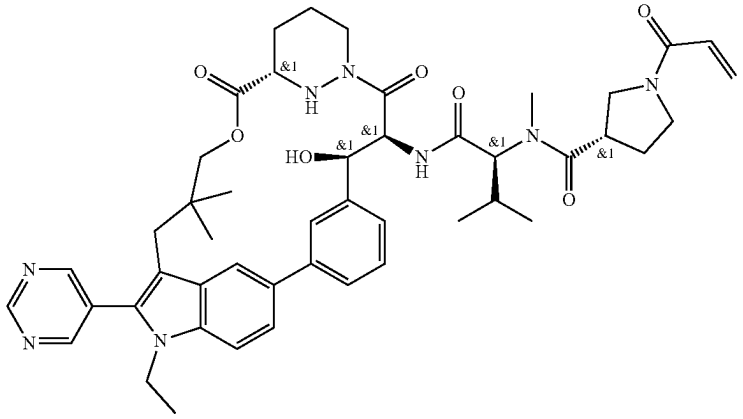
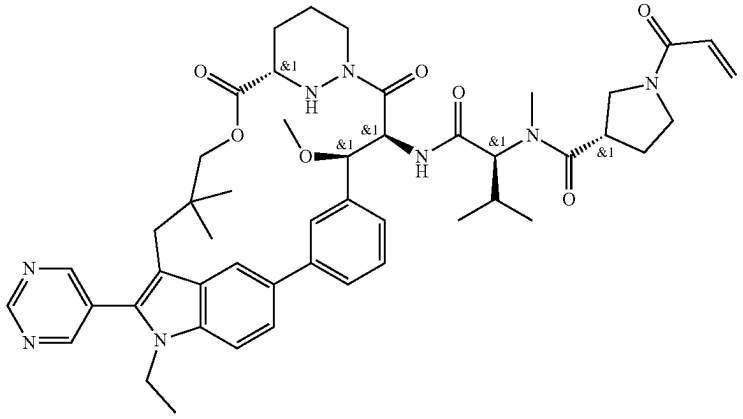
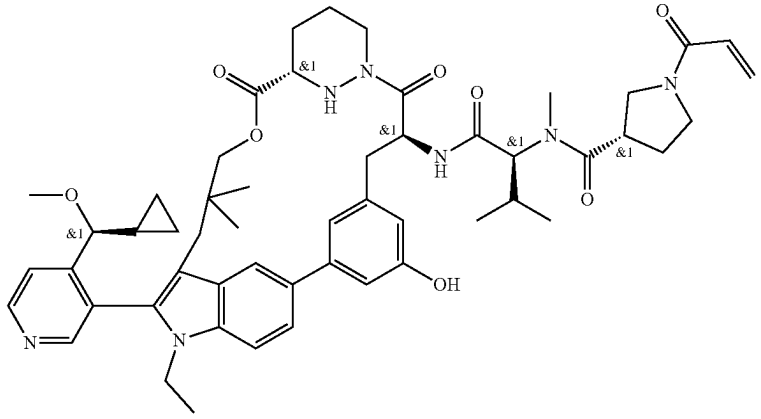
Certain Compounds of the Present Invention	
Ex#	Structure
A272	
A273	
A274	

TABLE 1-continued

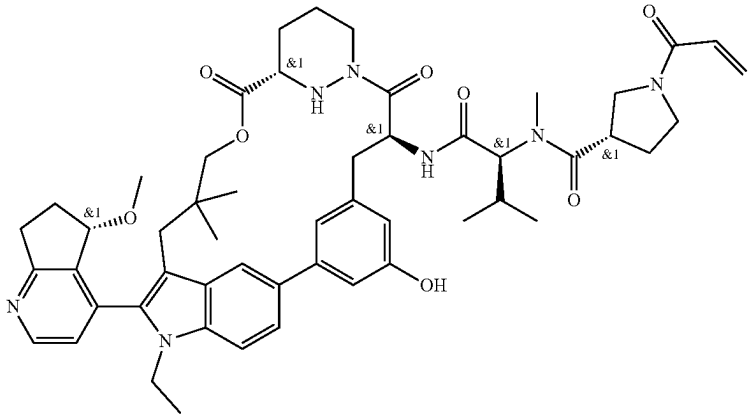
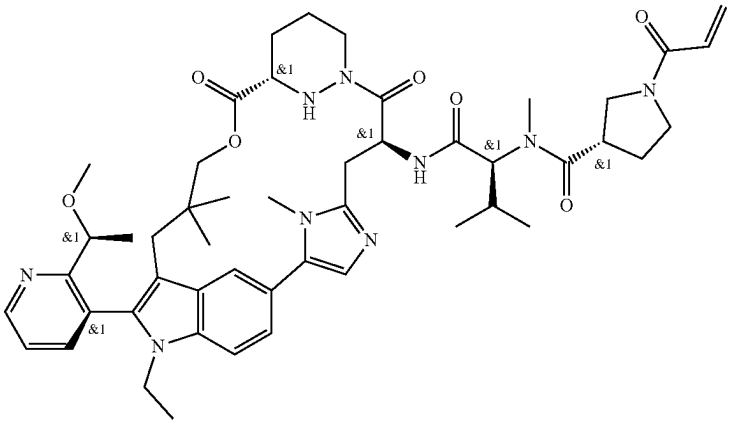
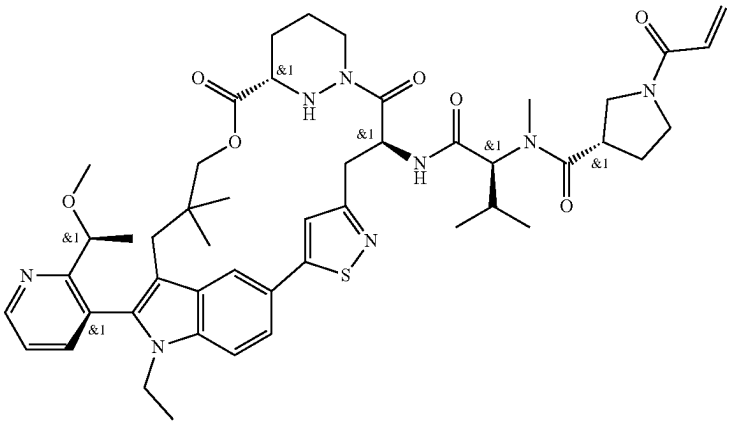
Certain Compounds of the Present Invention	
Ex#	Structure
A275	
A276	
A277	

TABLE 1-continued

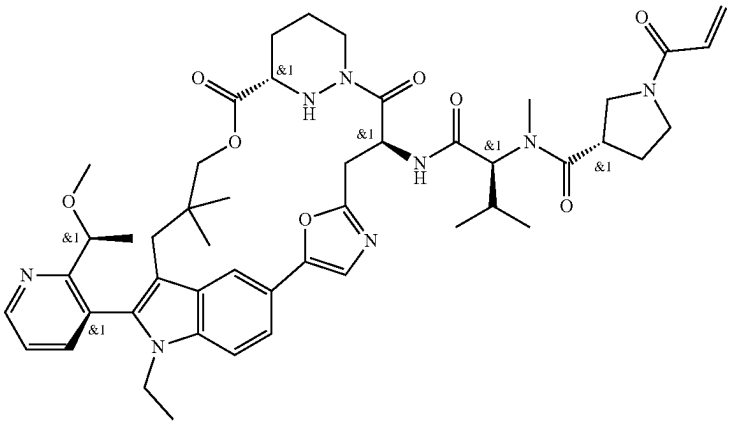
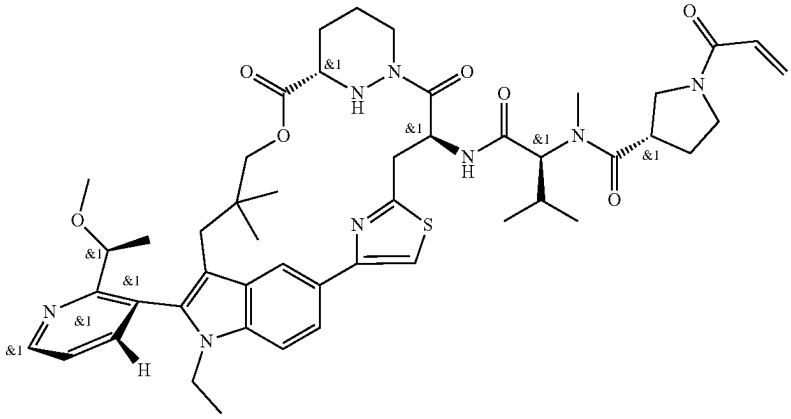
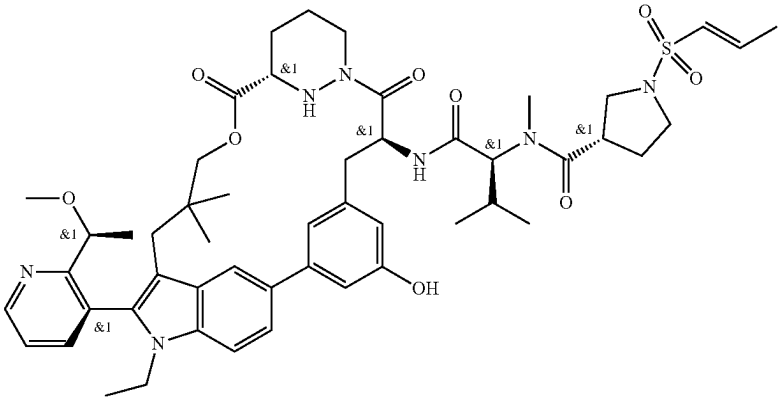
Certain Compounds of the Present Invention	
Ex#	Structure
A278	
A279	
A280	

TABLE 1-continued

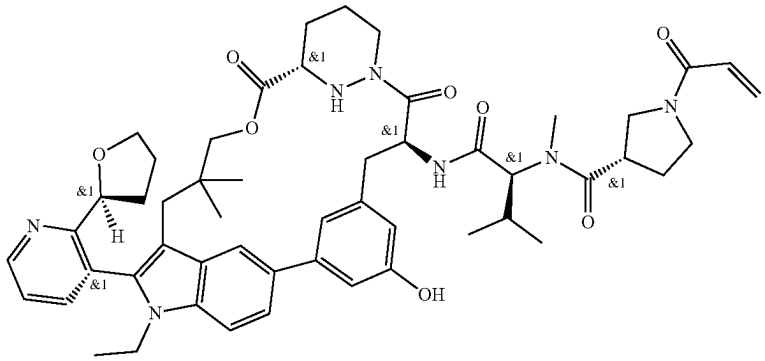
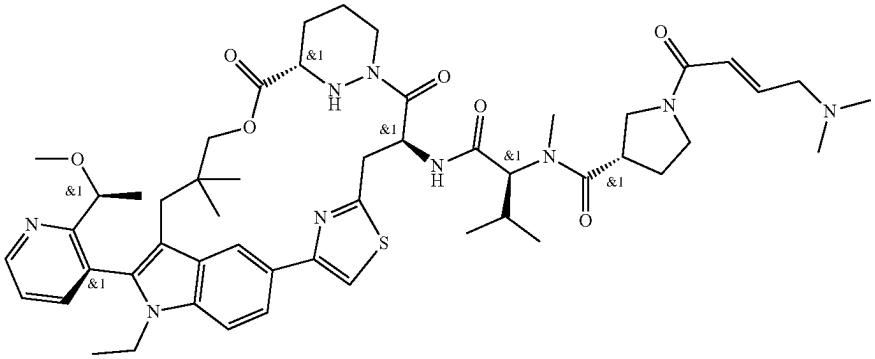
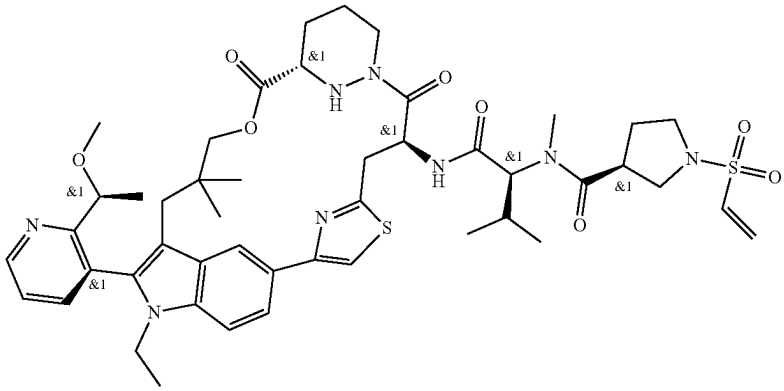
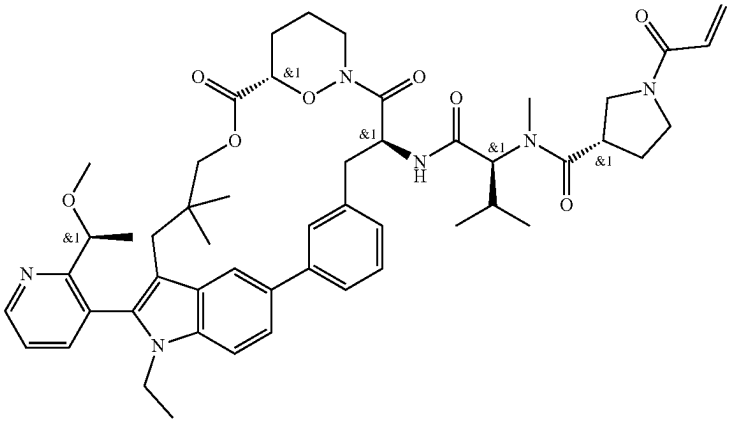
Certain Compounds of the Present Invention	
Ex#	Structure
A281	
A282	
A283	
A284	

TABLE 1-continued

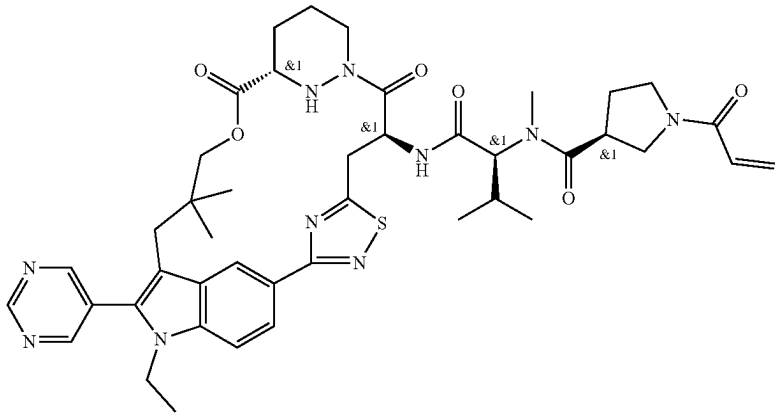
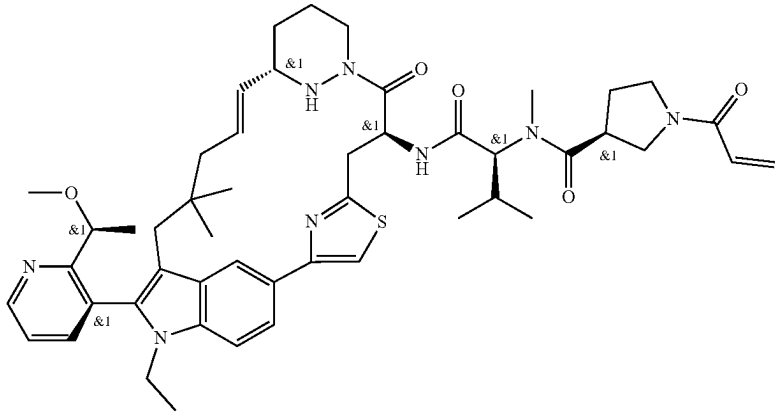
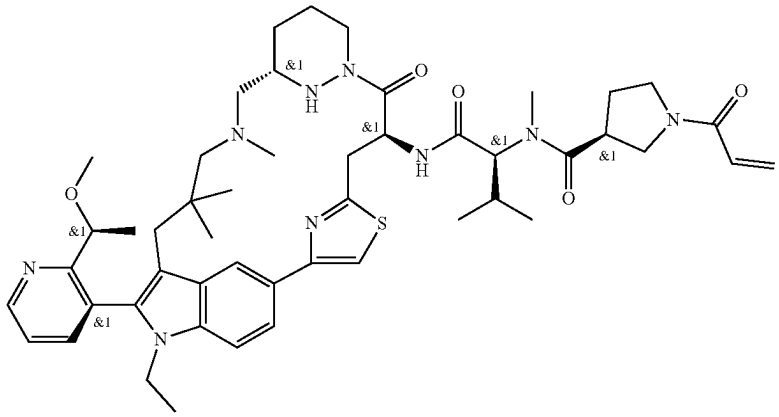
Certain Compounds of the Present Invention	
Ex#	Structure
A285	
A286	
A287	

TABLE 1-continued

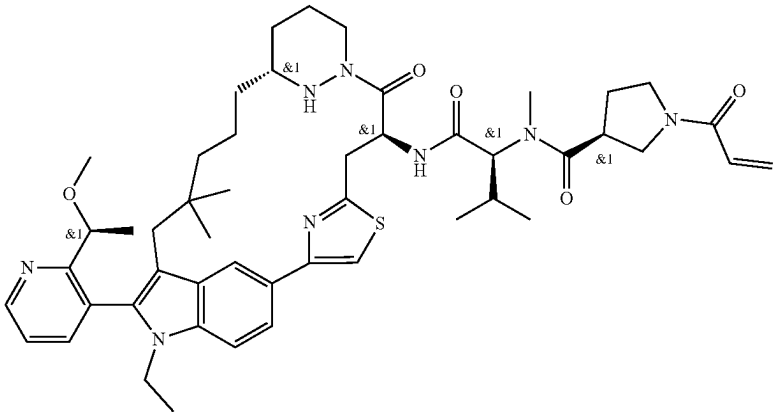
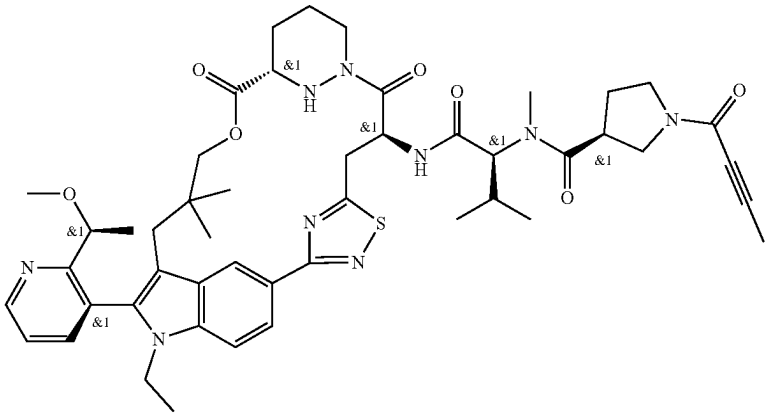
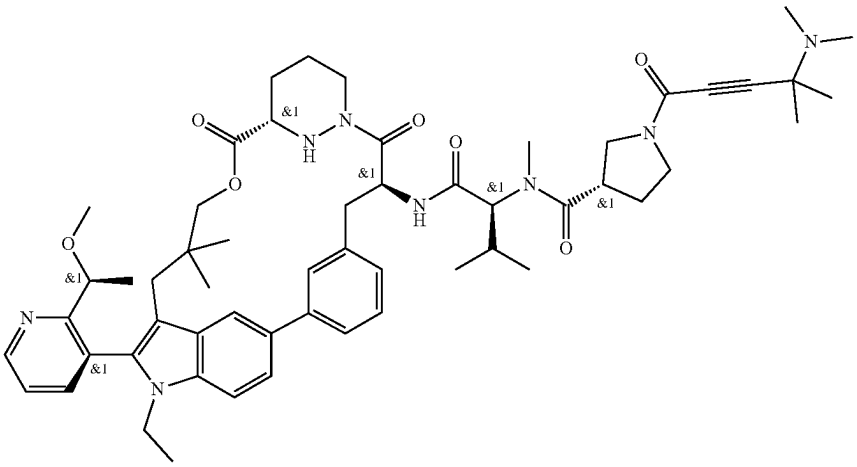
Certain Compounds of the Present Invention	
Ex#	Structure
A288	
A289	
A290	

TABLE 1-continued

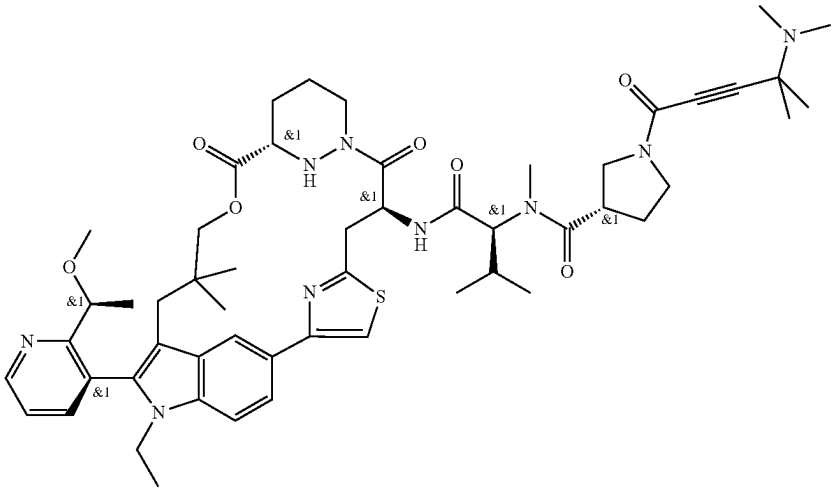
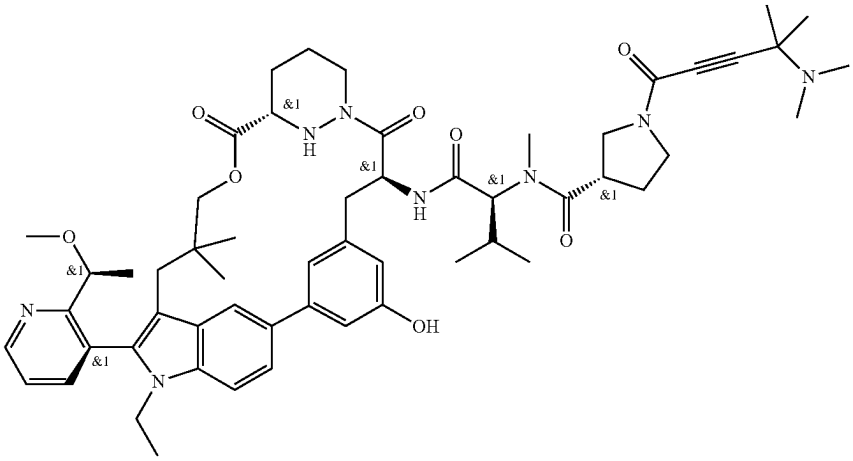
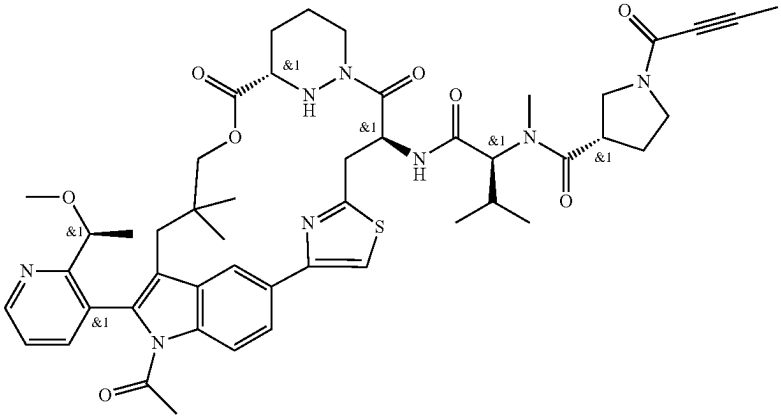
Certain Compounds of the Present Invention	
Ex#	Structure
A291	
A292	
A293	

TABLE 1-continued

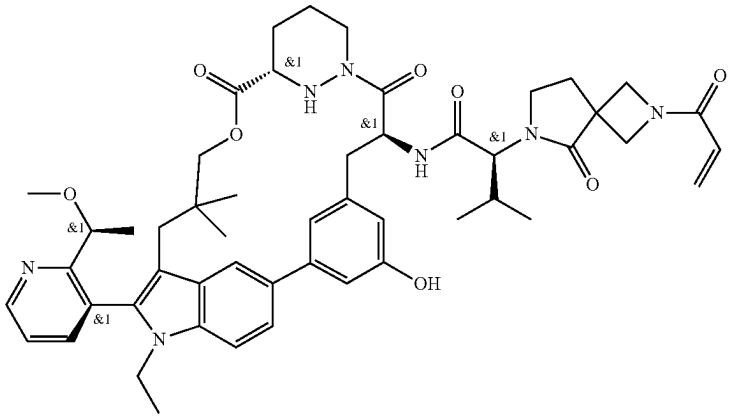
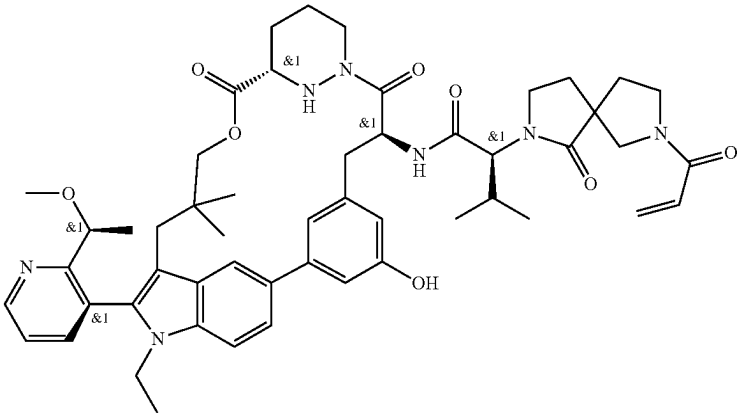
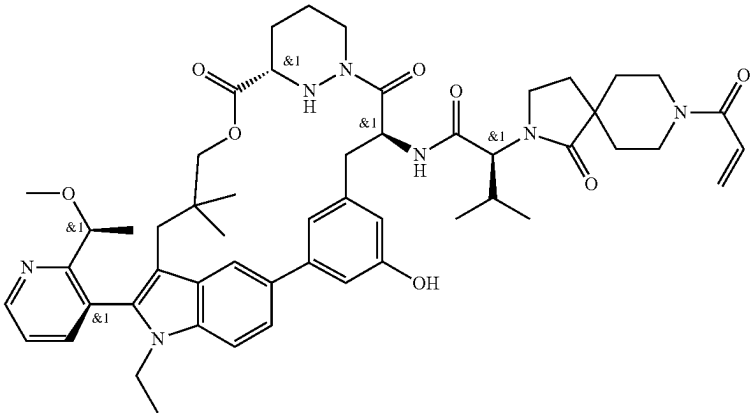
Certain Compounds of the Present Invention	
Ex#	Structure
A294	
A295	
A296	

TABLE 1-continued

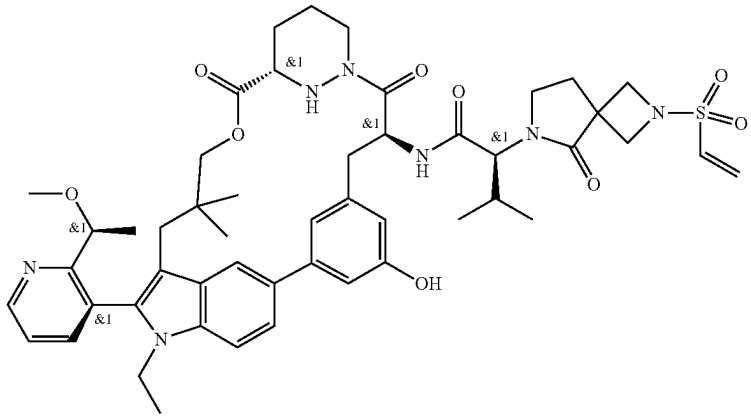
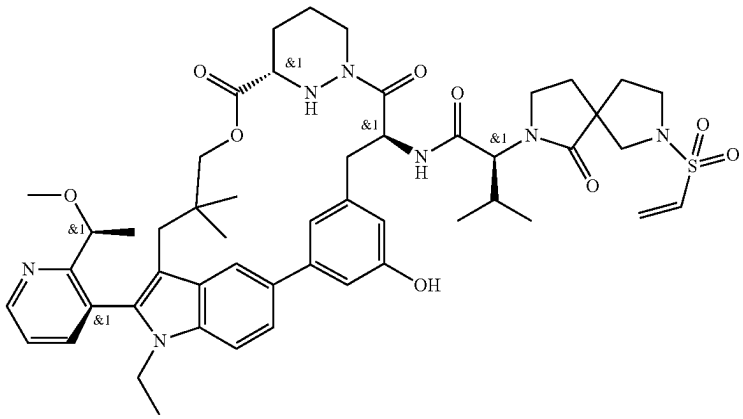
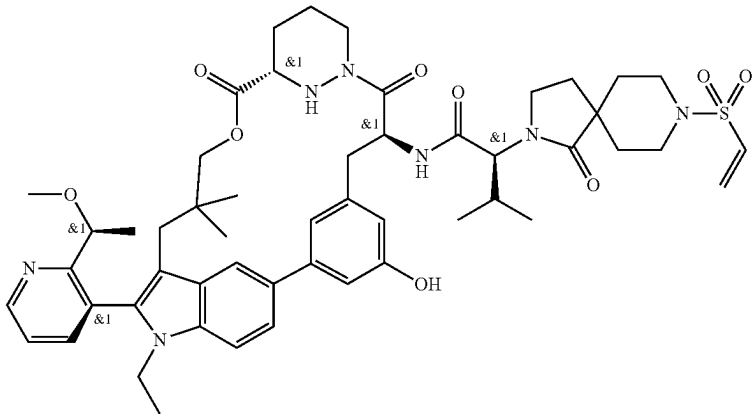
Certain Compounds of the Present Invention	
Ex#	Structure
A297	
A298	
A299	

TABLE 1-continued

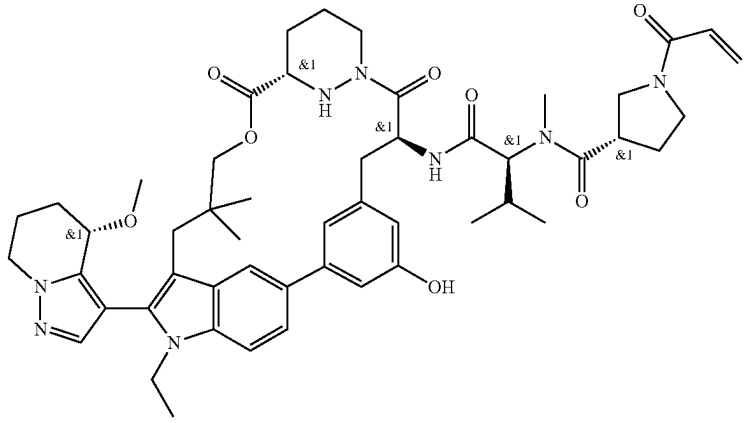
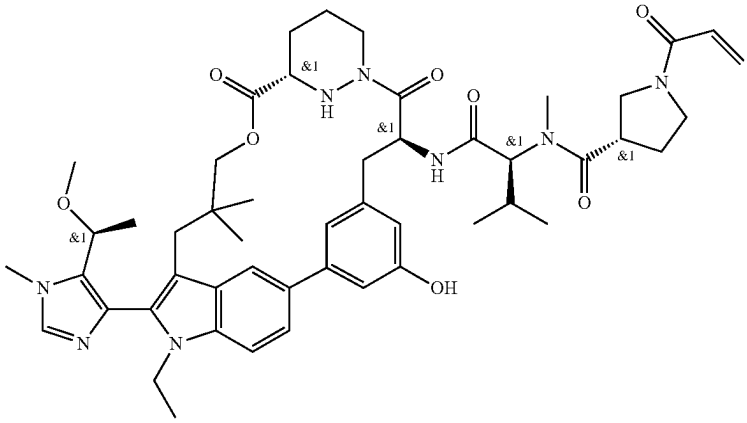
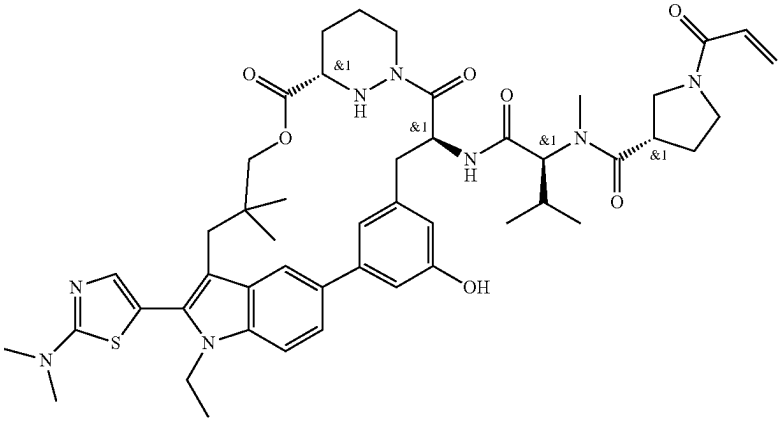
Certain Compounds of the Present Invention	
Ex#	Structure
A300	
A301	
A302	

TABLE 1-continued

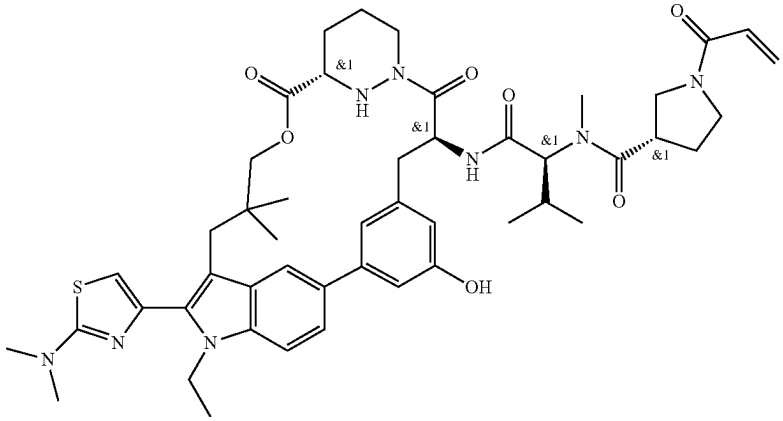
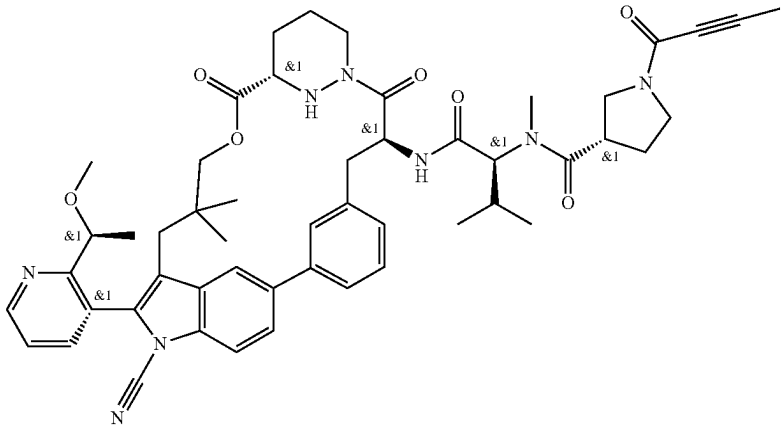
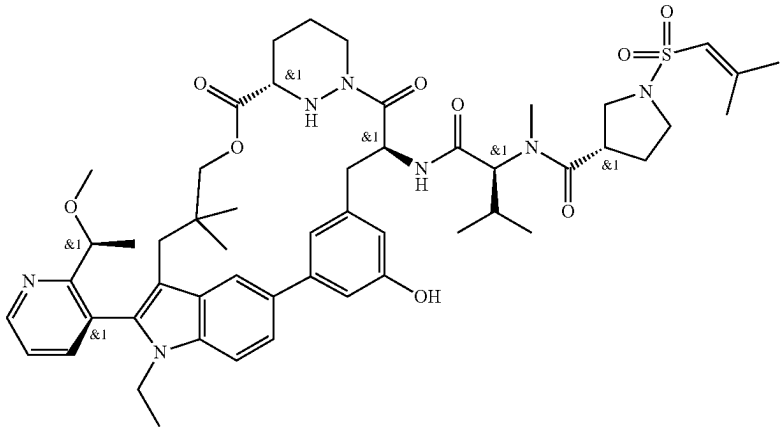
Certain Compounds of the Present Invention	
Ex#	Structure
A303	
A304	
A305	

TABLE 1-continued

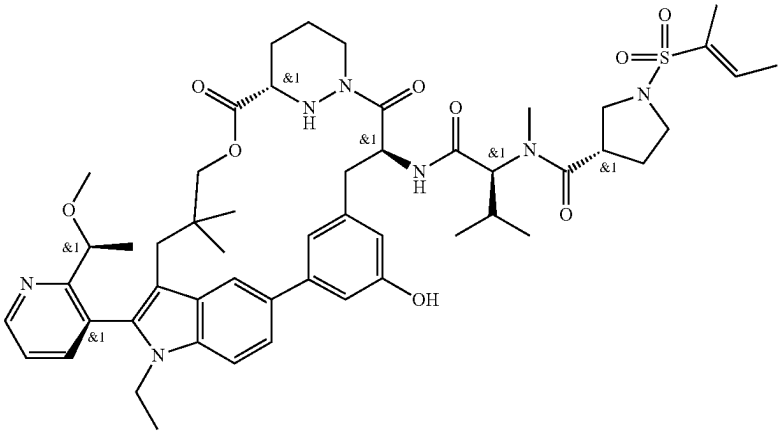
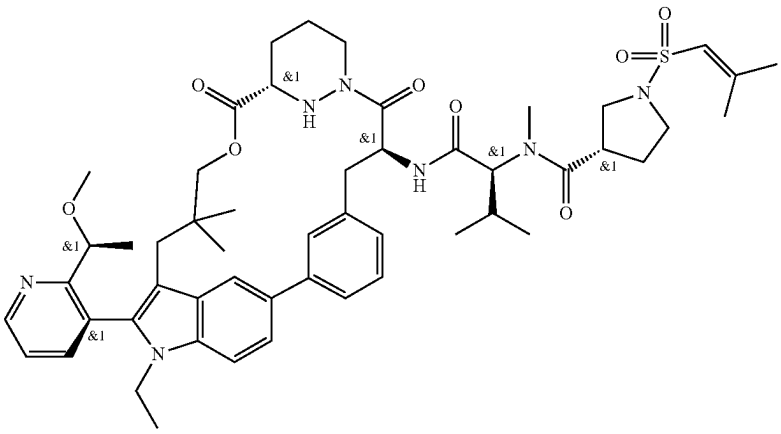
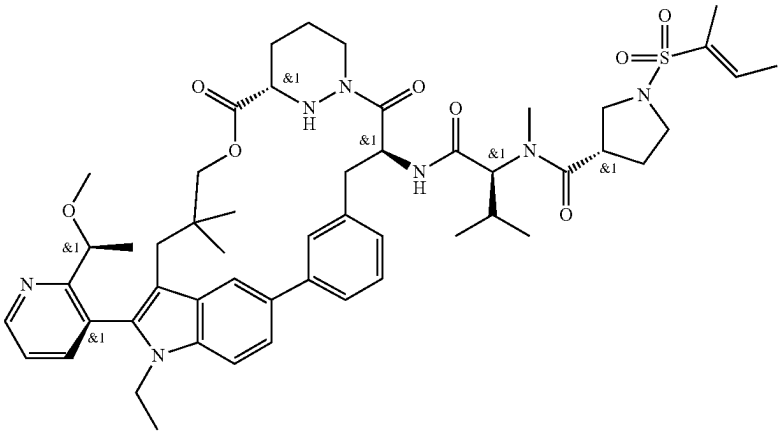
Certain Compounds of the Present Invention	
Ex#	Structure
A306	
A307	
A308	

TABLE 1-continued

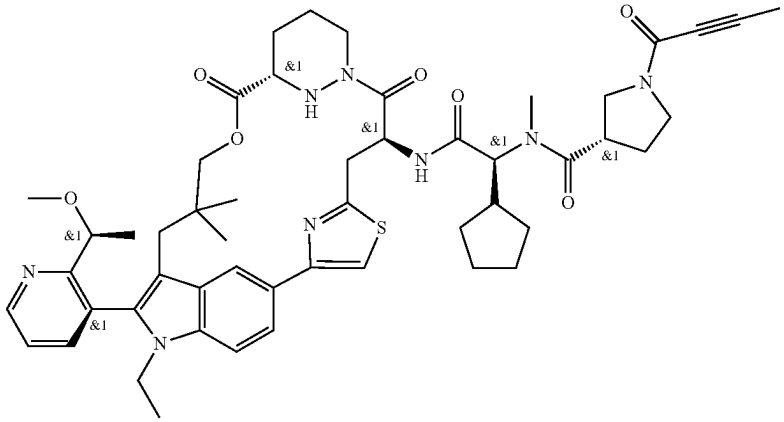
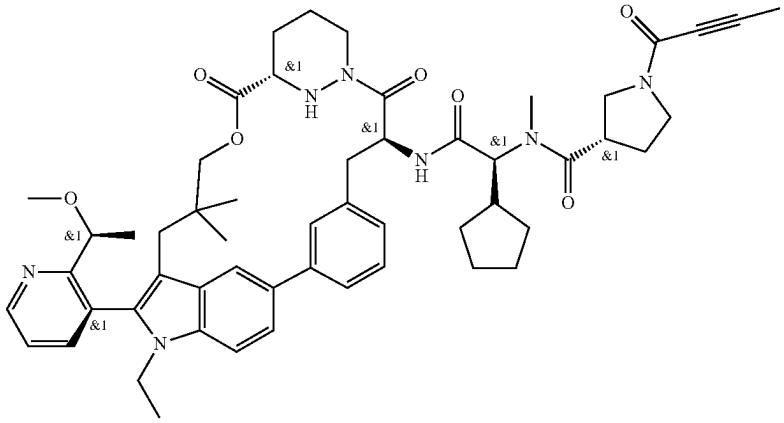
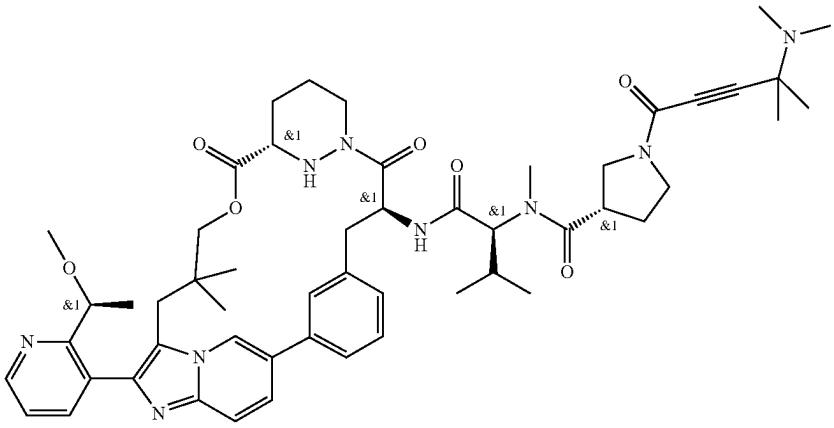
Certain Compounds of the Present Invention	
Ex#	Structure
A309	
A310	
A311	

TABLE 1-continued

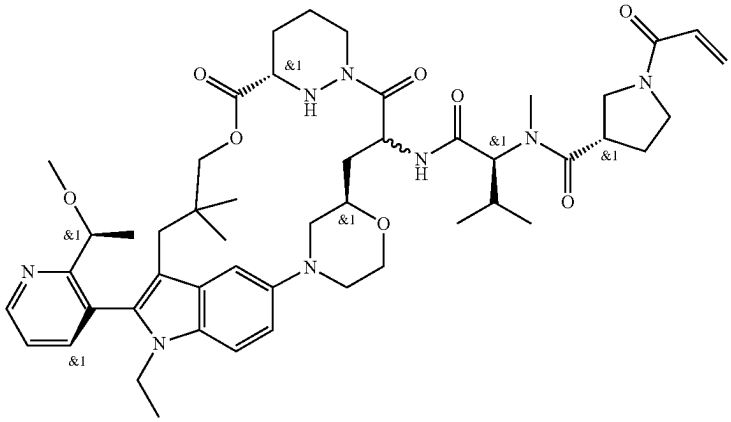
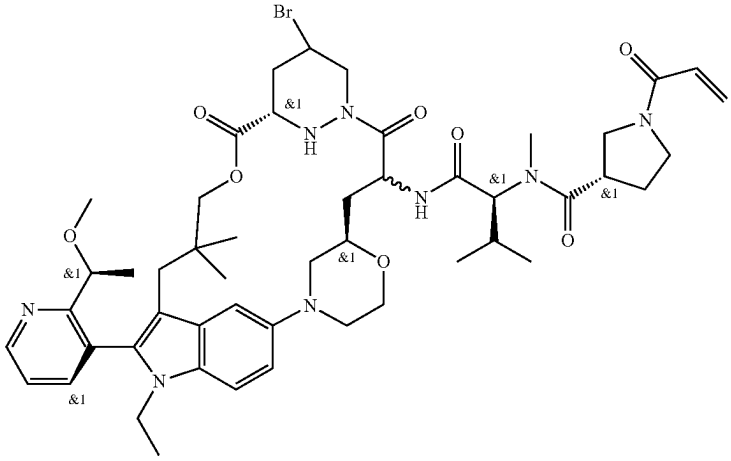
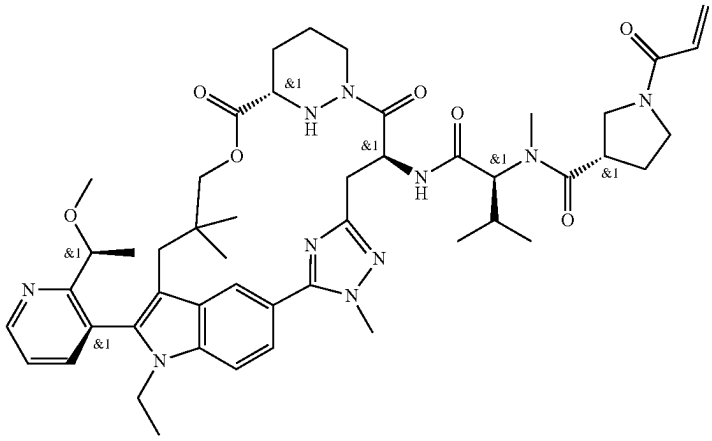
Certain Compounds of the Present Invention	
Ex#	Structure
A312	
A313	
A314	

TABLE 1-continued

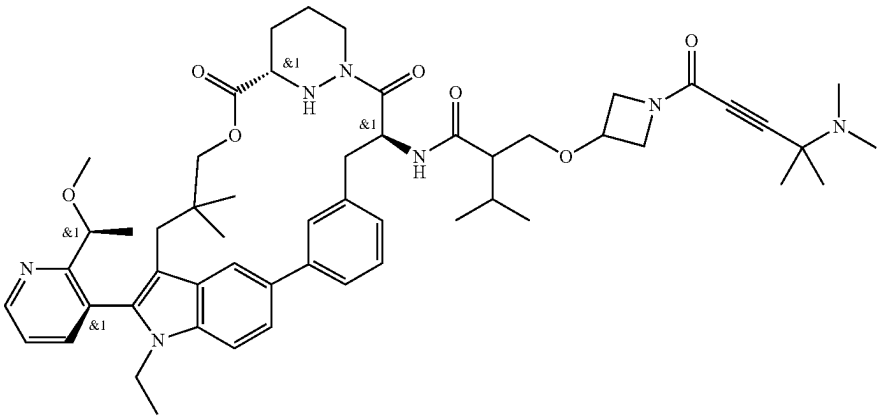
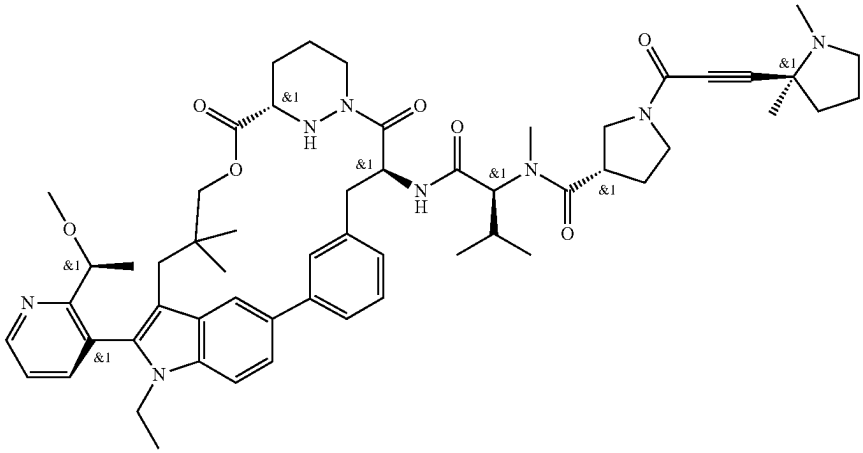
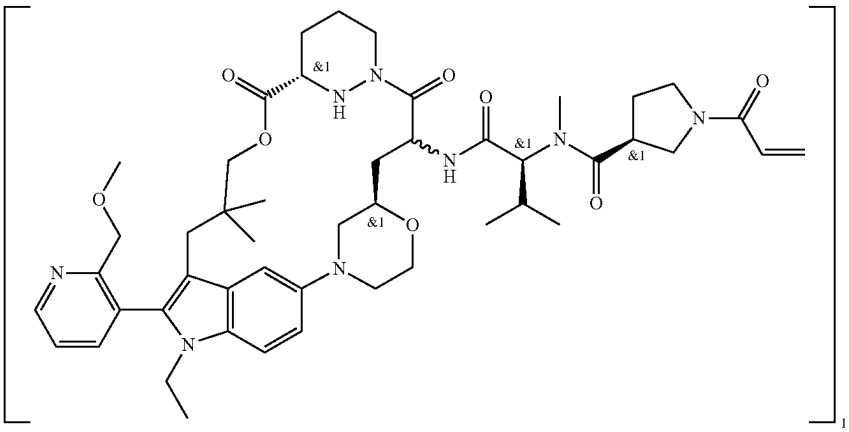
Certain Compounds of the Present Invention	
Ex#	Structure
A316	
A317	
A318	

TABLE 1-continued

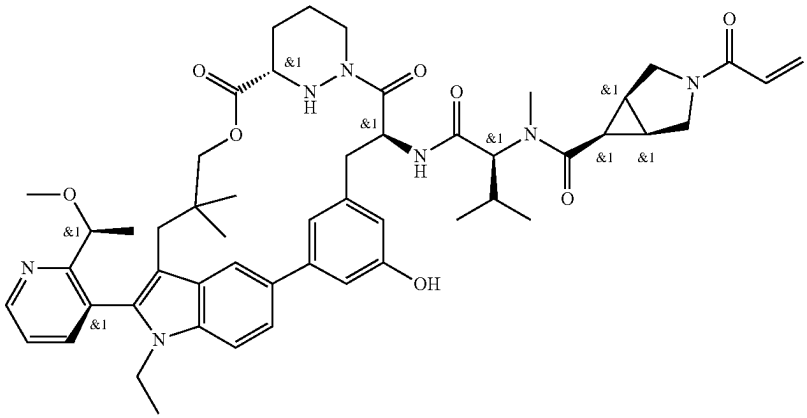
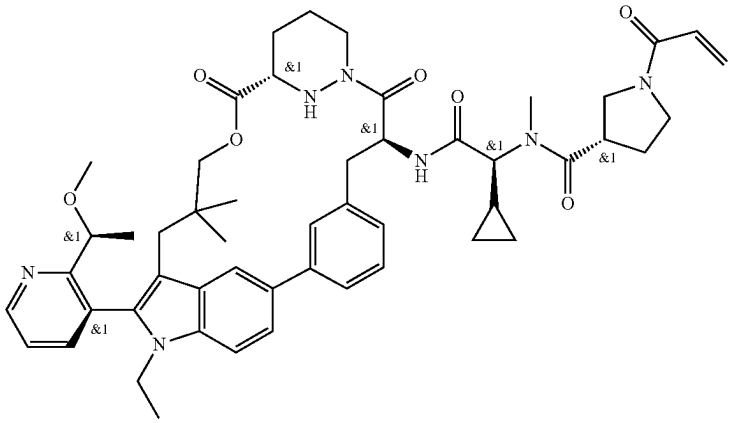
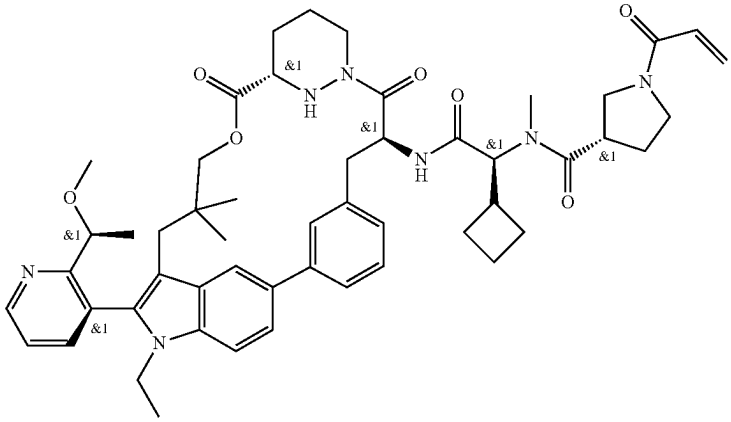
Certain Compounds of the Present Invention	
Ex#	Structure
A319	
A320	
A321	

TABLE 1-continued

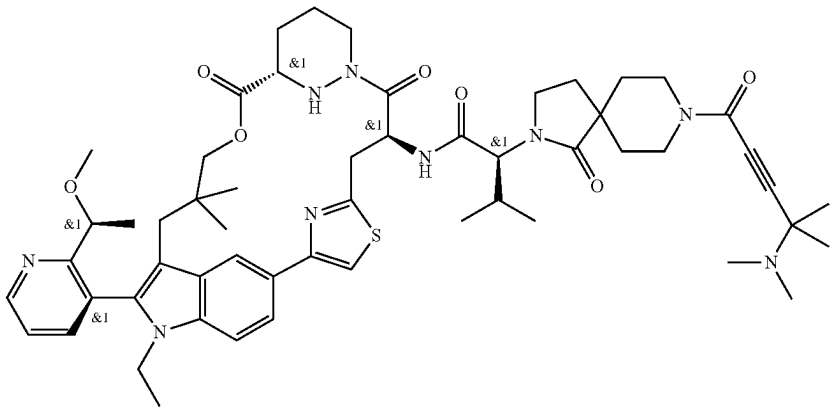
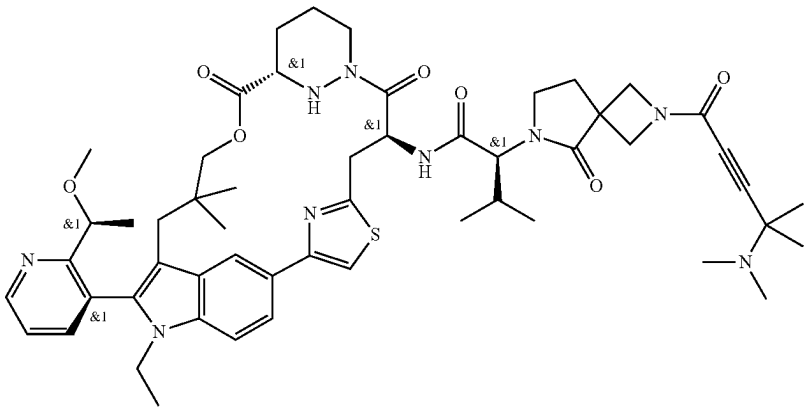
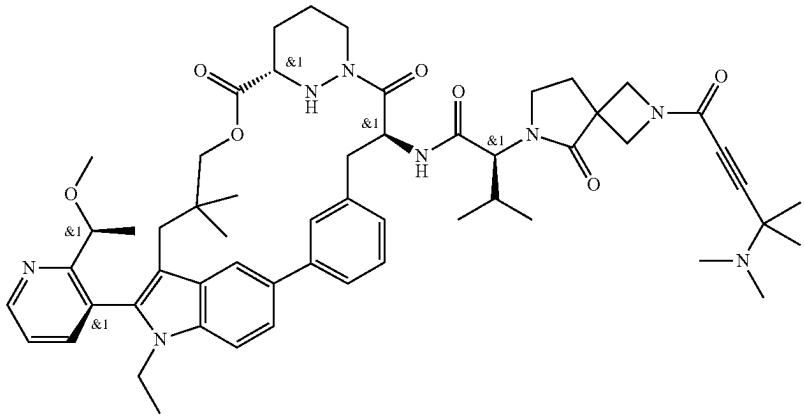
Certain Compounds of the Present Invention	
Ex#	Structure
A322	
A323	
A324	

TABLE 1-continued

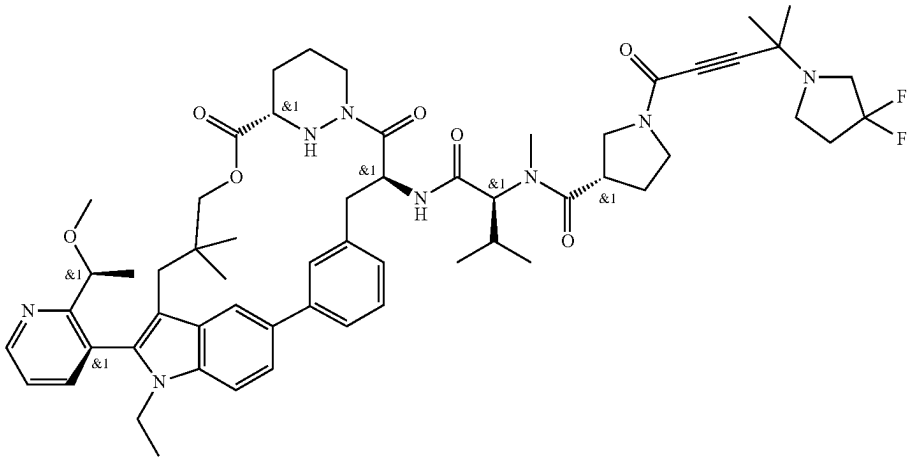
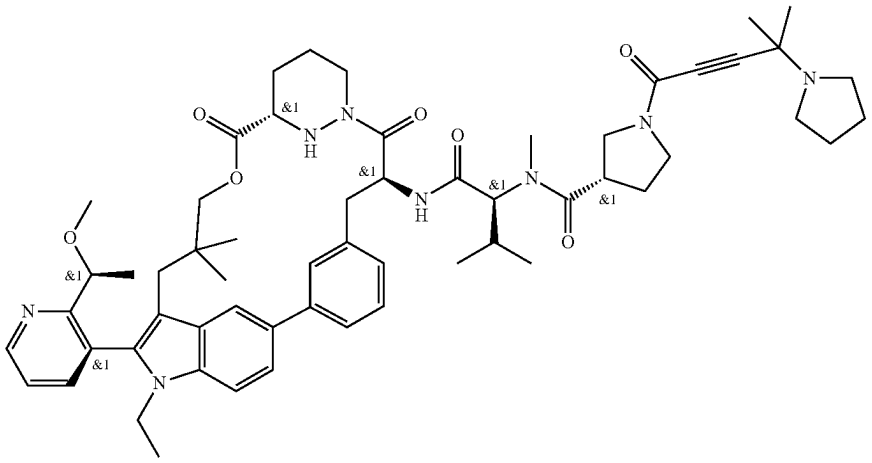
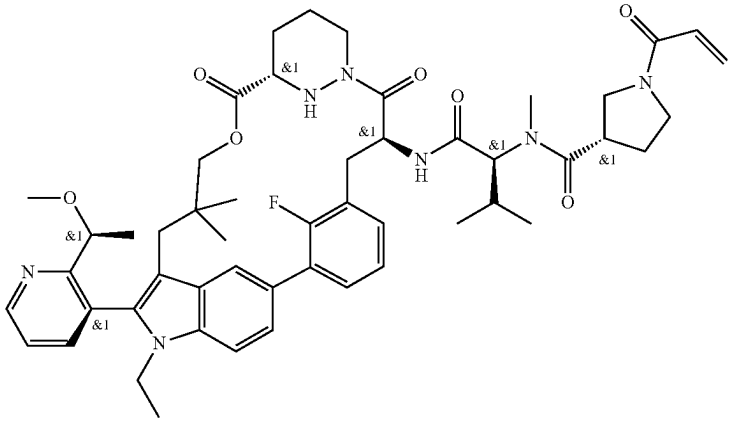
Certain Compounds of the Present Invention	
Ex#	Structure
A325	
A326	
A327	

TABLE 1-continued

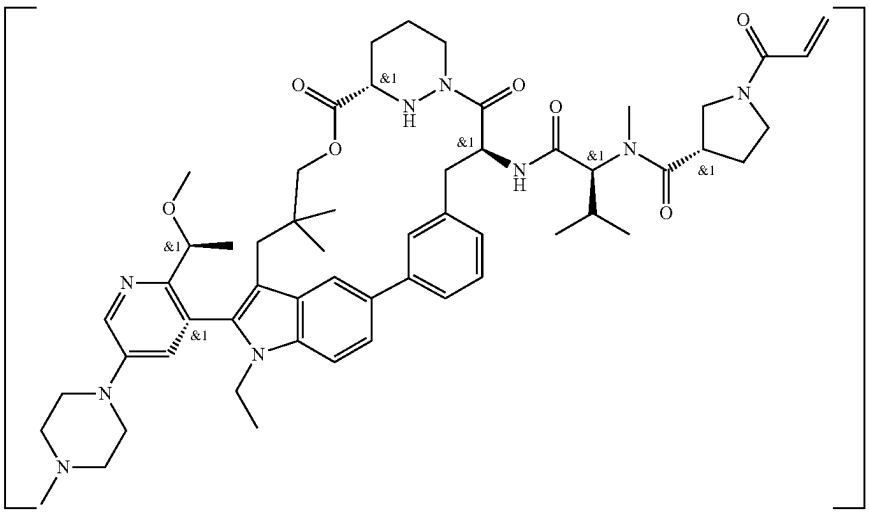
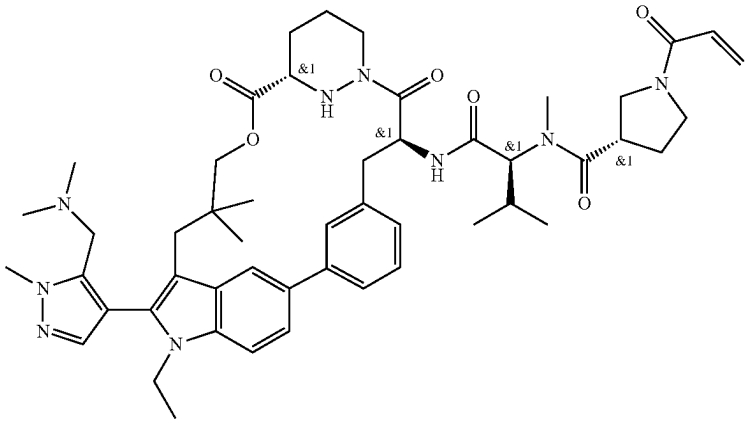
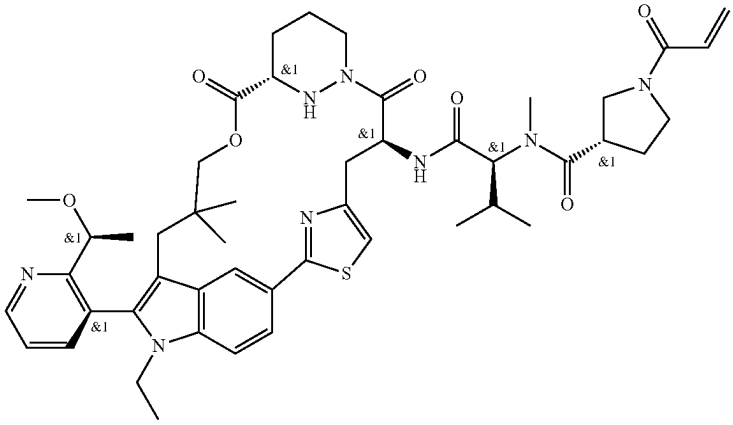
Certain Compounds of the Present Invention	
Ex#	Structure
A328	
A329	
A330	

TABLE 1-continued

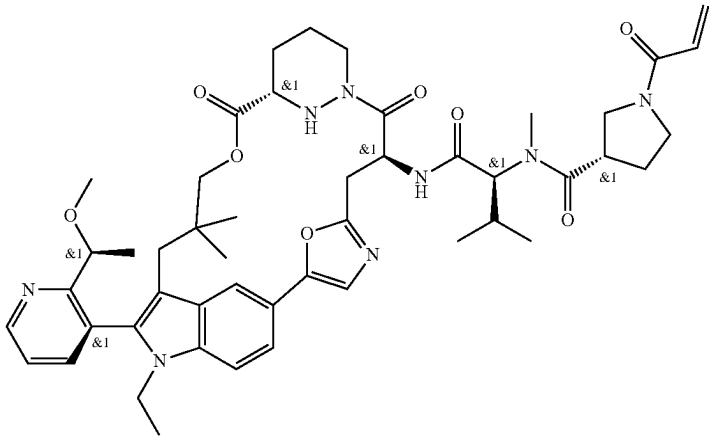
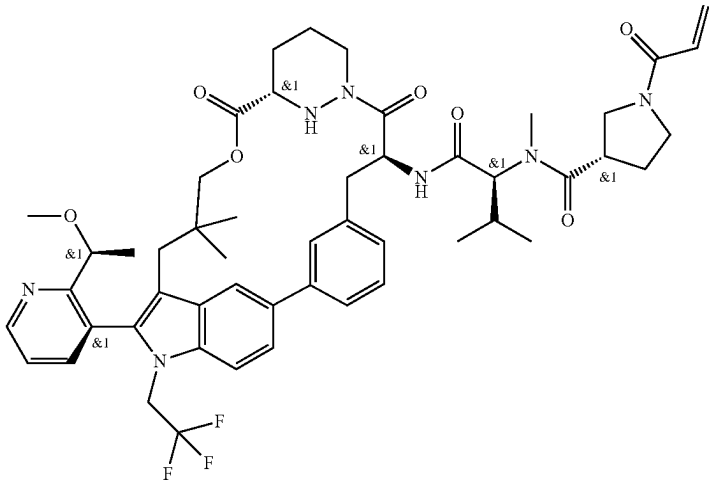
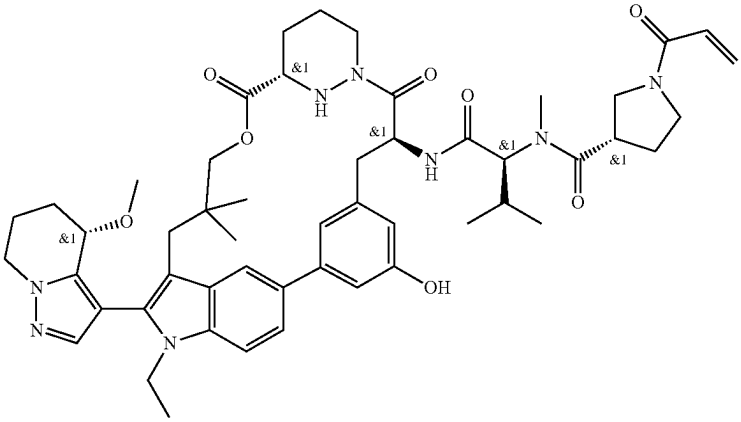
Certain Compounds of the Present Invention	
Ex#	Structure
A331	
A332	
A333	

TABLE 1-continued

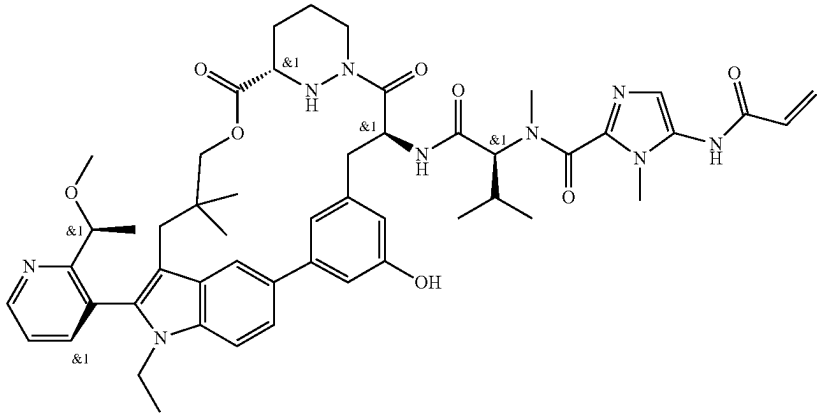
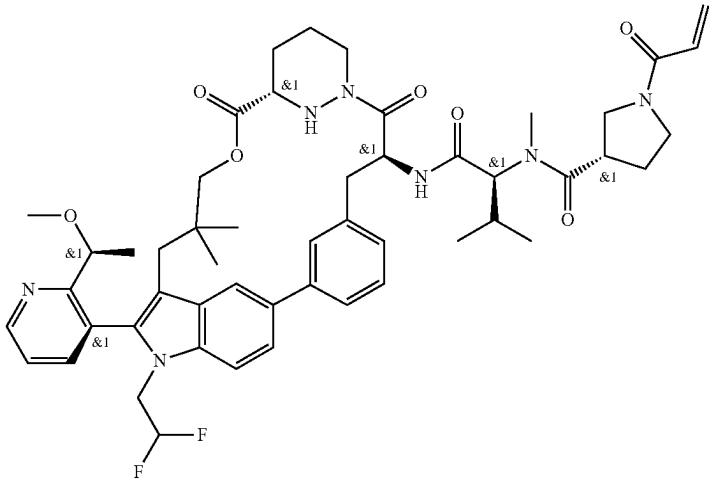
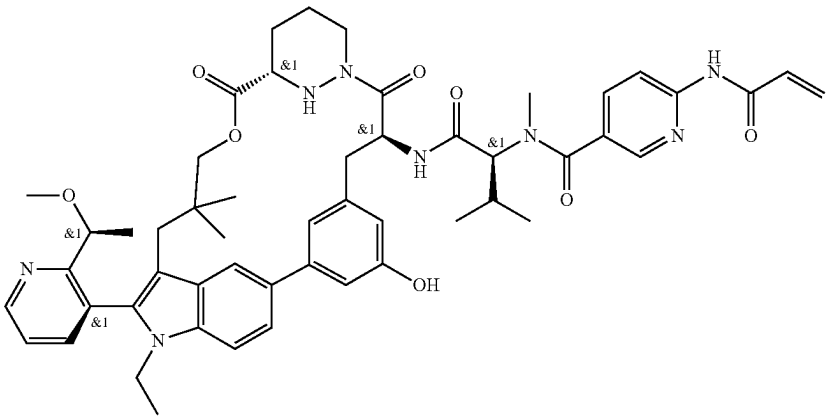
Certain Compounds of the Present Invention	
Ex#	Structure
A334	
A335	
A336	

TABLE 1-continued

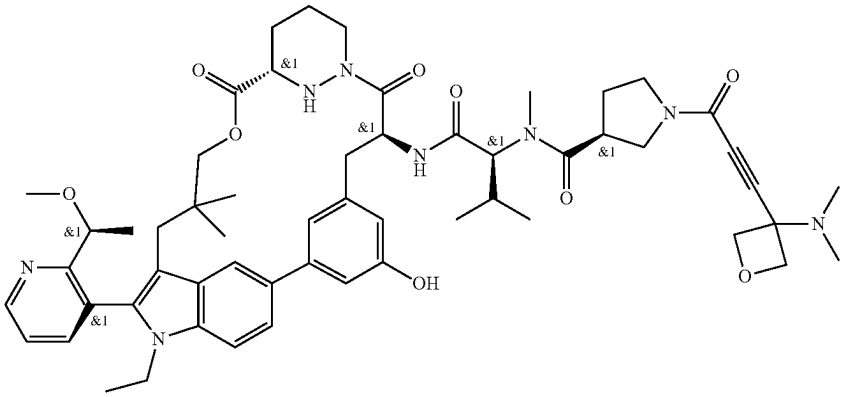
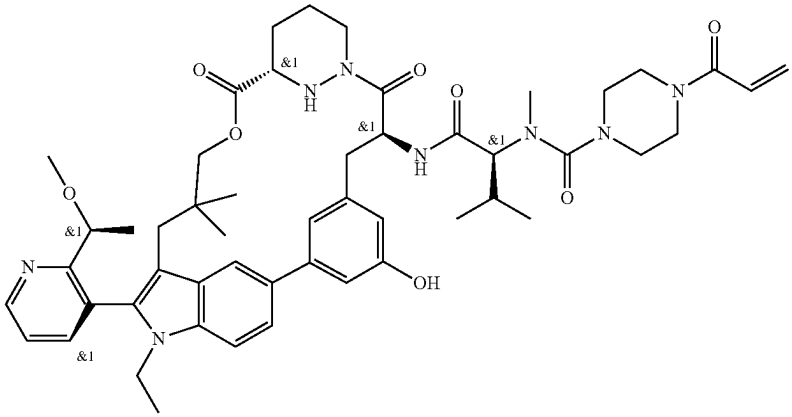
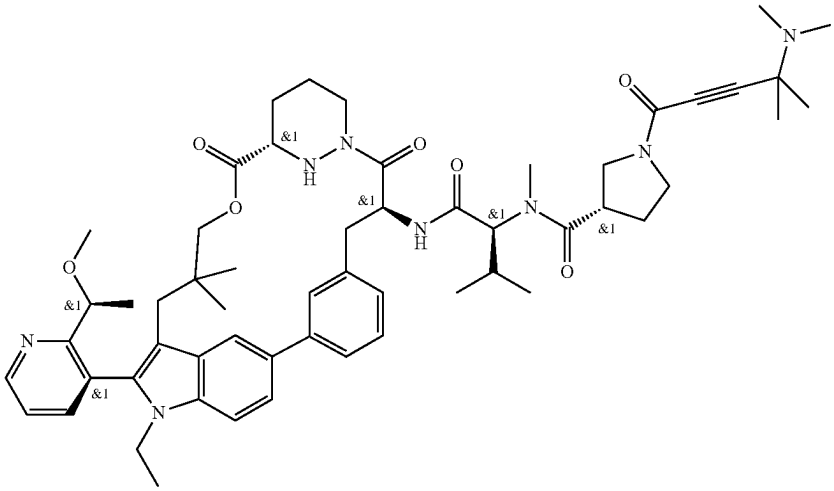
Certain Compounds of the Present Invention	
Ex#	Structure
A337	
A338	
A339	

TABLE 1-continued

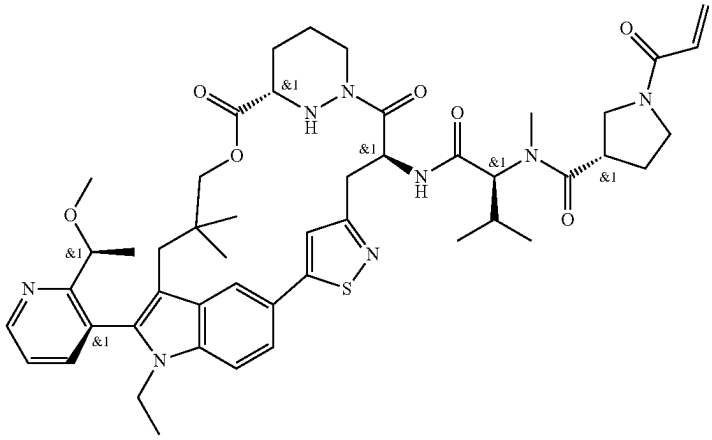
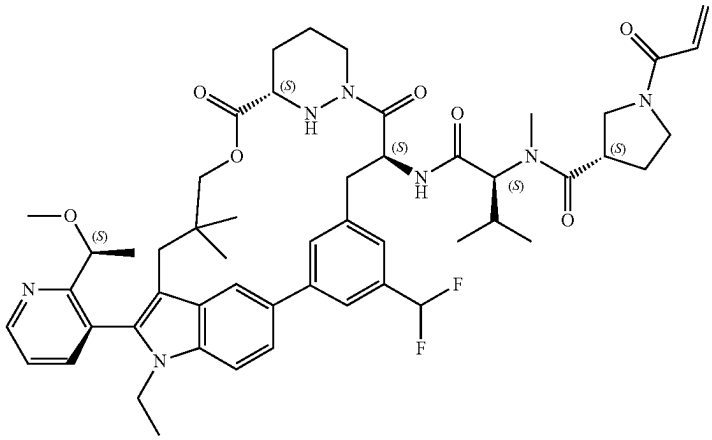
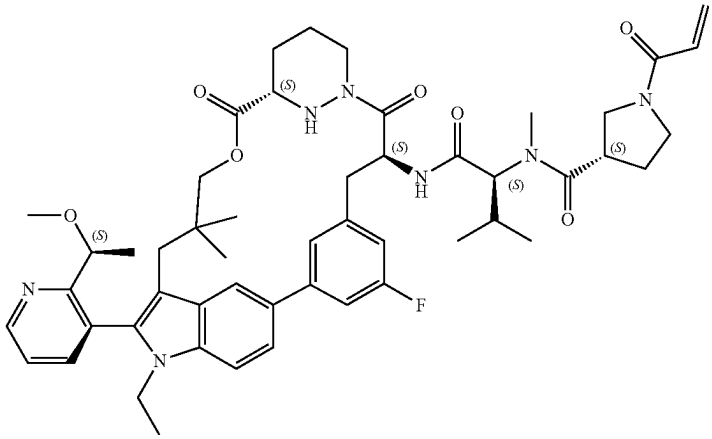
Certain Compounds of the Present Invention	
Ex#	Structure
A340	
A341	
A342	

TABLE 1-continued

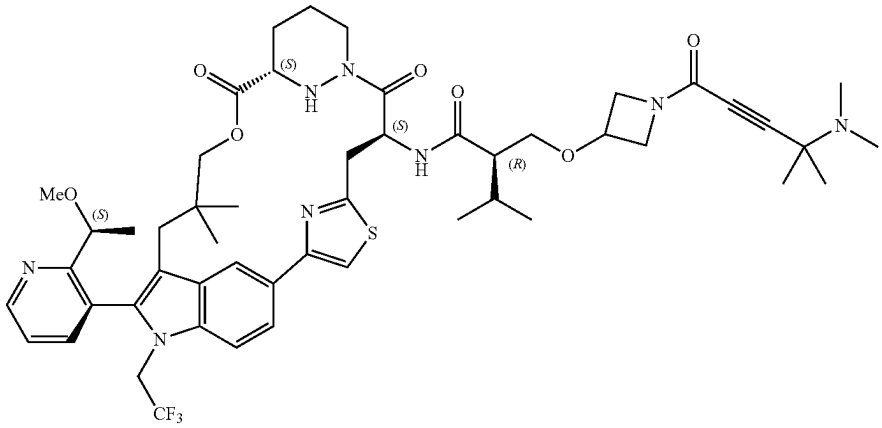
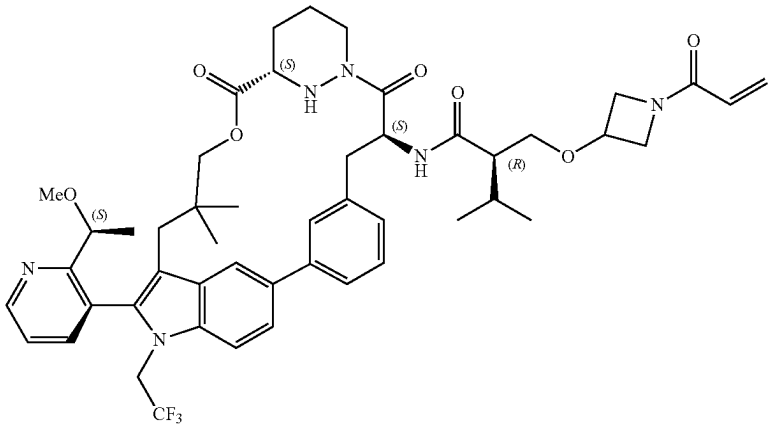
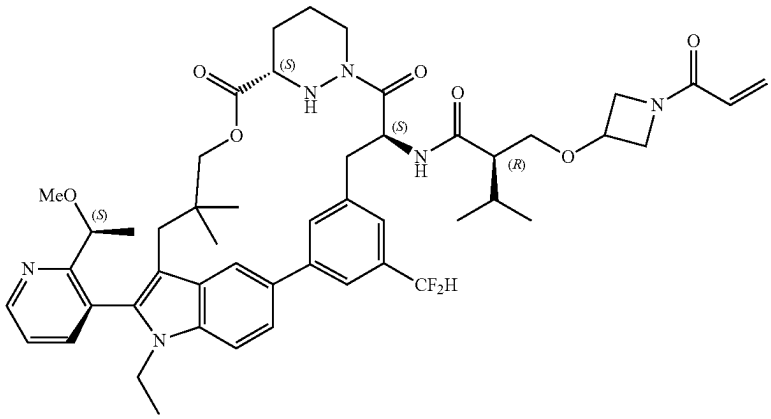
Certain Compounds of the Present Invention	
Ex#	Structure
A343	
A344	
A345	

TABLE 1-continued

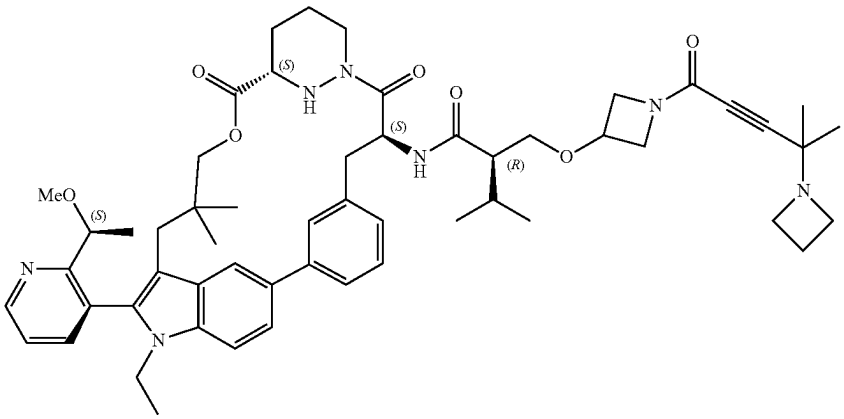
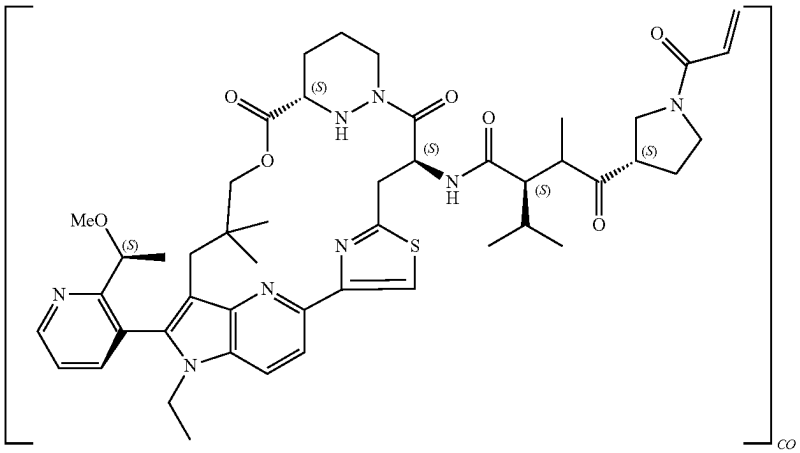
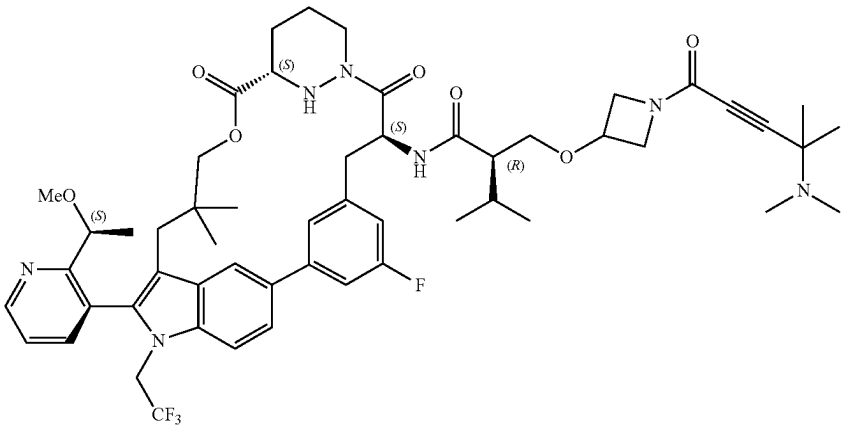
Certain Compounds of the Present Invention	
Ex#	Structure
A346	
A347	
A348	

TABLE 1-continued

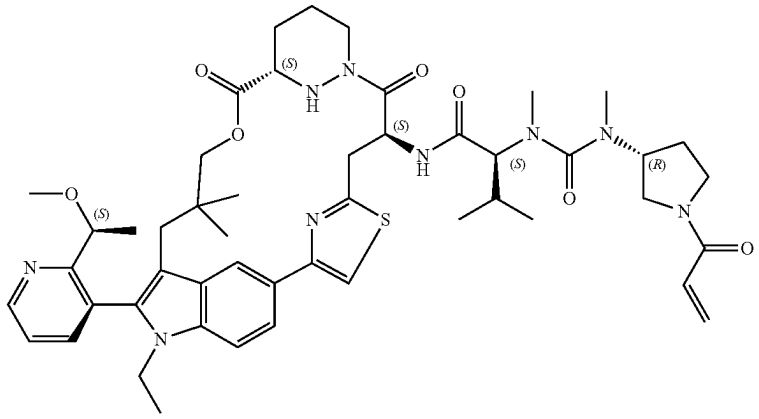
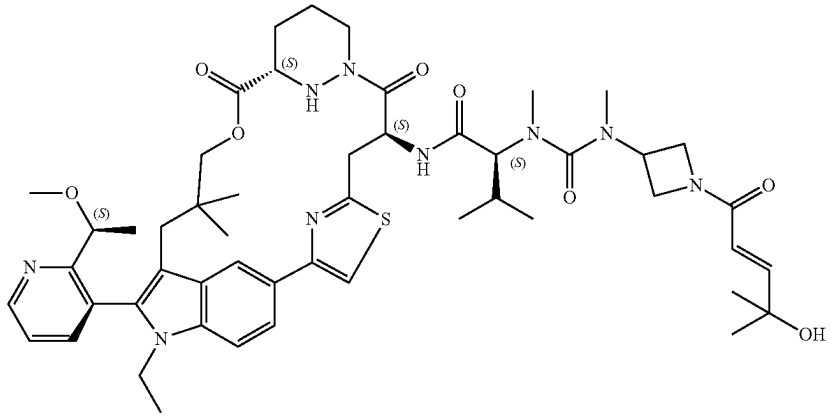
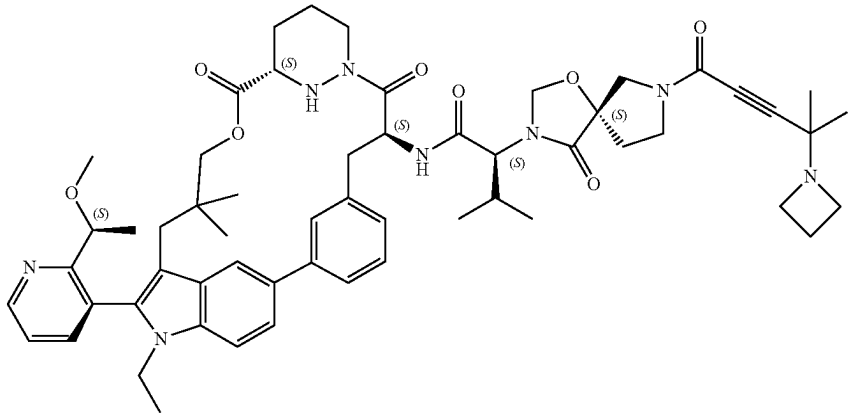
Certain Compounds of the Present Invention	
Ex#	Structure
A349	
A350	
A351	

TABLE 1-continued

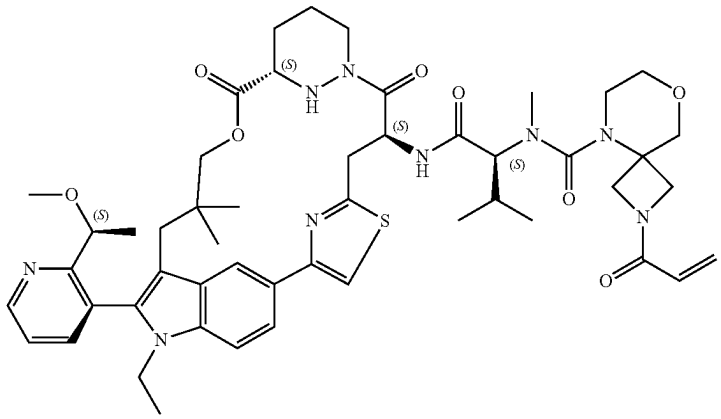
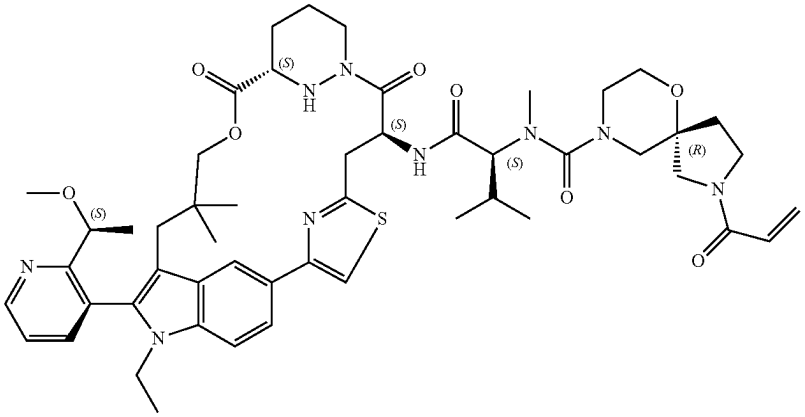
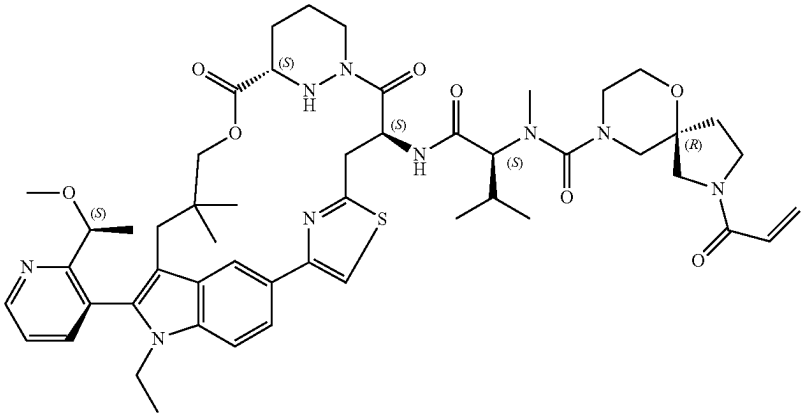
Certain Compounds of the Present Invention	
Ex#	Structure
A352	
A353	assumed 
A354	assumed 

TABLE 1-continued

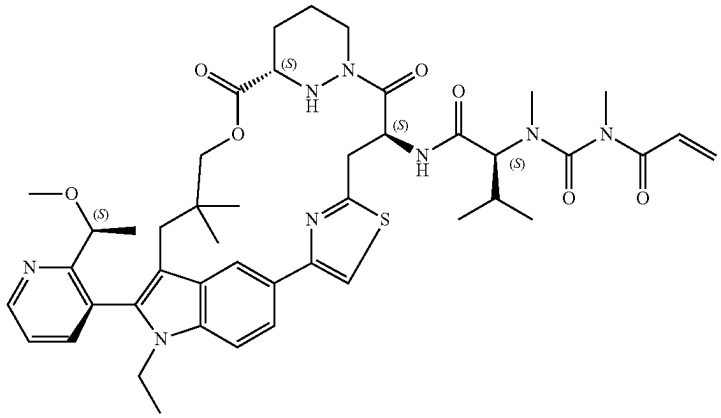
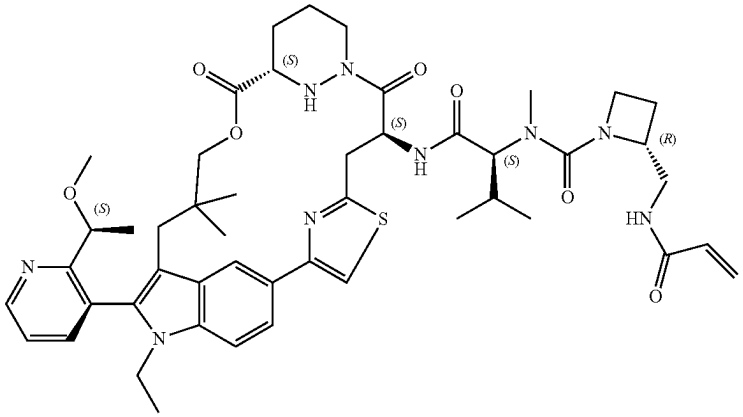
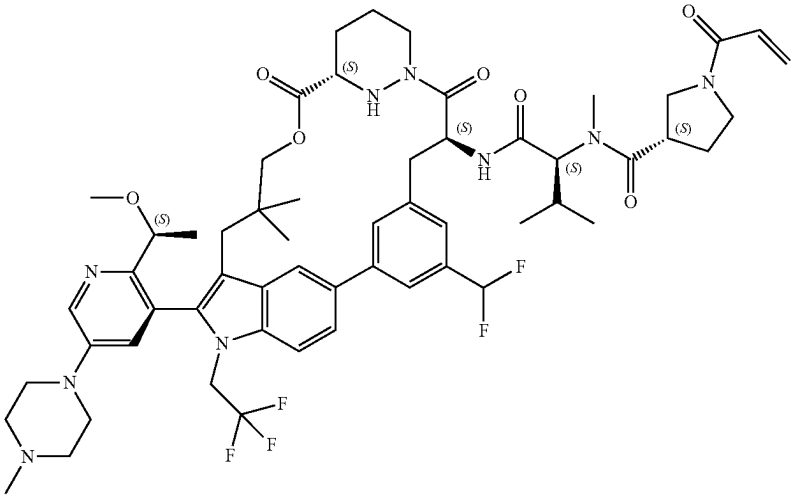
Certain Compounds of the Present Invention	
Ex#	Structure
A355	
A356	
A357	

TABLE 1-continued

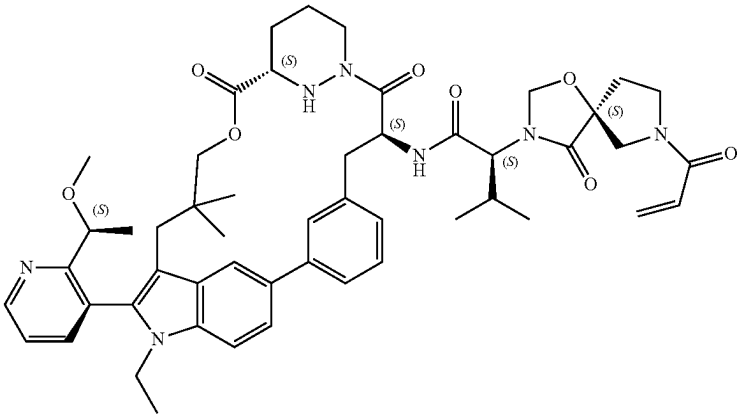
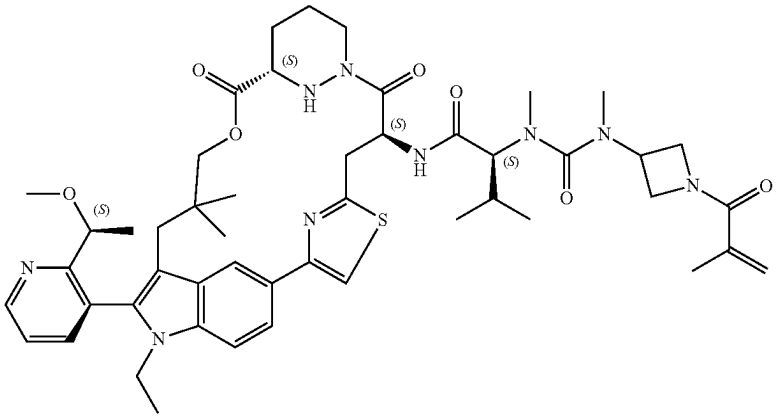
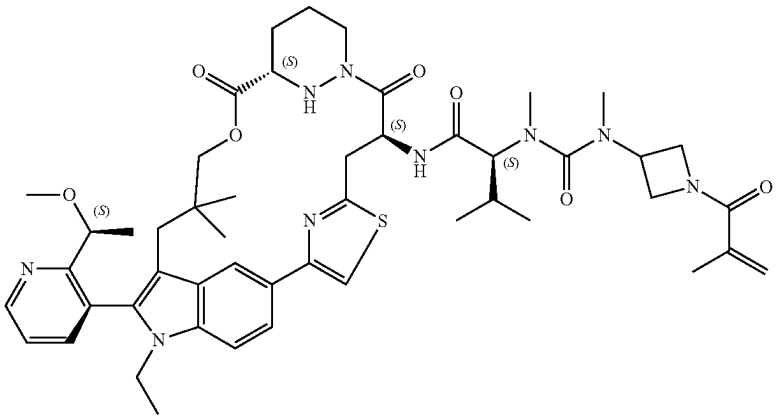
Certain Compounds of the Present Invention	
Ex#	Structure
A358	
A359	
A360	

TABLE 1-continued

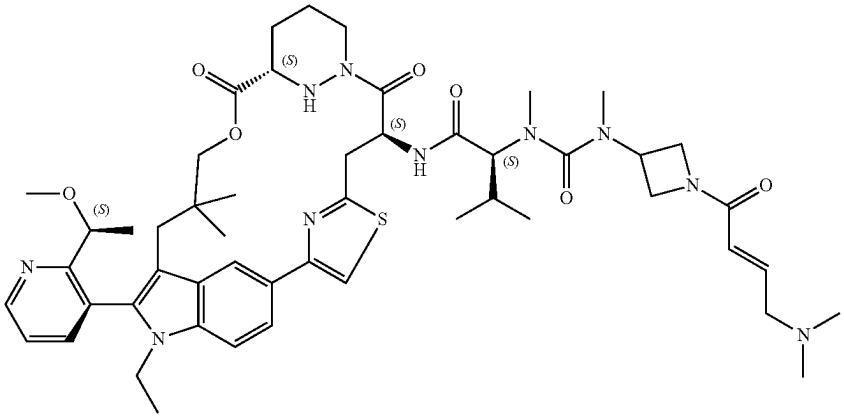
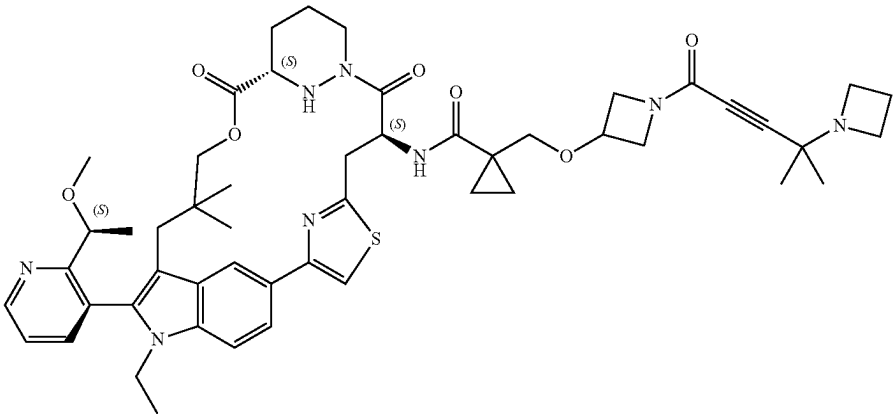
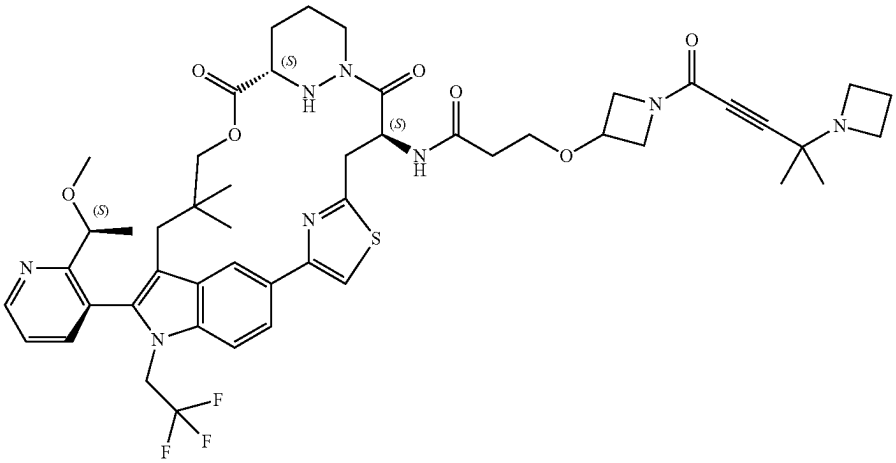
Certain Compounds of the Present Invention	
Ex#	Structure
A361	
A362	
A363	

TABLE 1-continued

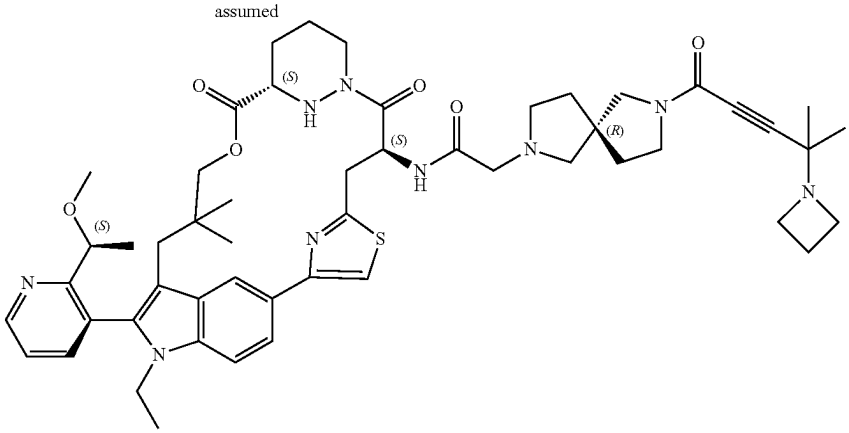
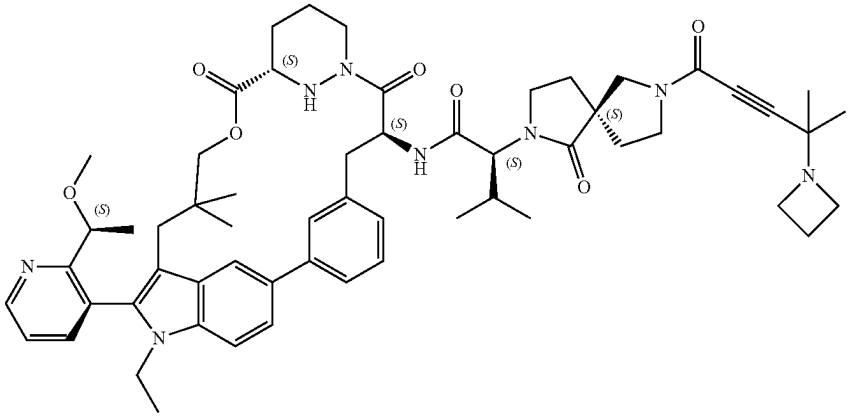
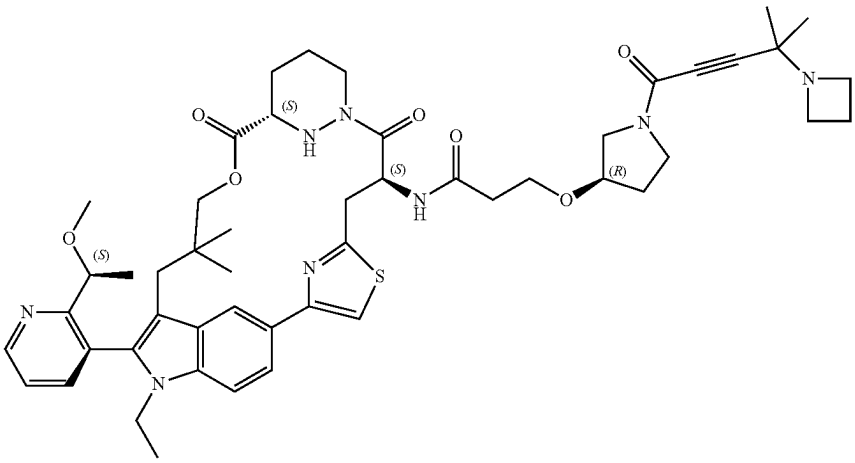
Certain Compounds of the Present Invention	
Ex#	Structure
A364	assumed 
A365	
A366	

TABLE 1-continued

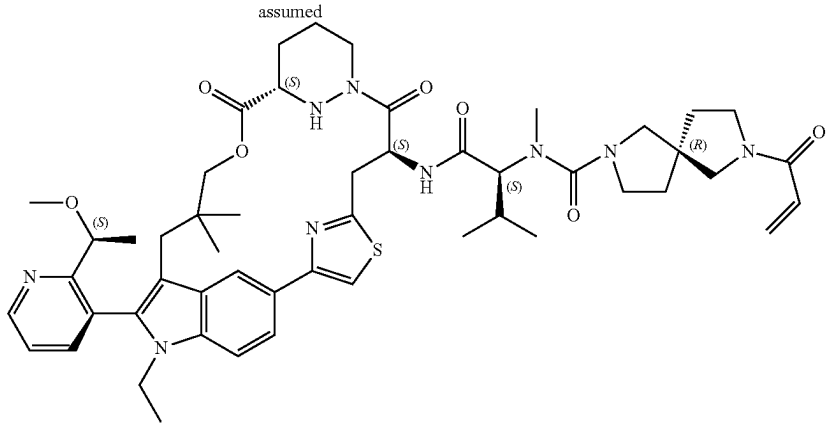
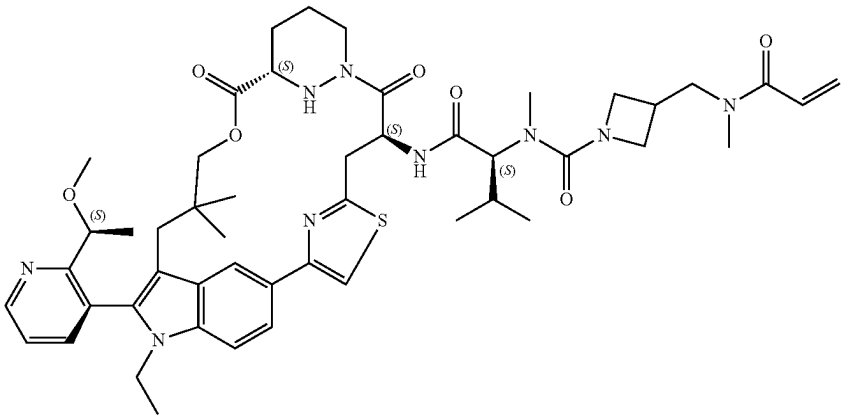
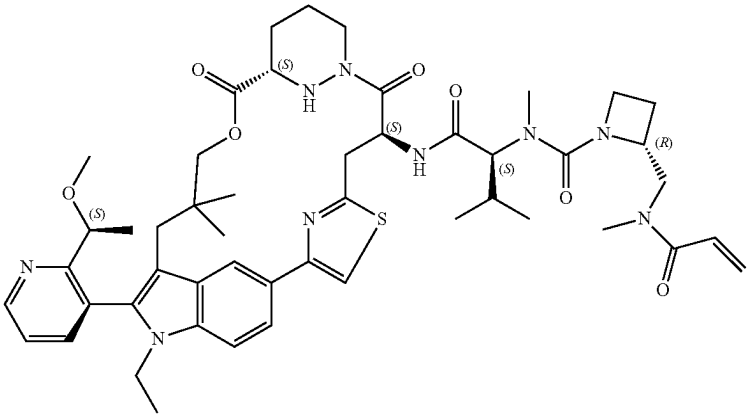
Certain Compounds of the Present Invention	
Ex#	Structure
A367	assumed 
A368	
A369	

TABLE 1-continued

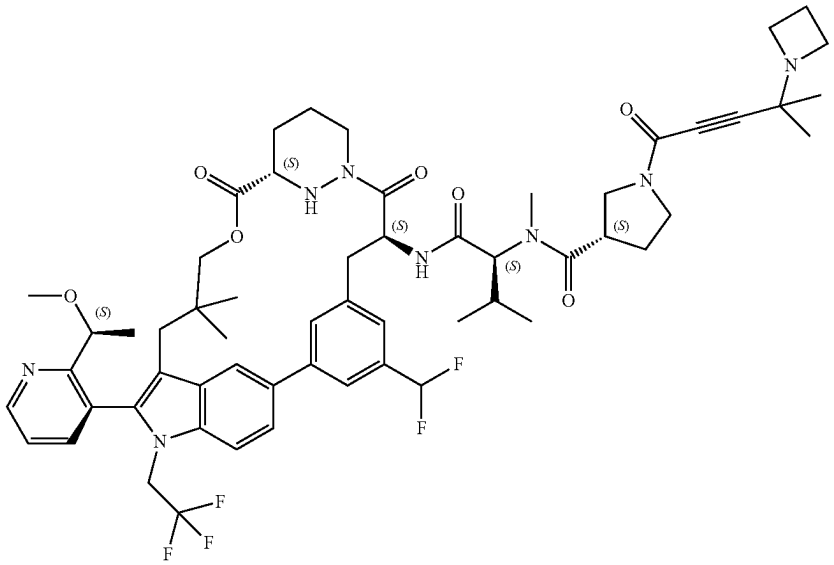
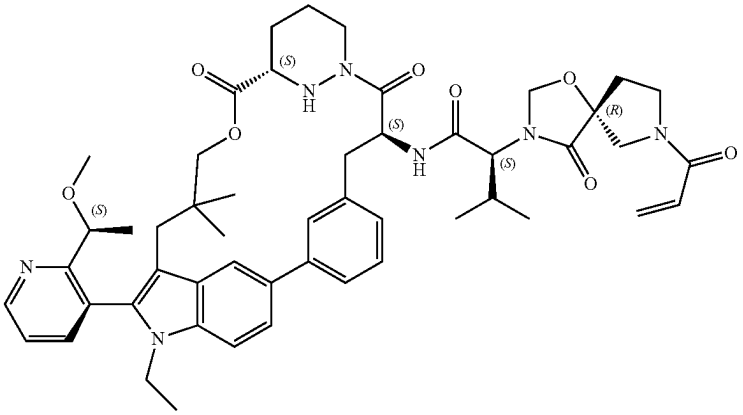
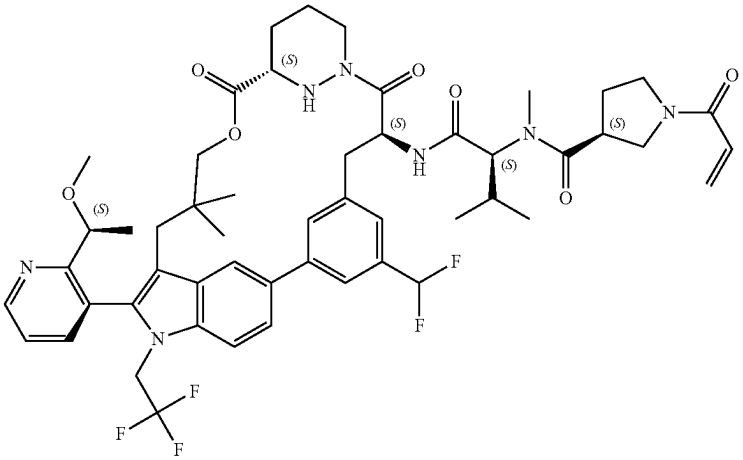
Certain Compounds of the Present Invention	
Ex#	Structure
A370	
A371	
A372	

TABLE 1-continued

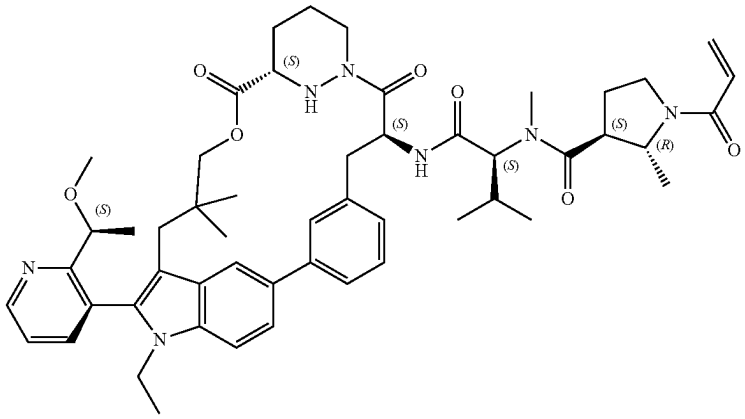
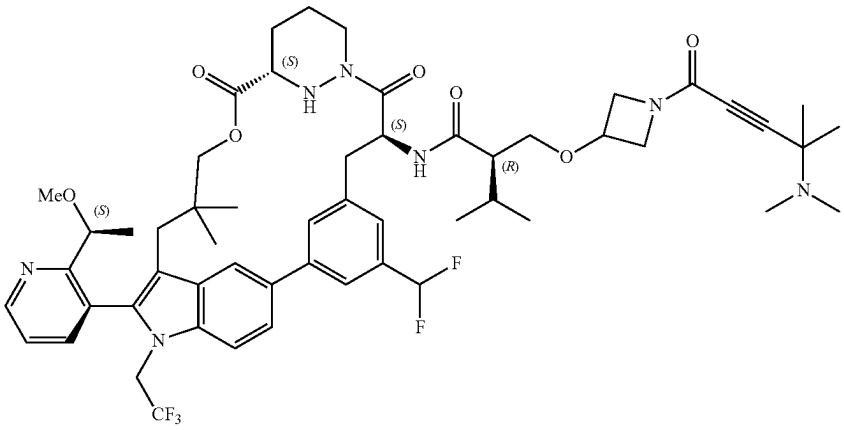
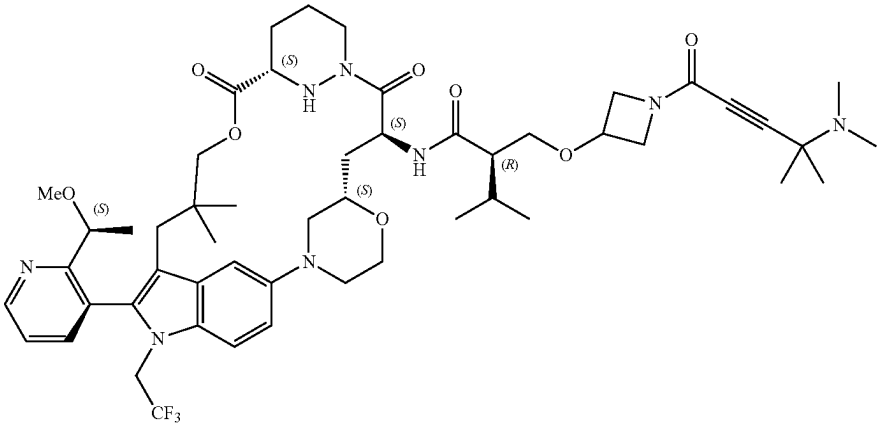
Certain Compounds of the Present Invention	
Ex#	Structure
A373	
A374	
A375	

TABLE 1-continued

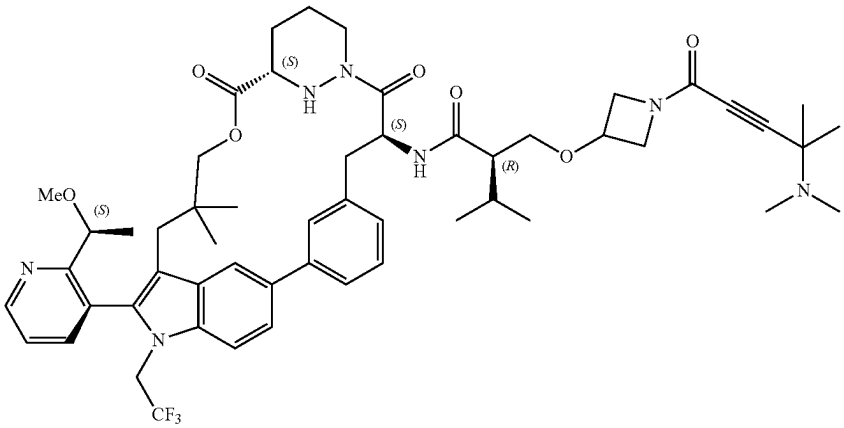
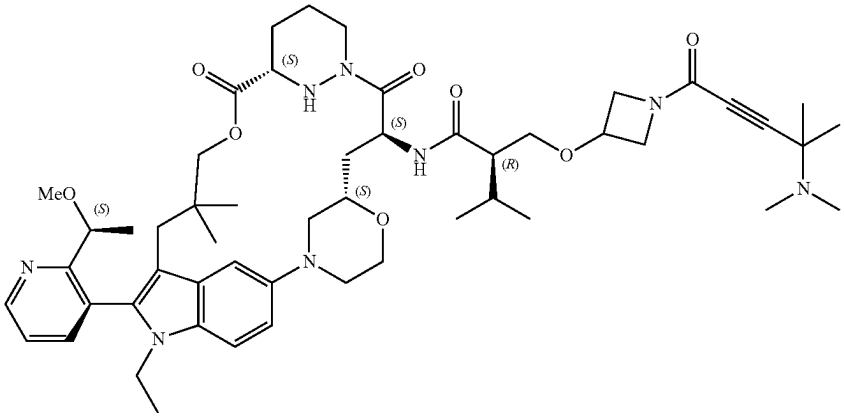
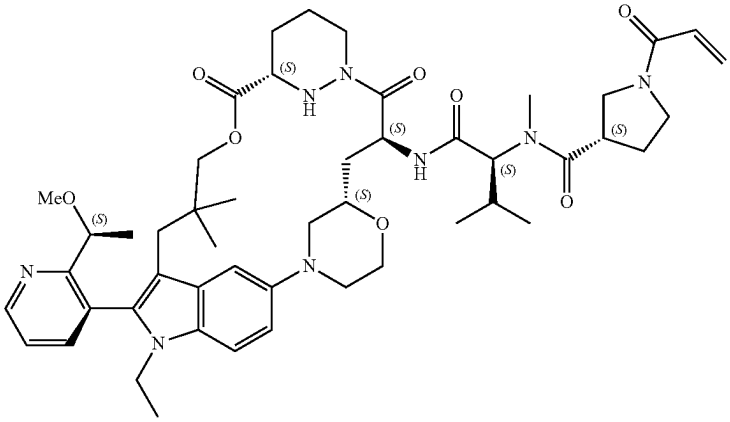
Certain Compounds of the Present Invention	
Ex#	Structure
A376	
A377	
A378	

TABLE 1-continued

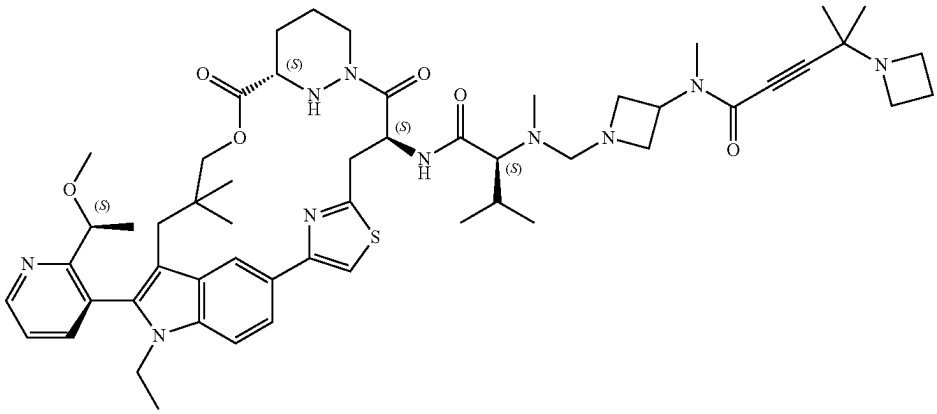
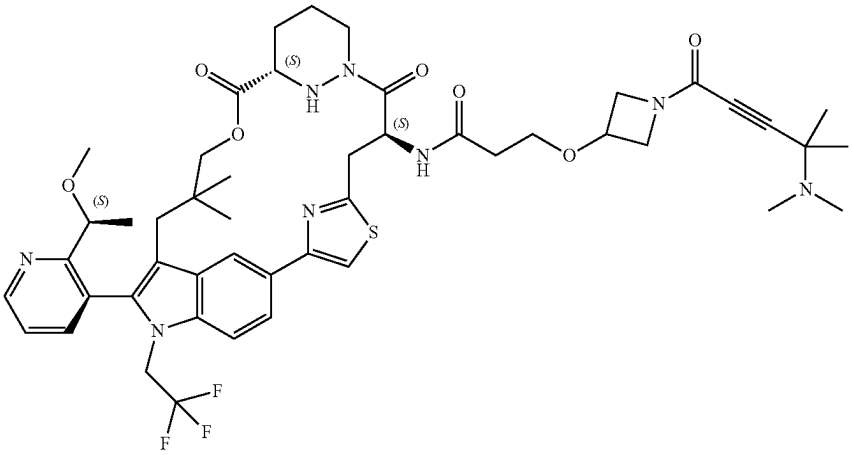
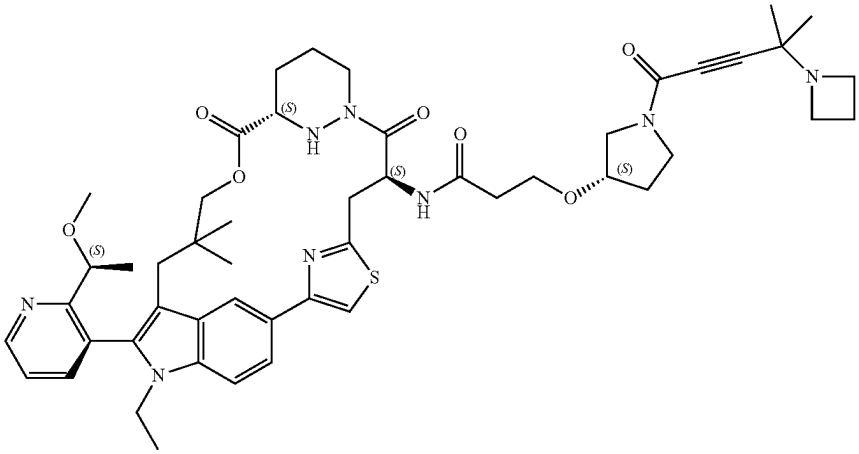
Certain Compounds of the Present Invention	
Ex#	Structure
A379	
A380	
A381	

TABLE 1-continued

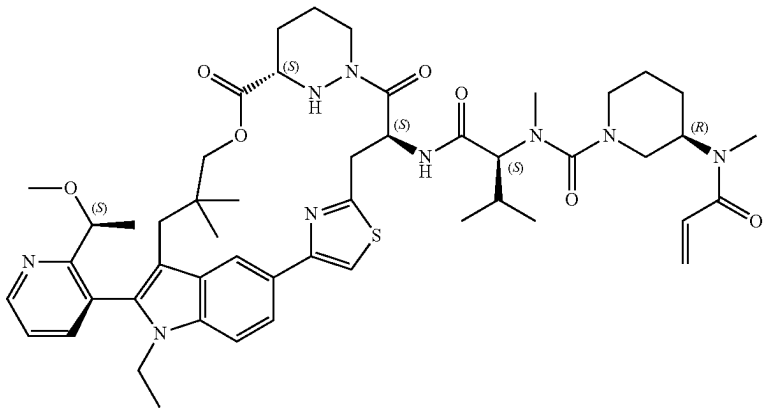
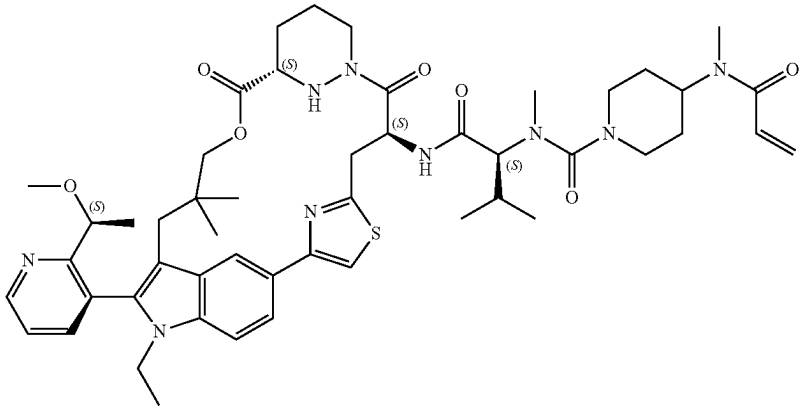
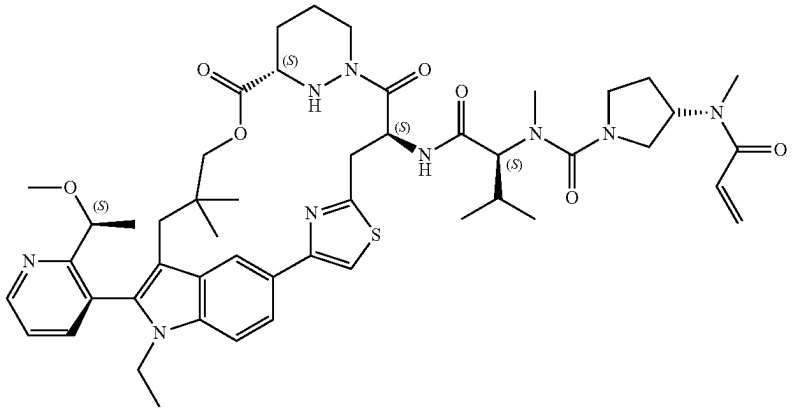
Certain Compounds of the Present Invention	
Ex#	Structure
A382	
A383	
A384	

TABLE 1-continued

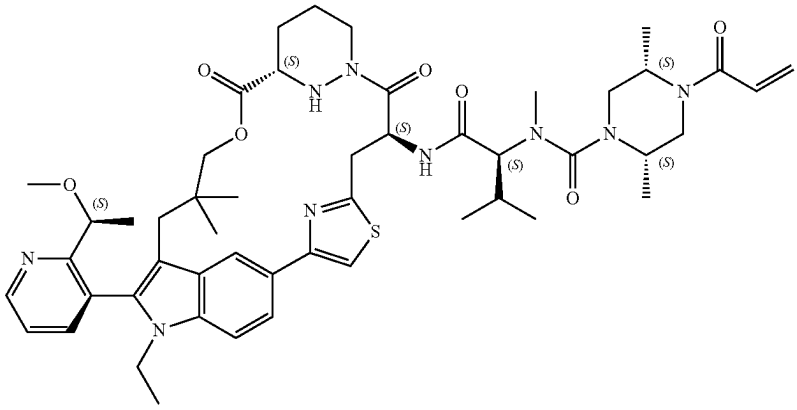
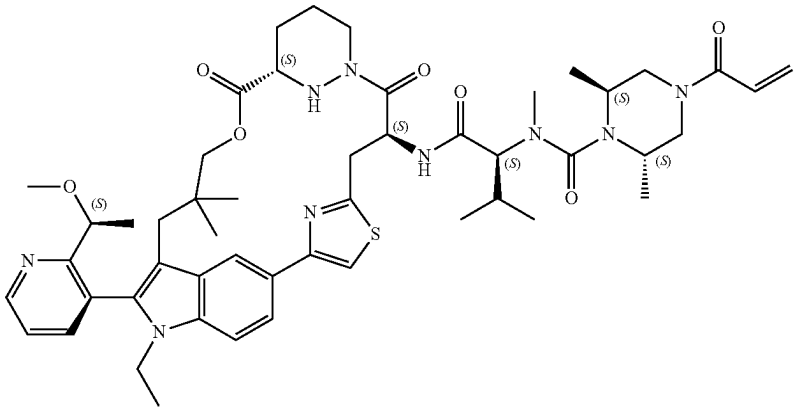
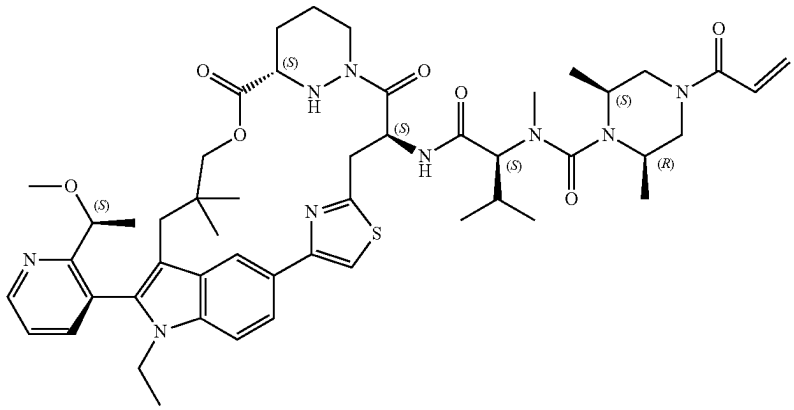
Certain Compounds of the Present Invention	
Ex#	Structure
A385	
A386	
A387	

TABLE 1-continued

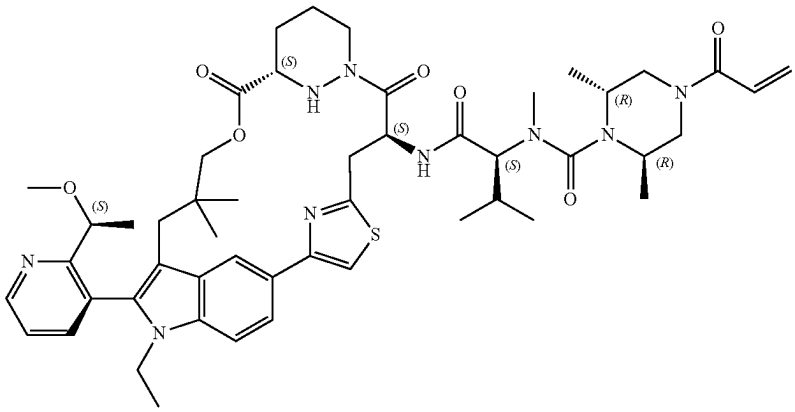
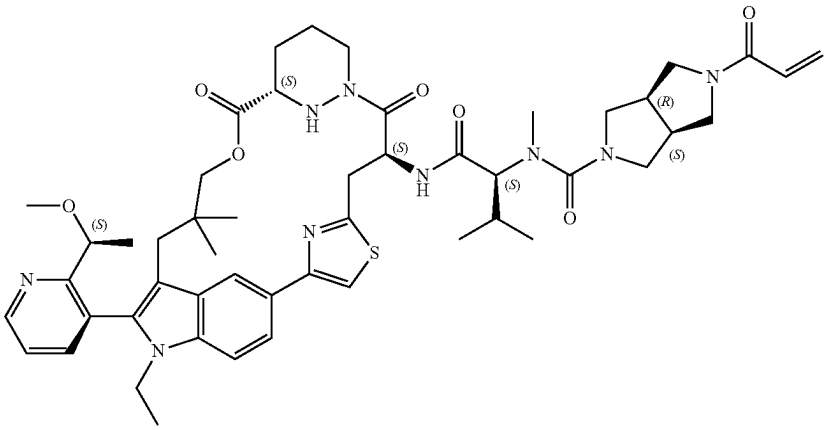
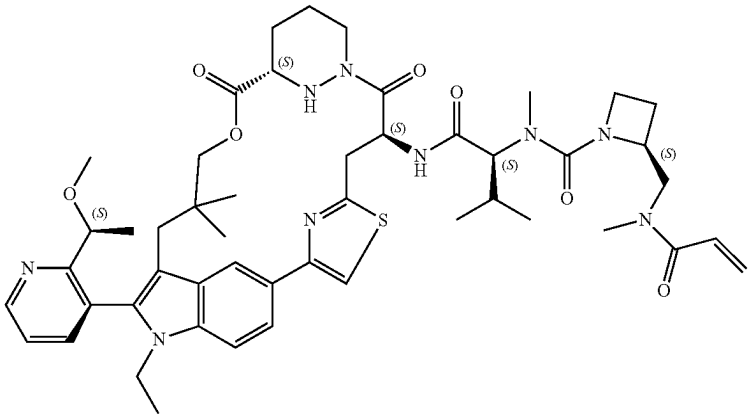
Certain Compounds of the Present Invention	
Ex#	Structure
A388	
A389	
A390	

TABLE 1-continued

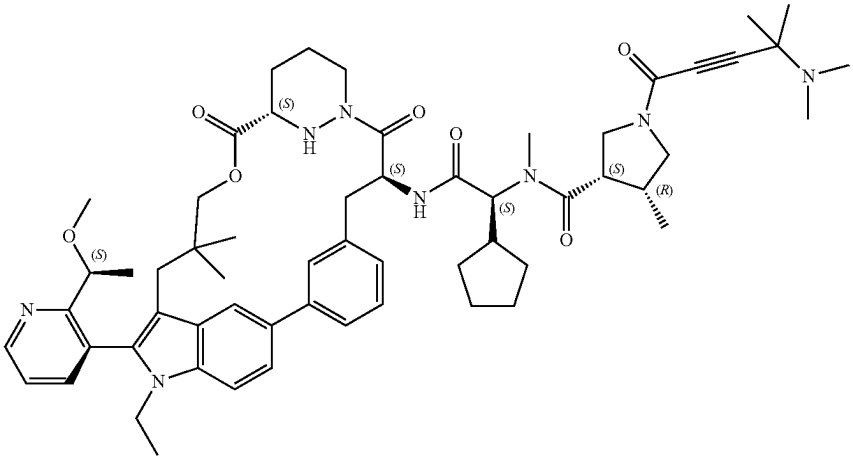
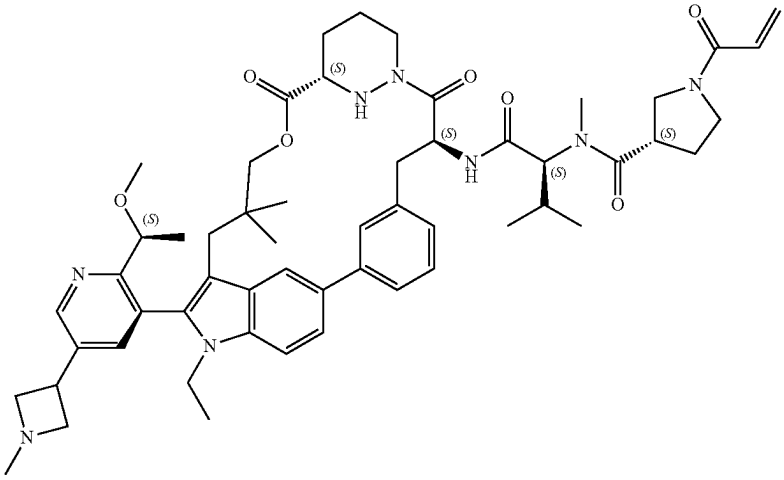
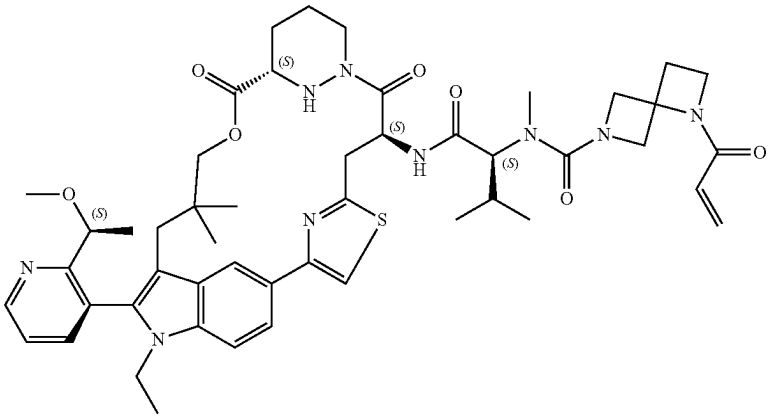
Certain Compounds of the Present Invention	
Ex#	Structure
A391	
A392	
A393	

TABLE 1-continued

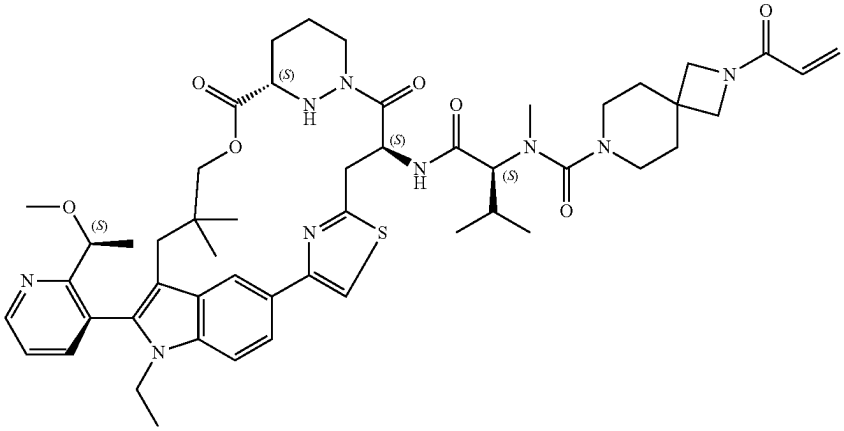
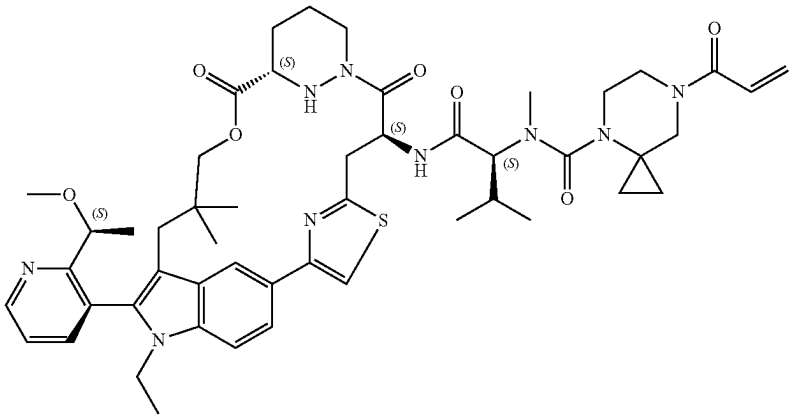
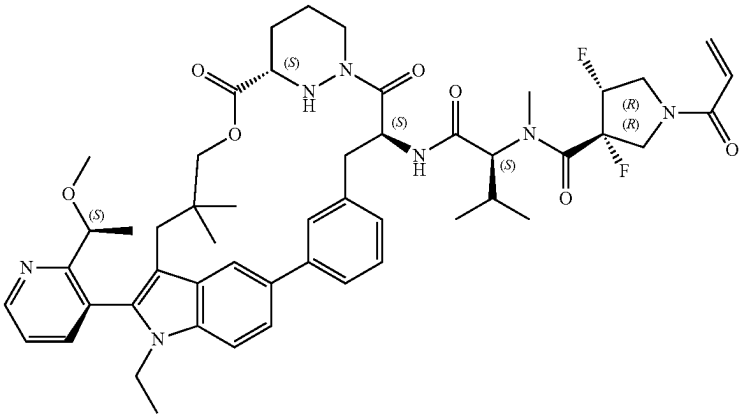
Certain Compounds of the Present Invention	
Ex#	Structure
A394	
A395	
A396	

TABLE 1-continued

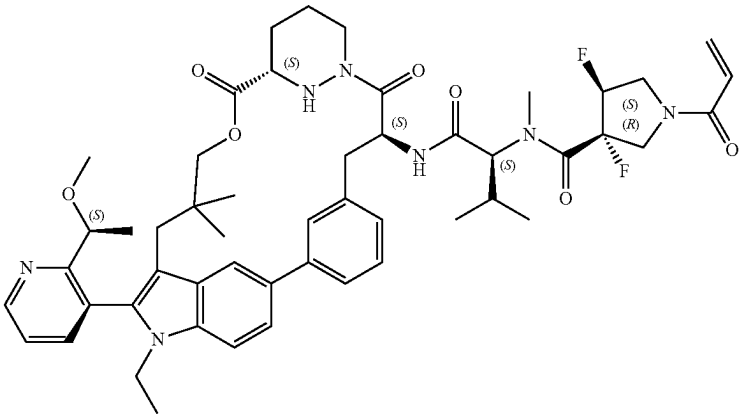
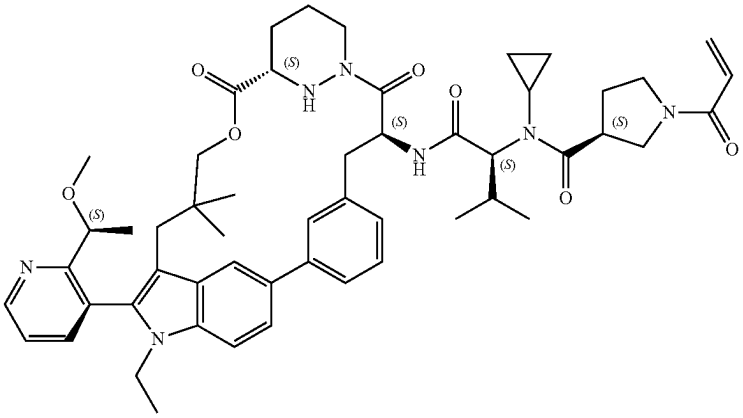
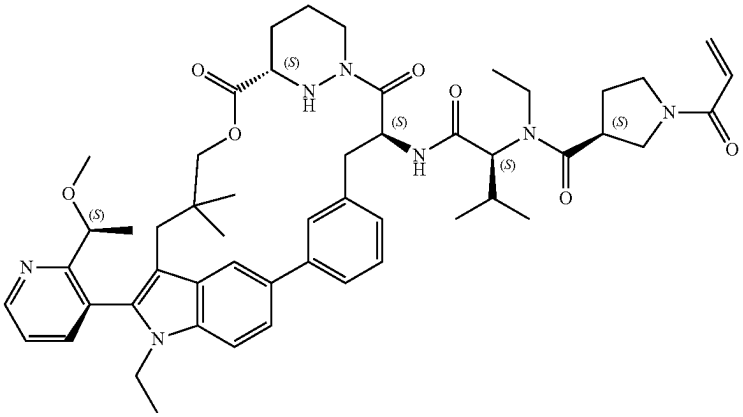
Certain Compounds of the Present Invention	
Ex#	Structure
A397	
A398	
A399	

TABLE 1-continued

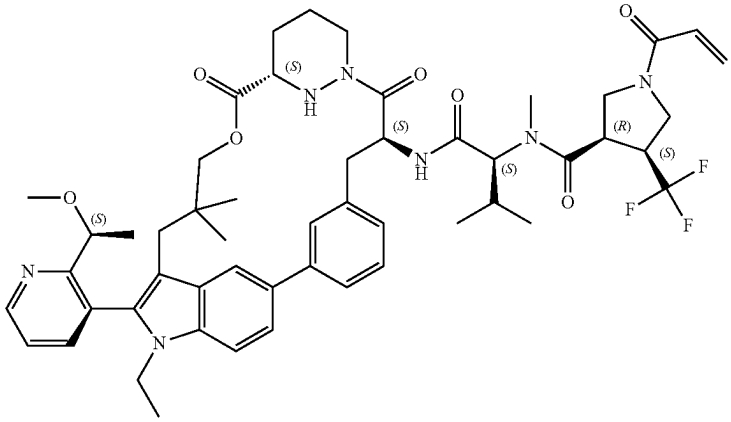
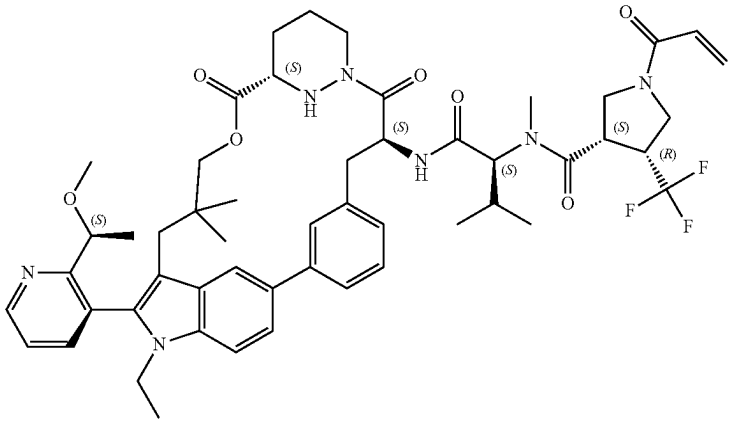
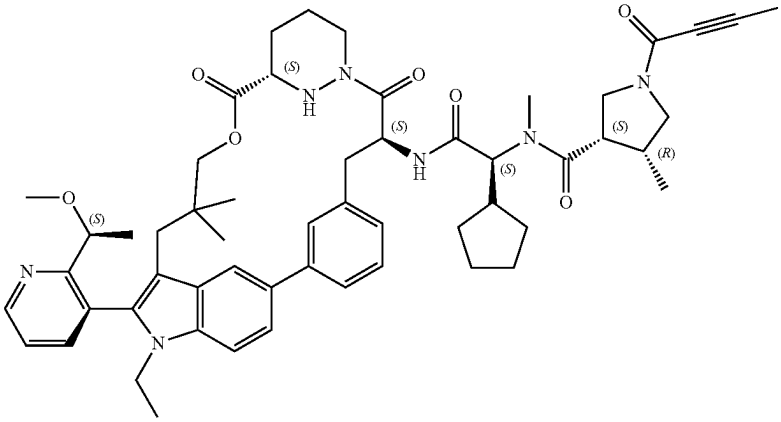
Certain Compounds of the Present Invention	
Ex#	Structure
A400	
A401	
A402	

TABLE 1-continued

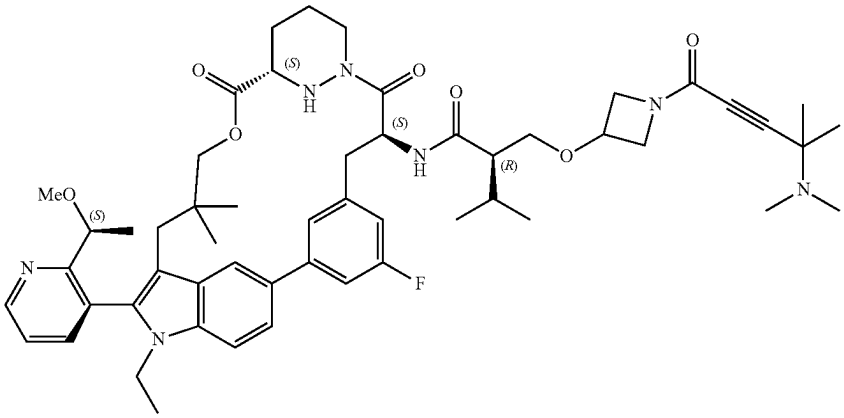
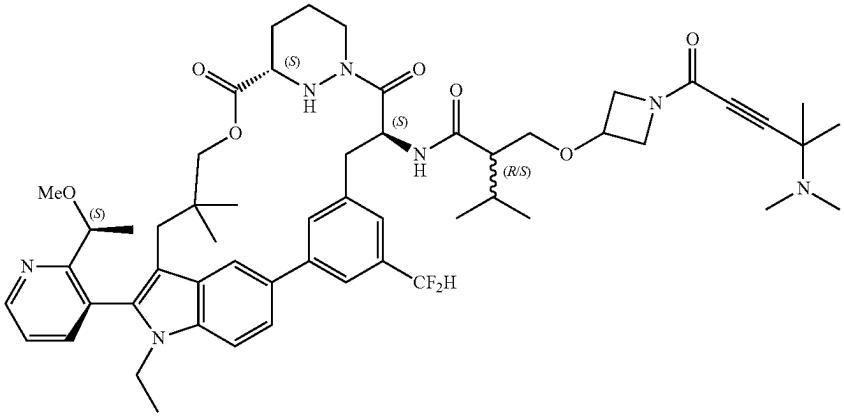
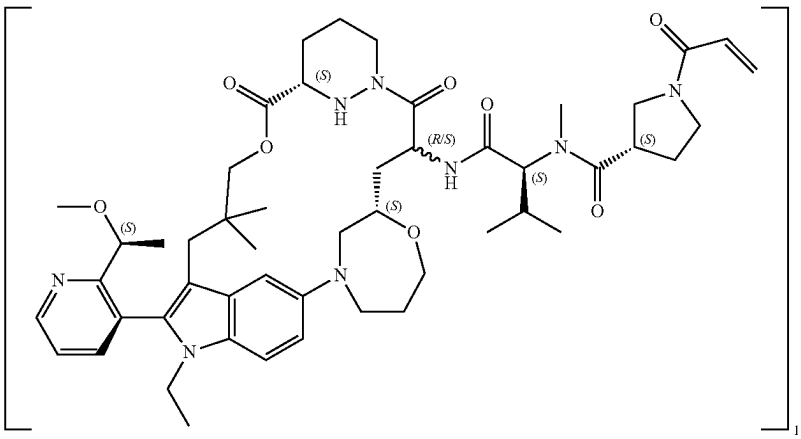
Certain Compounds of the Present Invention	
Ex#	Structure
A403	
A404	
A405	

TABLE 1-continued

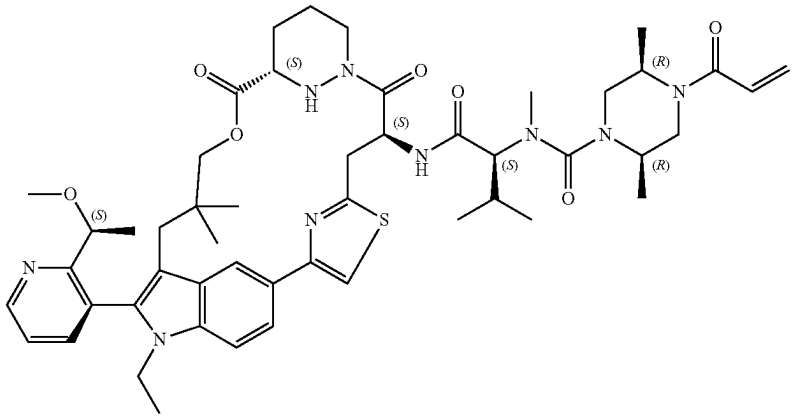
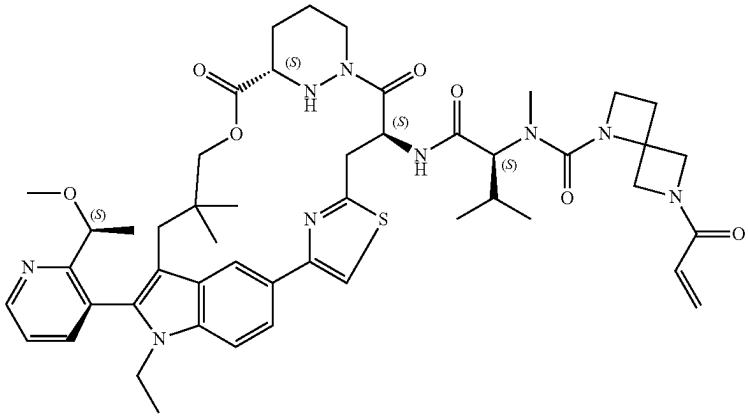
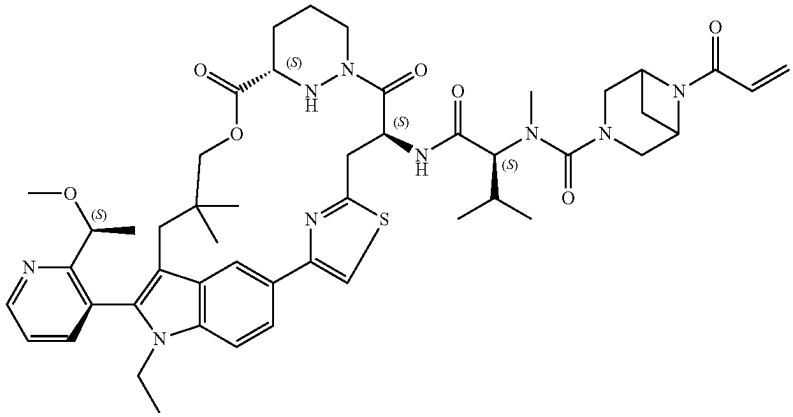
Certain Compounds of the Present Invention	
Ex#	Structure
A406	
A407	
A408	

TABLE 1-continued

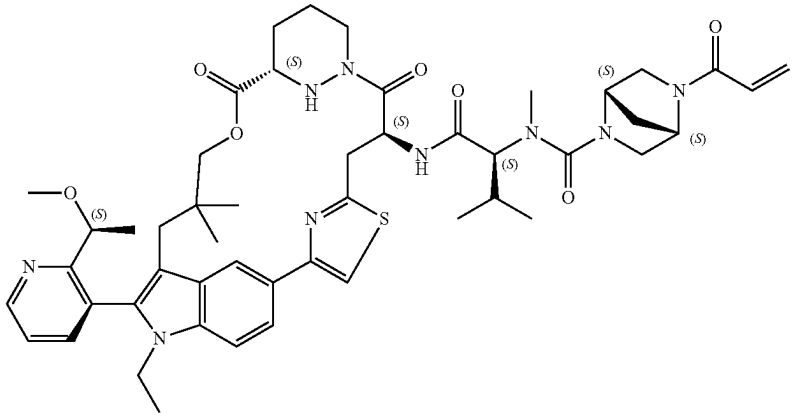
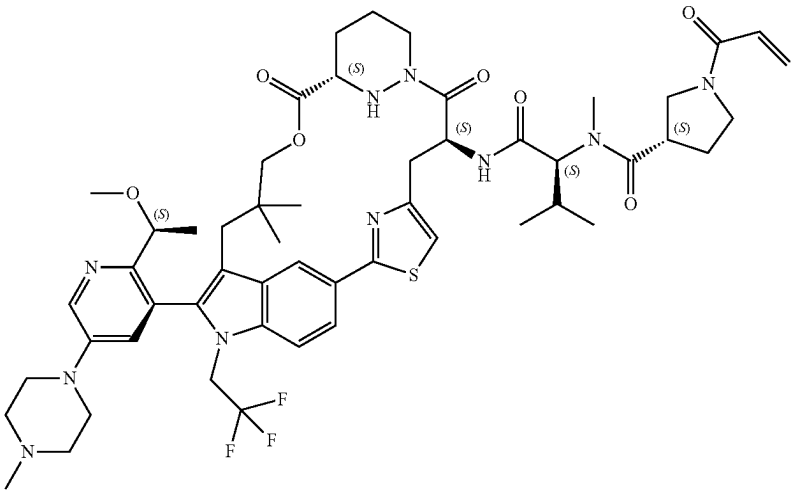
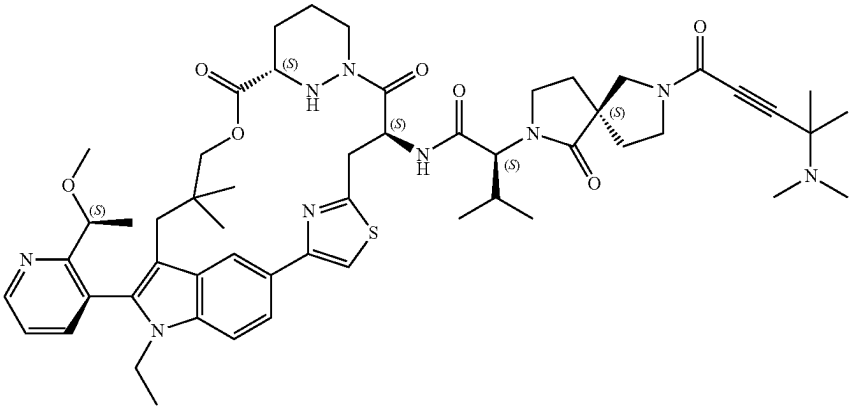
Certain Compounds of the Present Invention	
Ex#	Structure
A409	
A410	
A411	assumed 

TABLE 1-continued

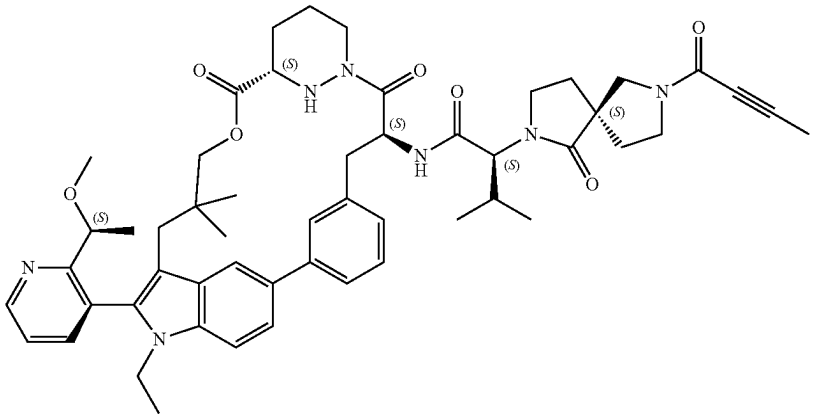
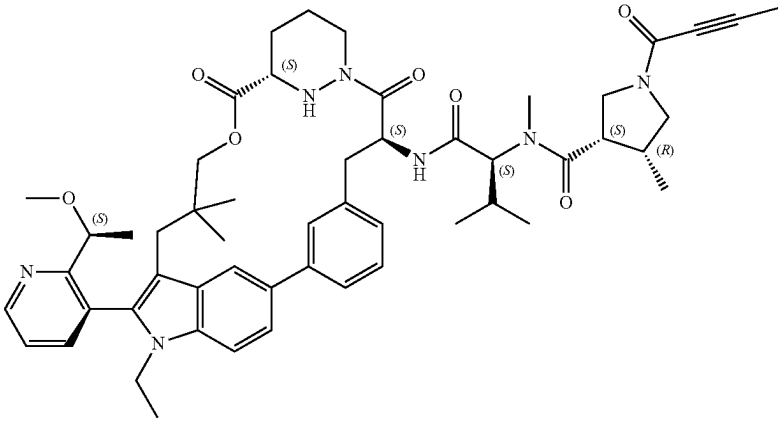
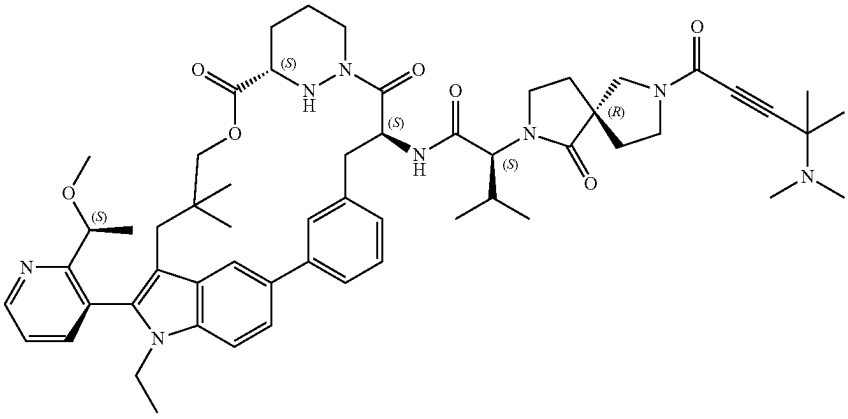
Certain Compounds of the Present Invention	
Ex#	Structure
A412	assumed 
A413	
A414	

TABLE 1-continued

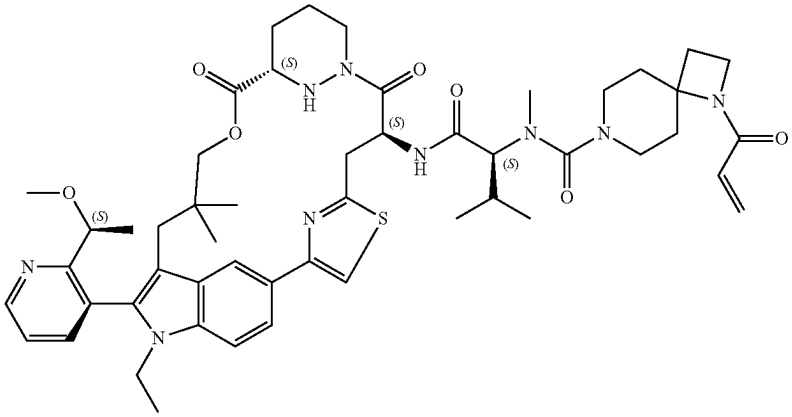
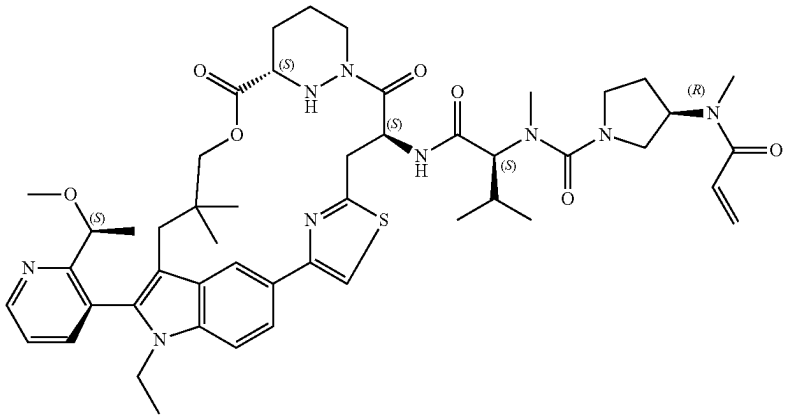
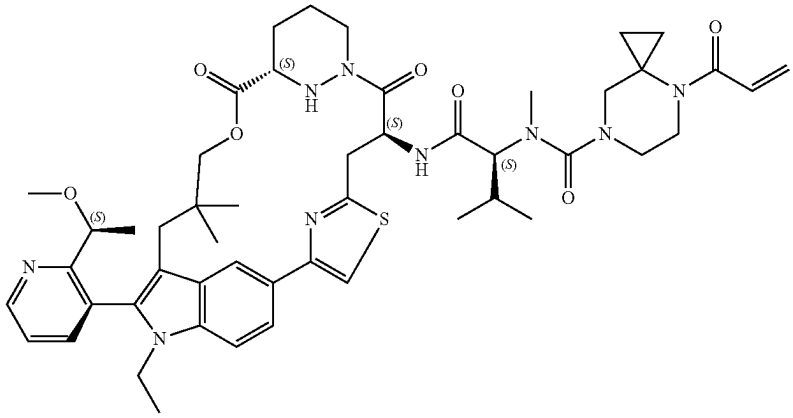
Certain Compounds of the Present Invention	
Ex#	Structure
A415	
A416	
A417	

TABLE 1-continued

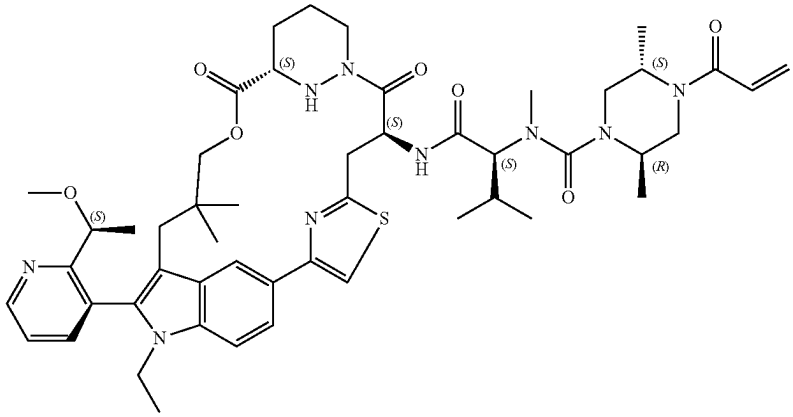
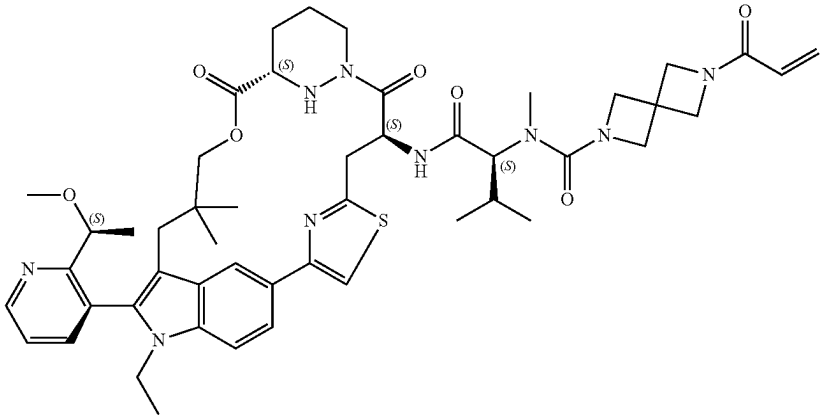
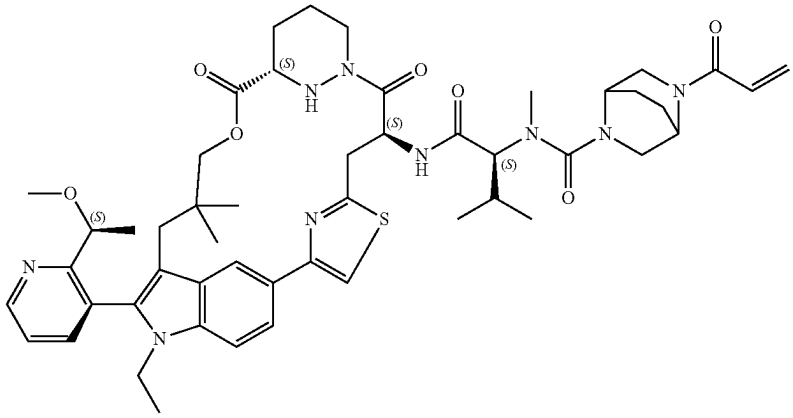
Certain Compounds of the Present Invention	
Ex#	Structure
A418	
A419	
A420	

TABLE 1-continued

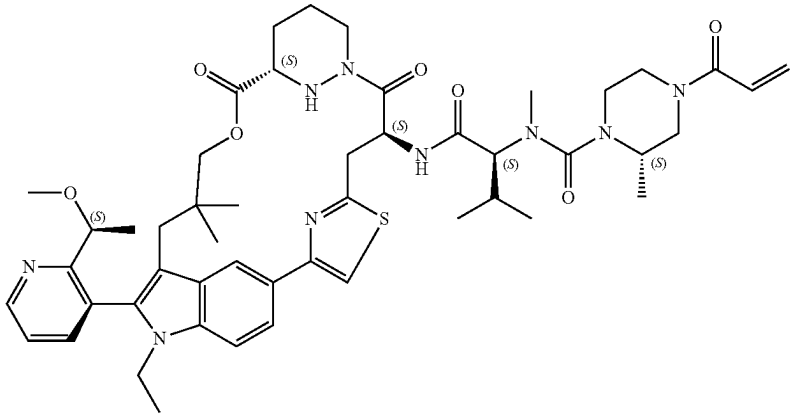
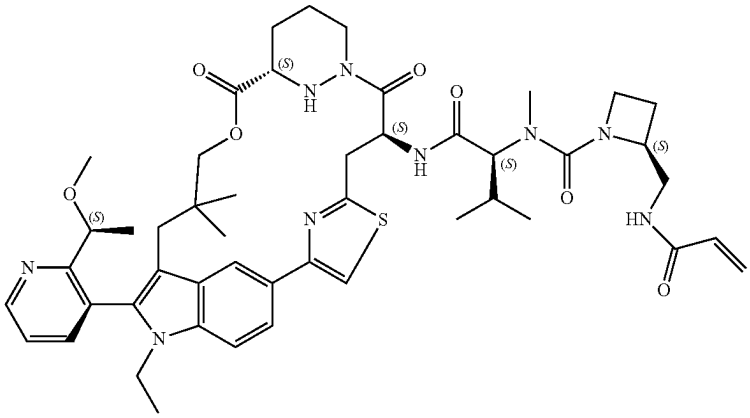
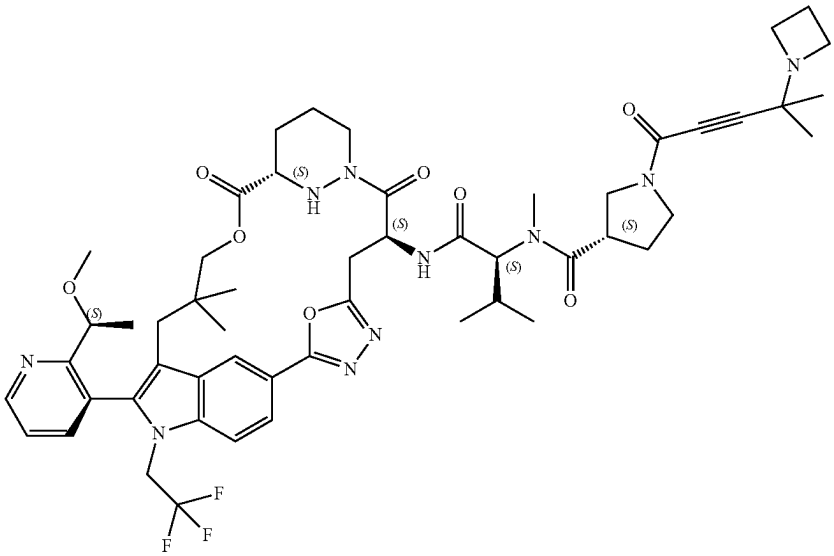
Certain Compounds of the Present Invention	
Ex#	Structure
A421	
A422	
A423	

TABLE 1-continued

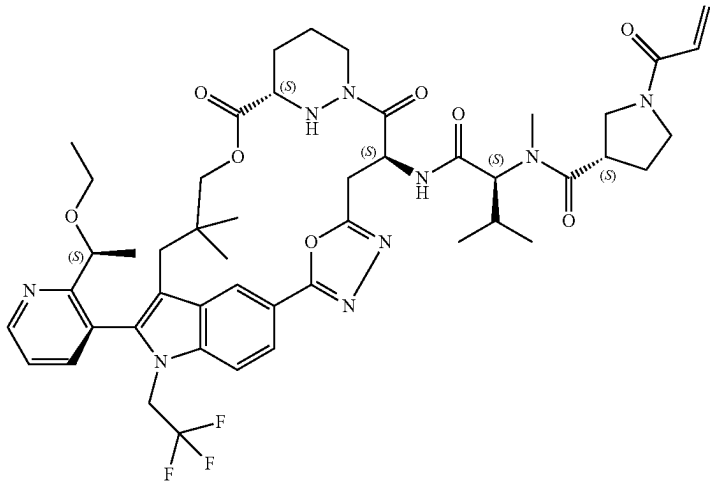
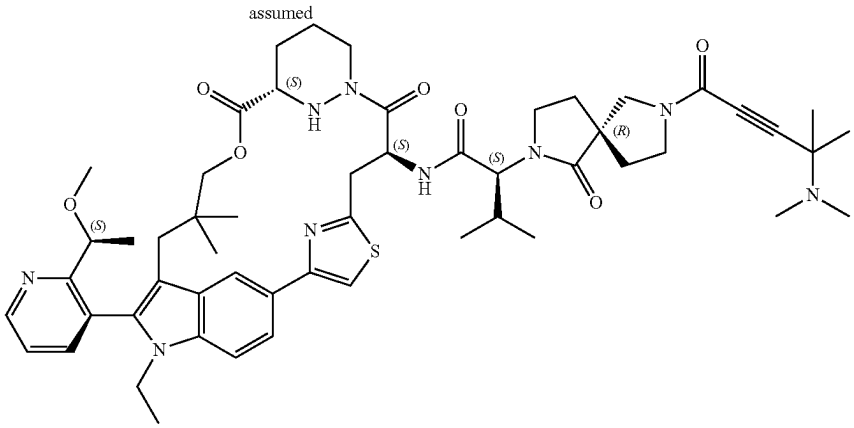
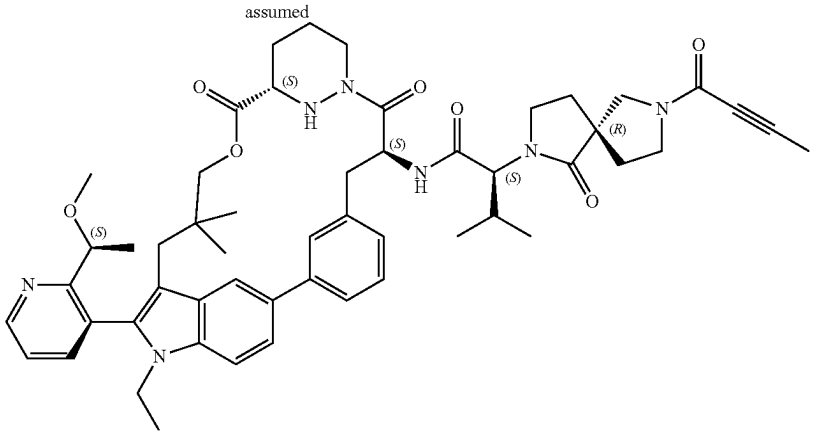
Certain Compounds of the Present Invention	
Ex#	Structure
A424	
A425	assumed 
A426	assumed 

TABLE 1-continued

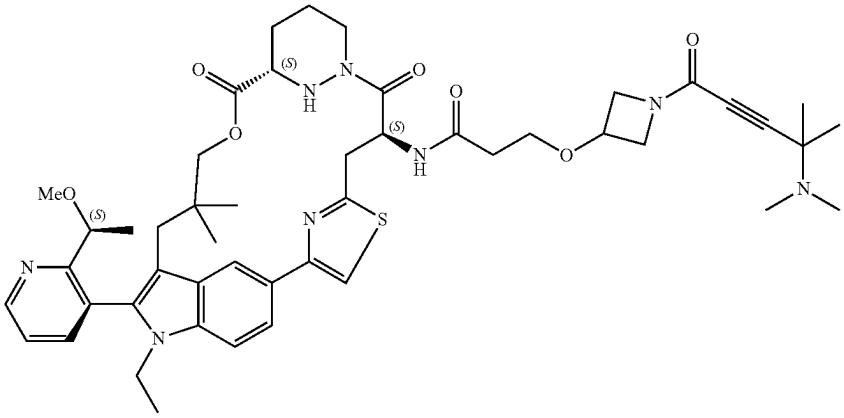
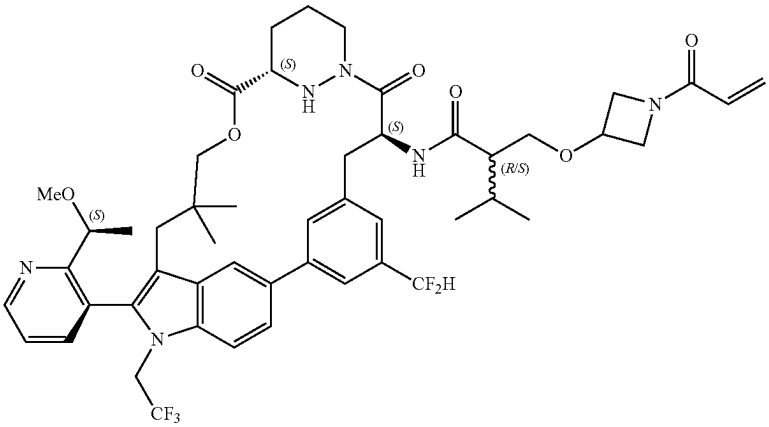
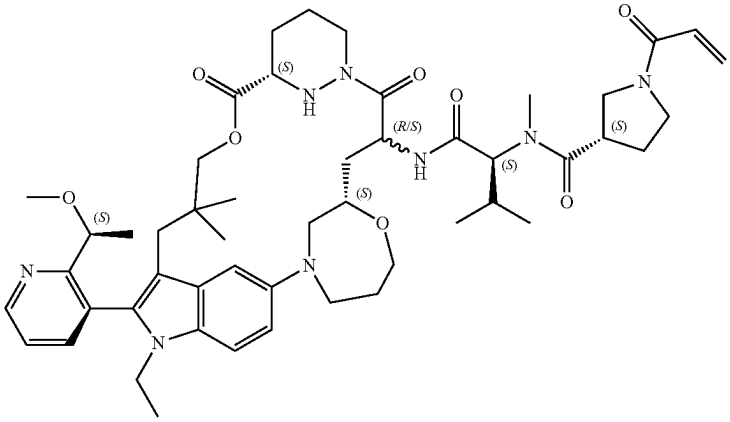
Certain Compounds of the Present Invention	
Ex#	Structure
A427	
A428	
A429	

TABLE 1-continued

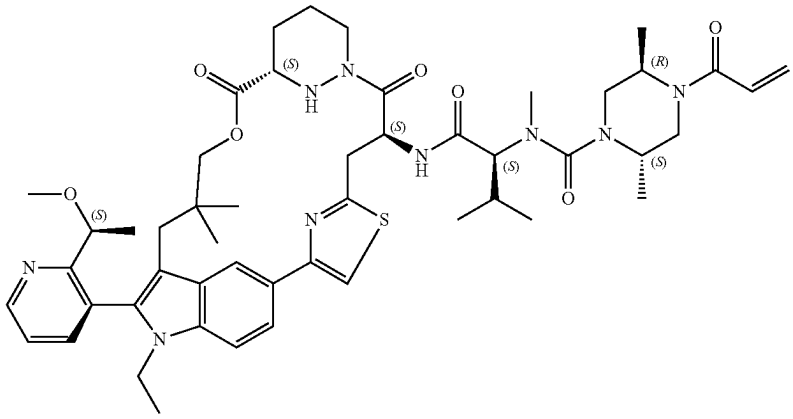
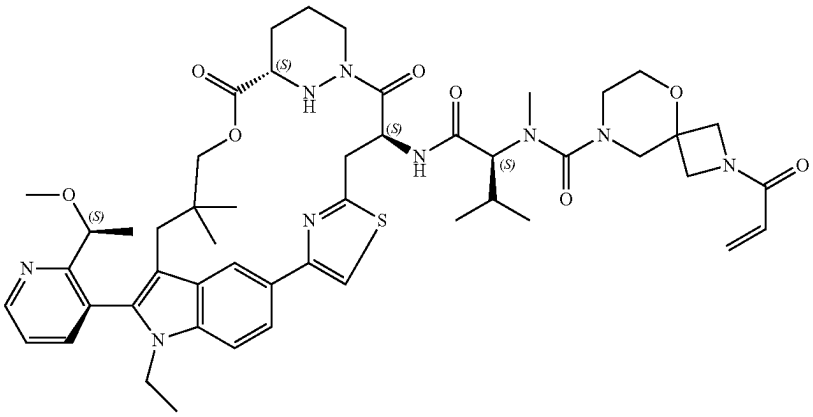
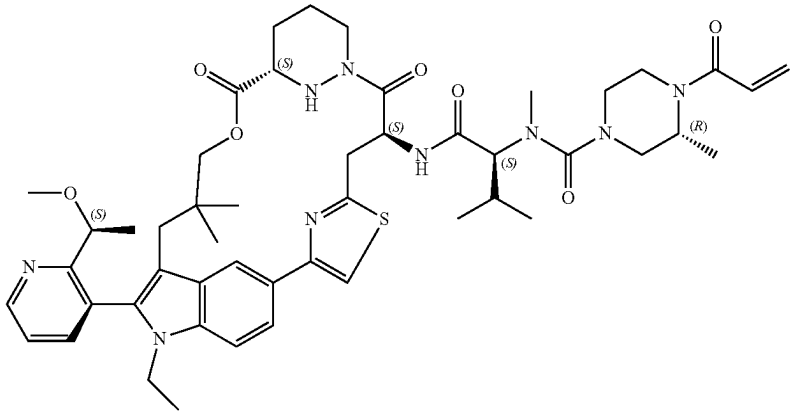
Certain Compounds of the Present Invention	
Ex#	Structure
A430	
A431	
A432	

TABLE 1-continued

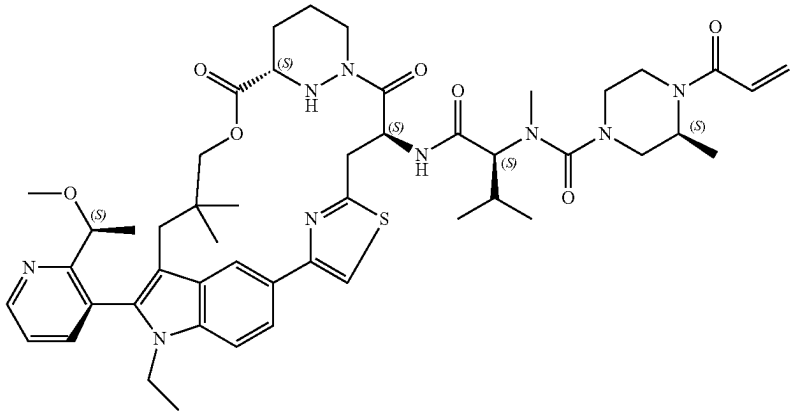
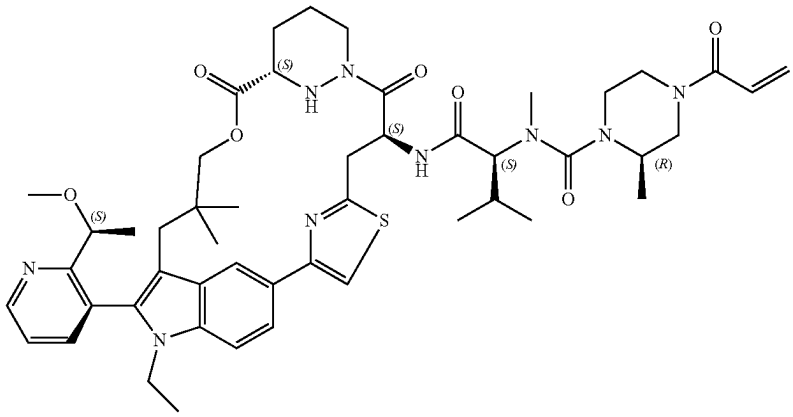
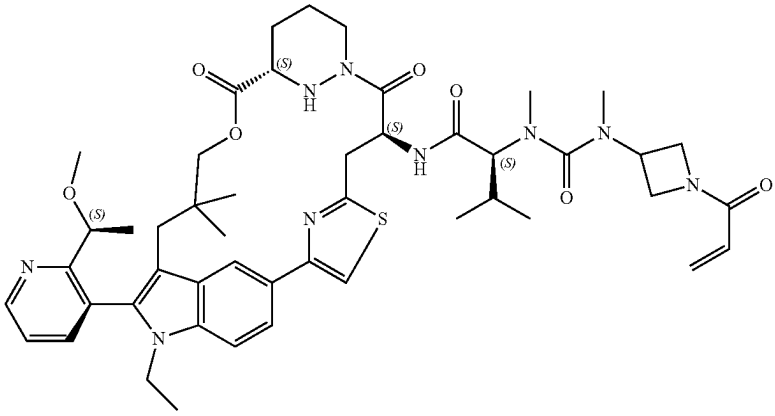
Certain Compounds of the Present Invention	
Ex#	Structure
A433	
A334	
A435	

TABLE 1-continued

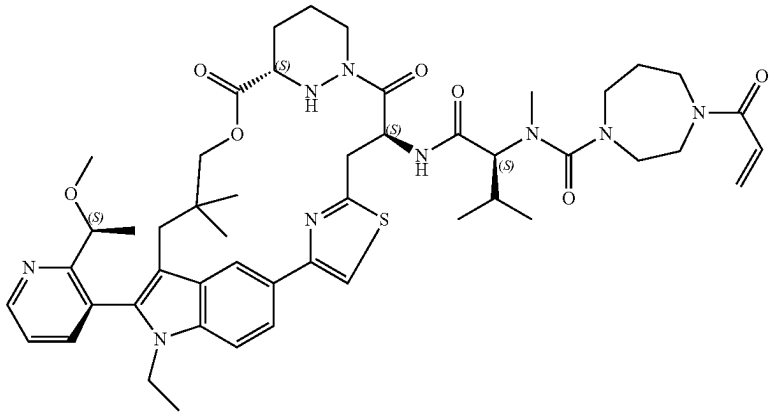
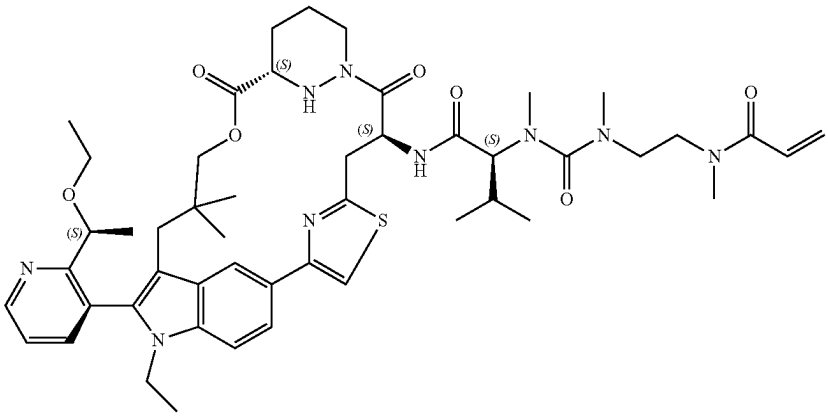
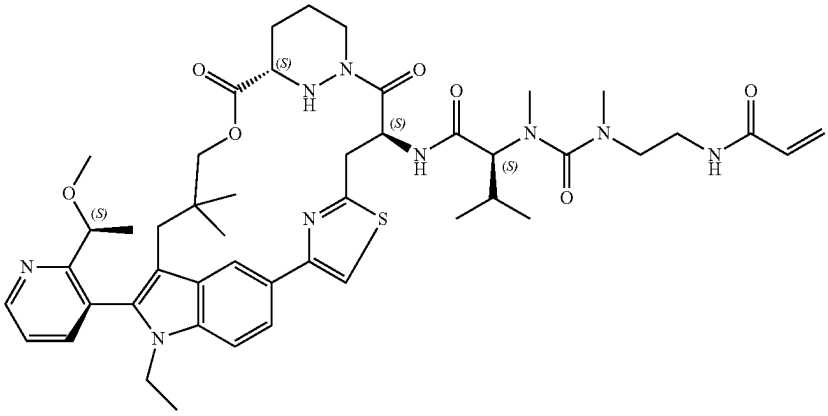
Certain Compounds of the Present Invention	
Ex#	Structure
A436	
A437	
A438	

TABLE 1-continued

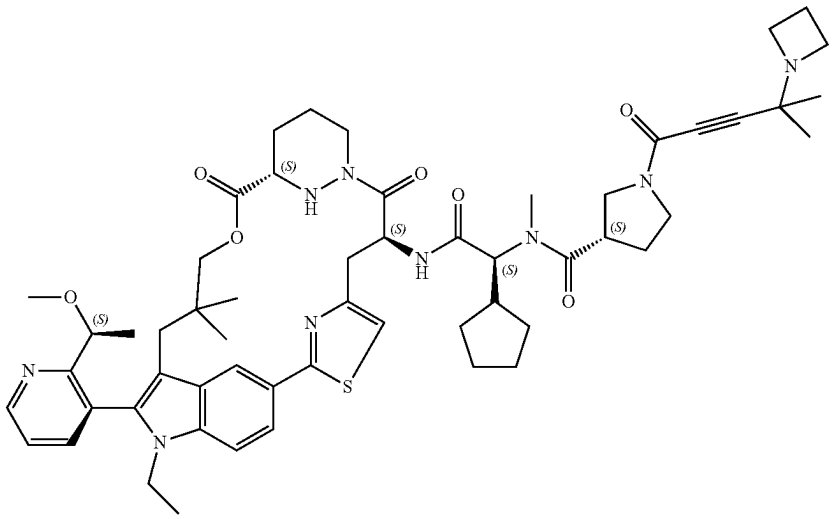
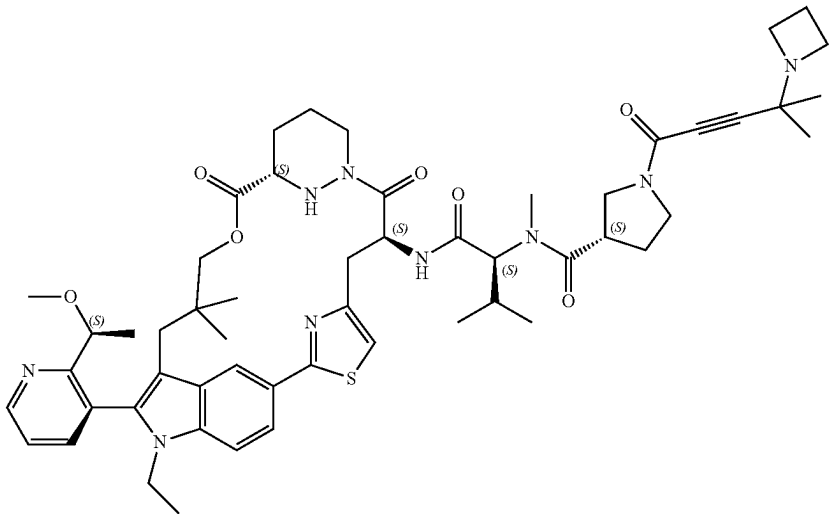
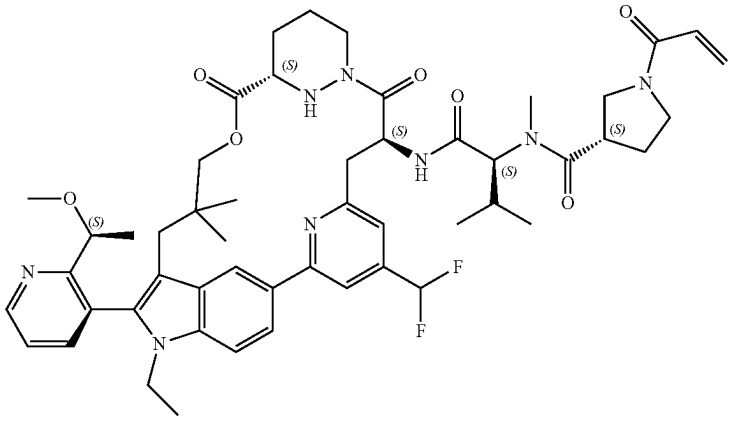
Certain Compounds of the Present Invention	
Ex#	Structure
A439	
A440	
A441	

TABLE 1-continued

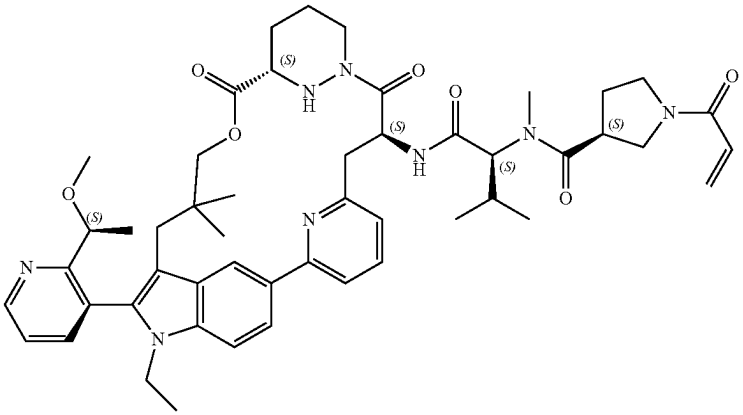
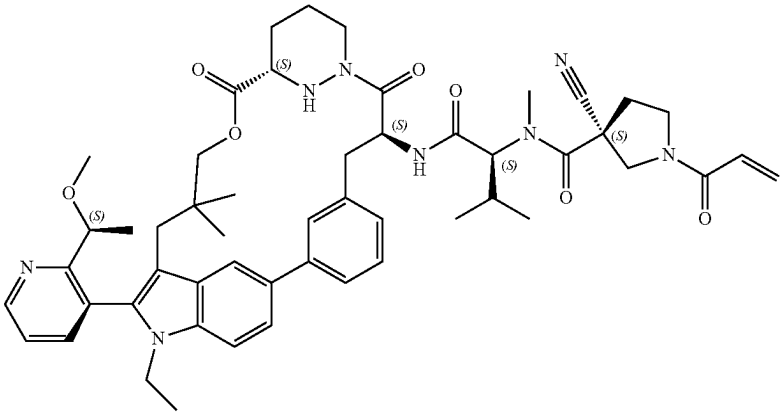
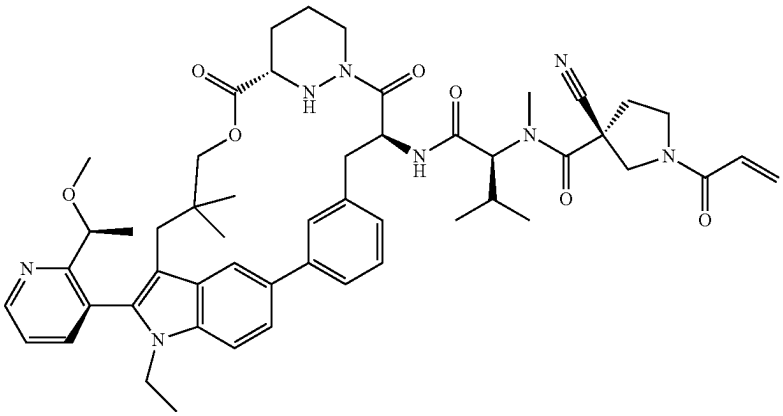
Certain Compounds of the Present Invention	
Ex#	Structure
A442	
A443	
A444	

TABLE 1-continued

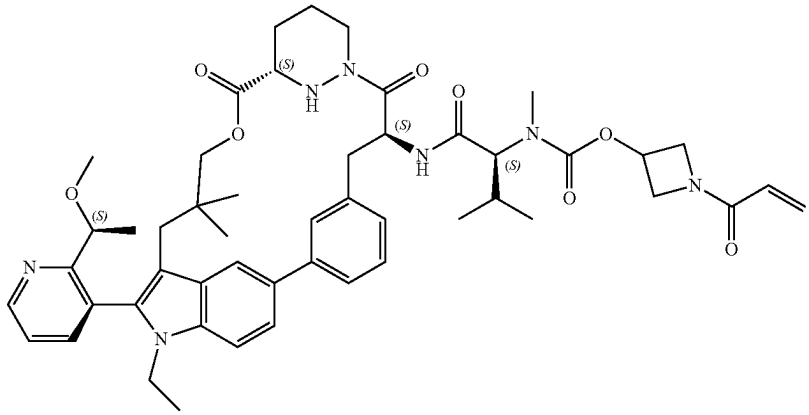
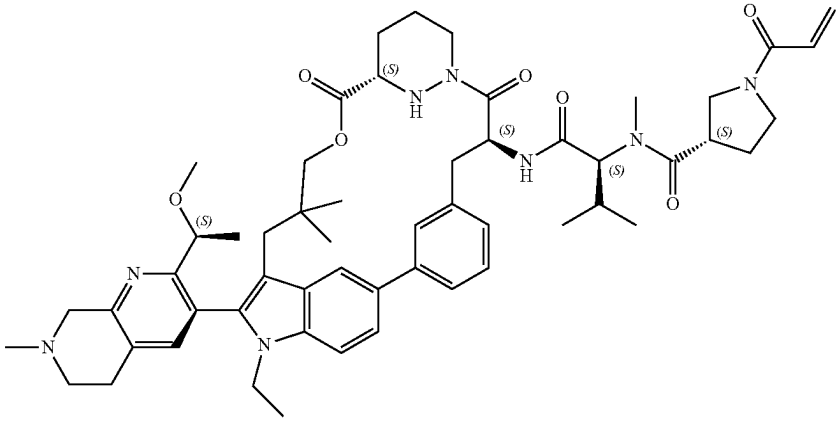
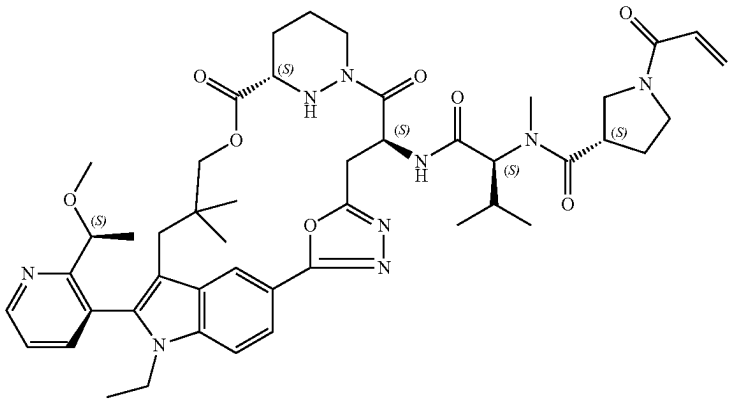
Certain Compounds of the Present Invention	
Ex#	Structure
A445	
A446	
A447	

TABLE 1-continued

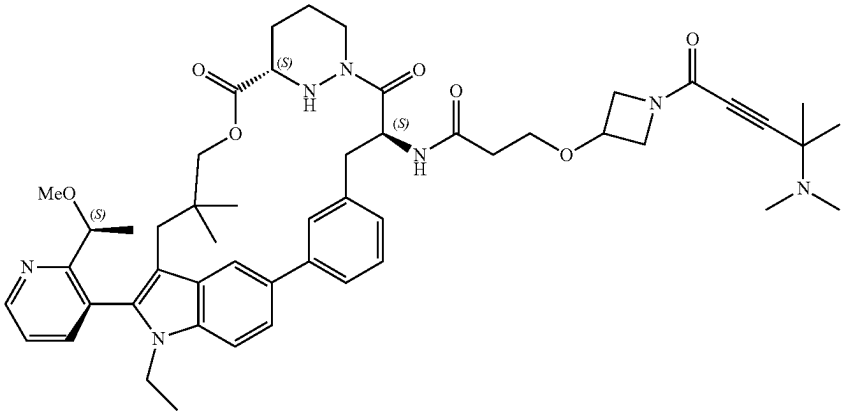
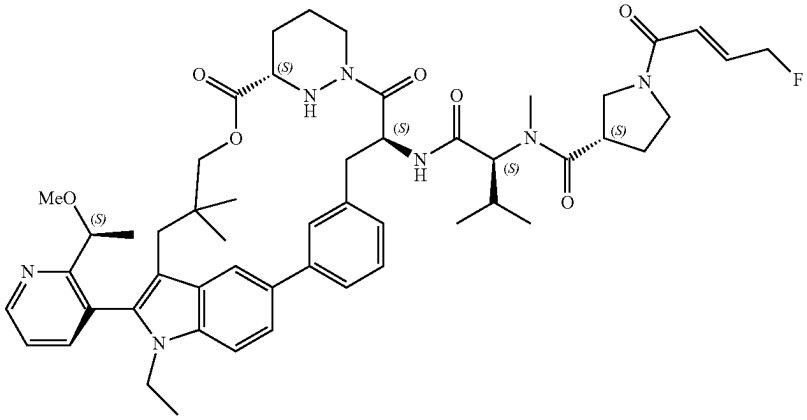
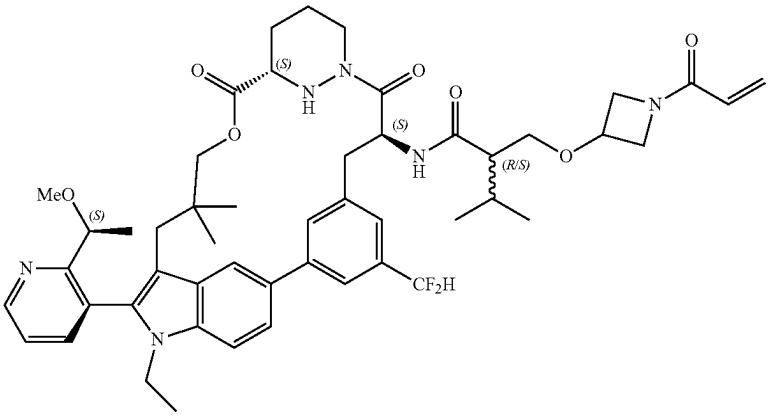
Certain Compounds of the Present Invention	
Ex#	Structure
A448	
A449	
A450	

TABLE 1-continued

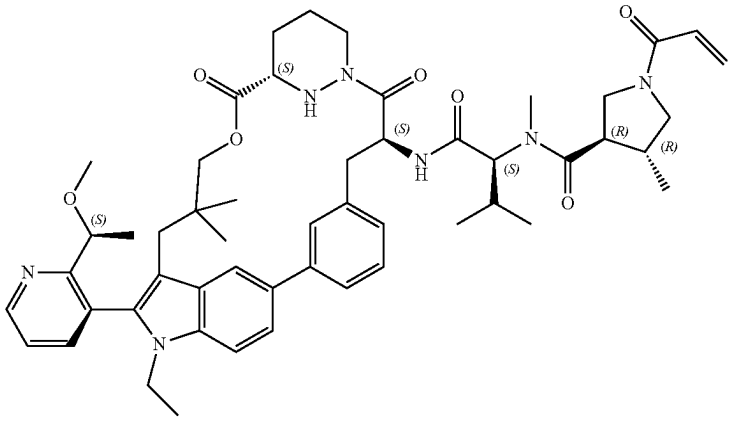
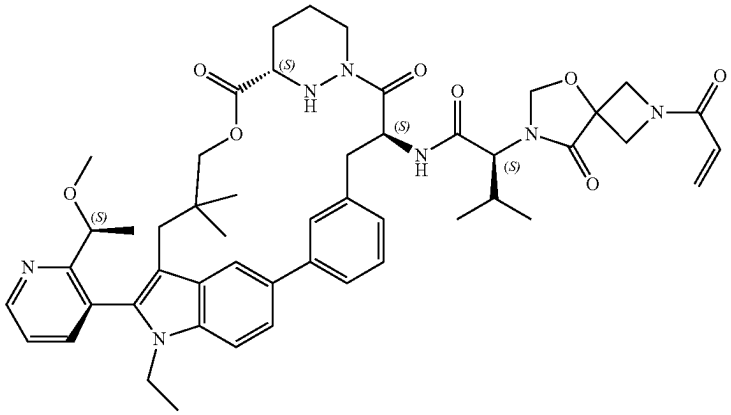
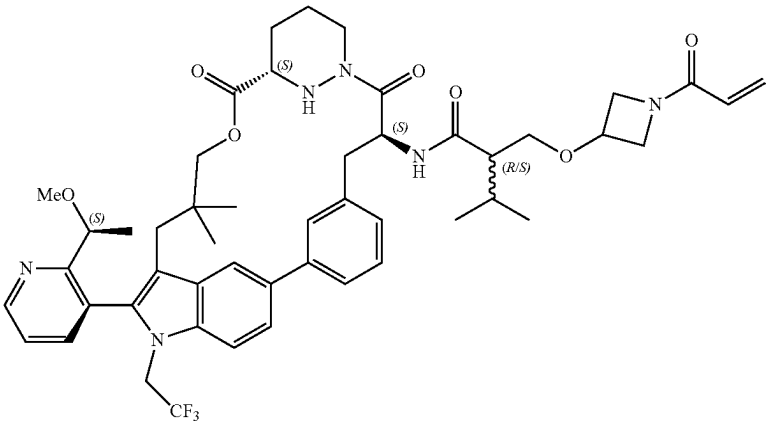
Certain Compounds of the Present Invention	
Ex#	Structure
A451	
A452	
A453	

TABLE 1-continued

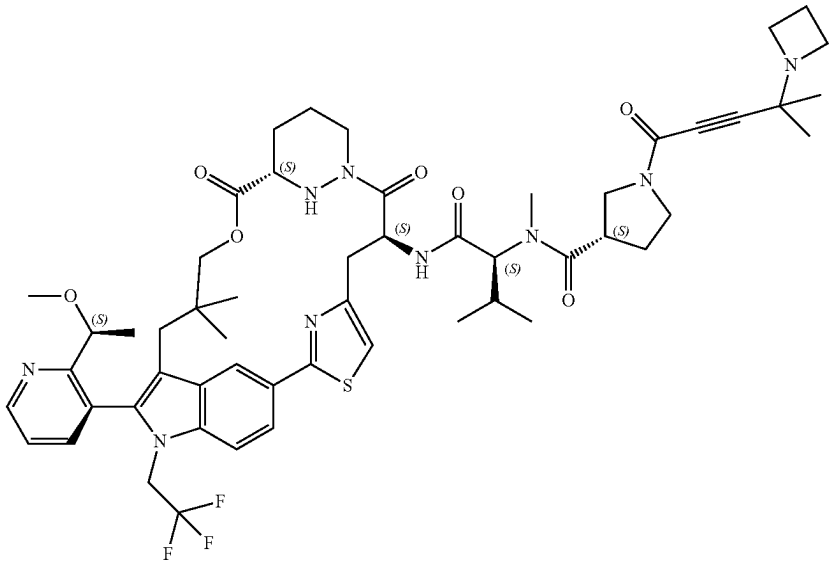
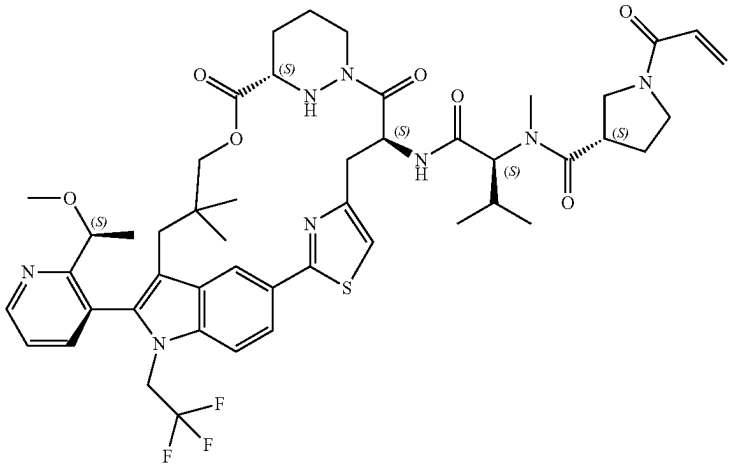
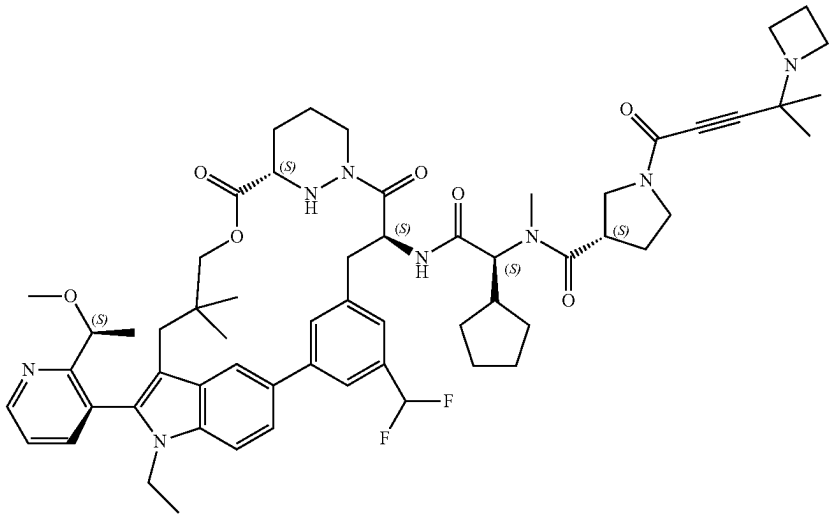
Certain Compounds of the Present Invention	
Ex#	Structure
A454	
A455	
A456	

TABLE 1-continued

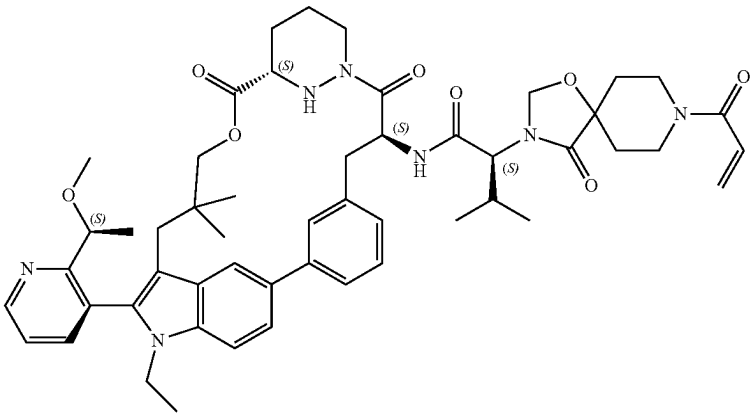
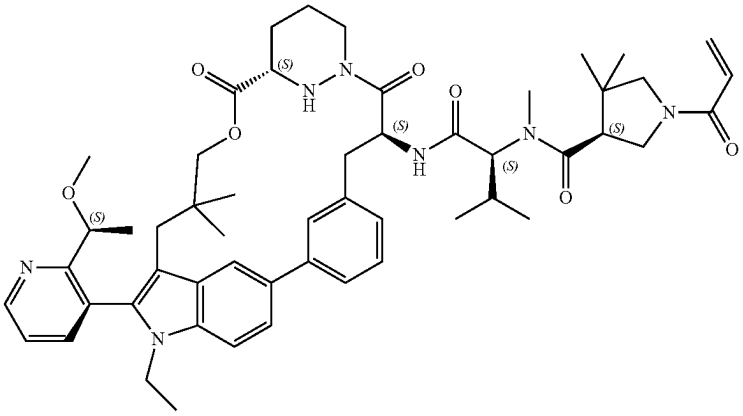
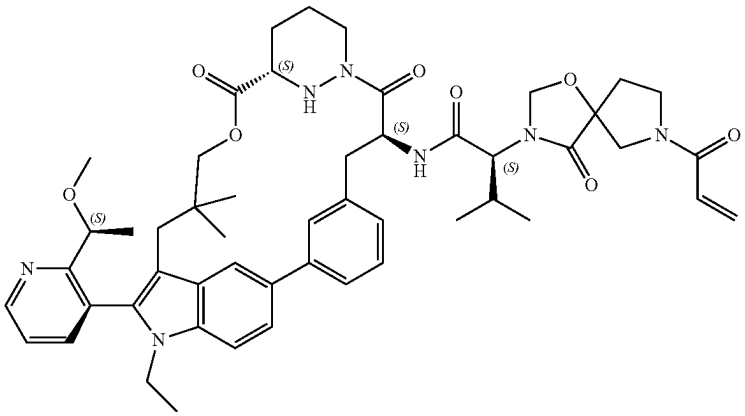
Certain Compounds of the Present Invention	
Ex#	Structure
A457	
A458	
A459	

TABLE 1-continued

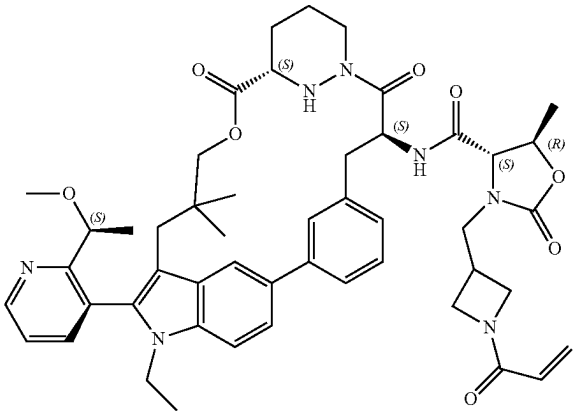
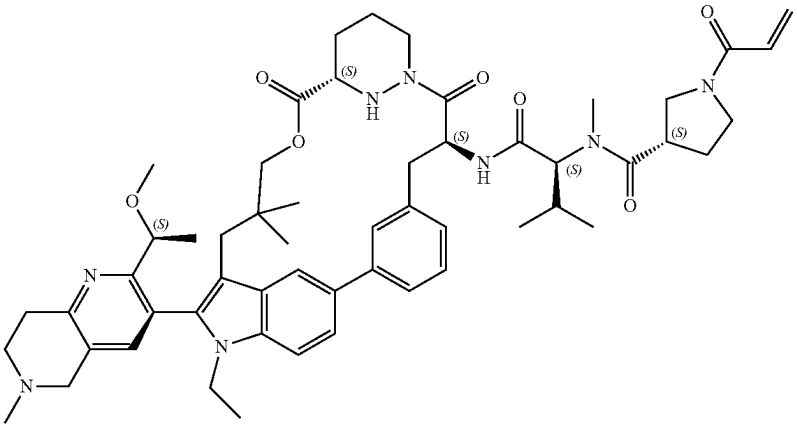
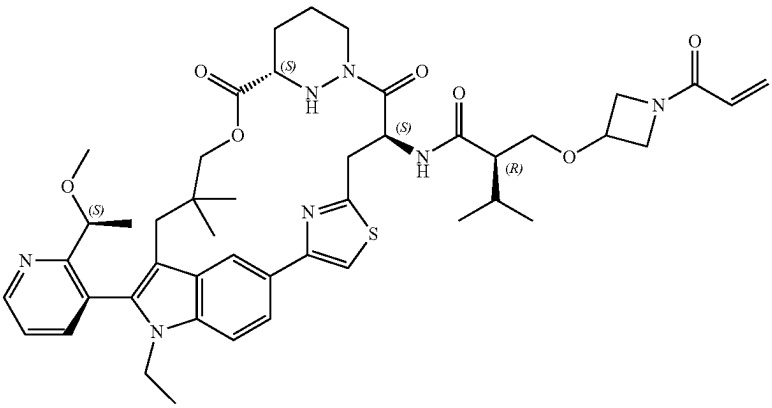
Certain Compounds of the Present Invention	
Ex#	Structure
A460	
A461	
A462	

TABLE 1-continued

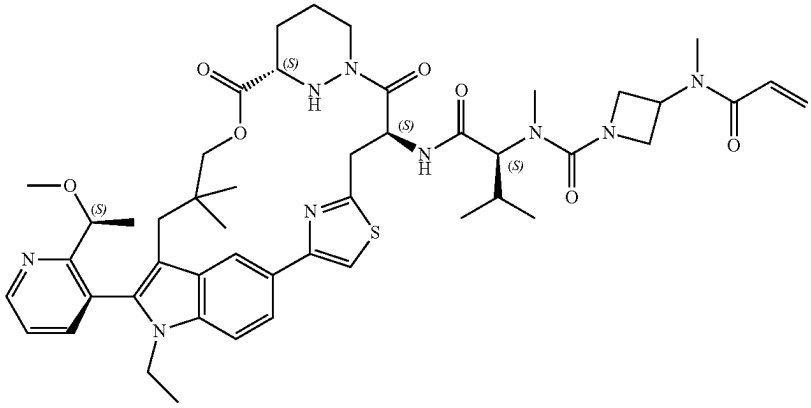
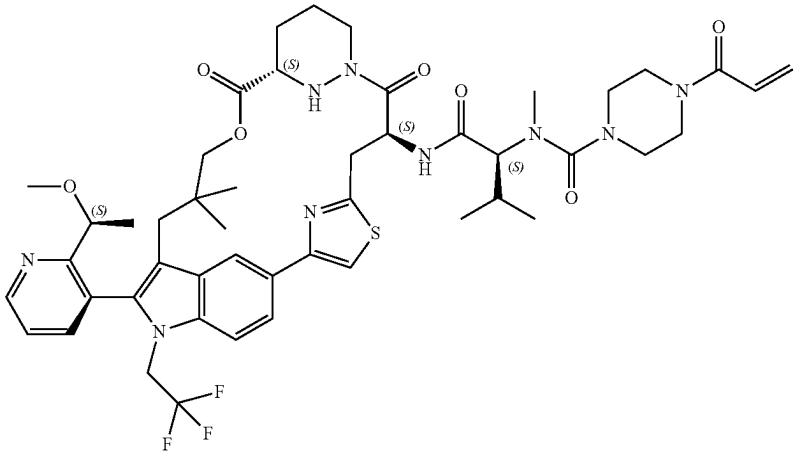
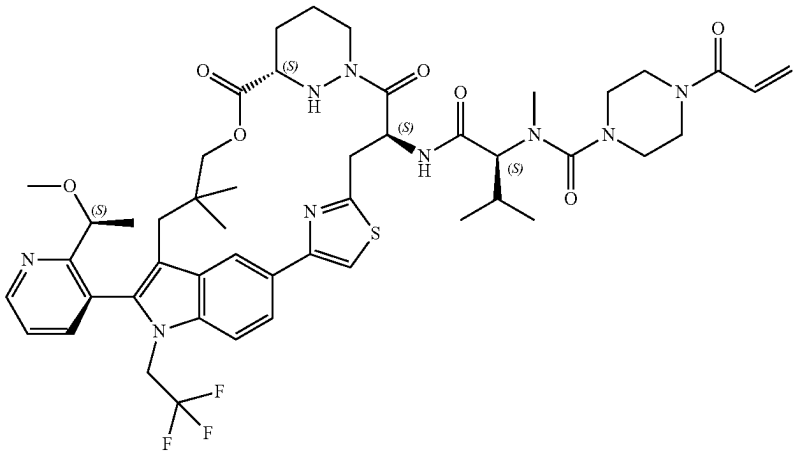
Certain Compounds of the Present Invention	
Ex#	Structure
A463	
A464	
A465	

TABLE 1-continued

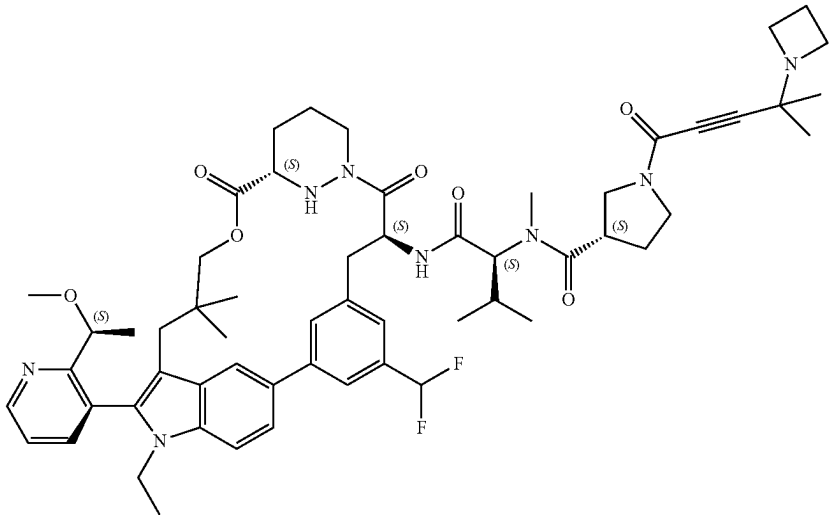
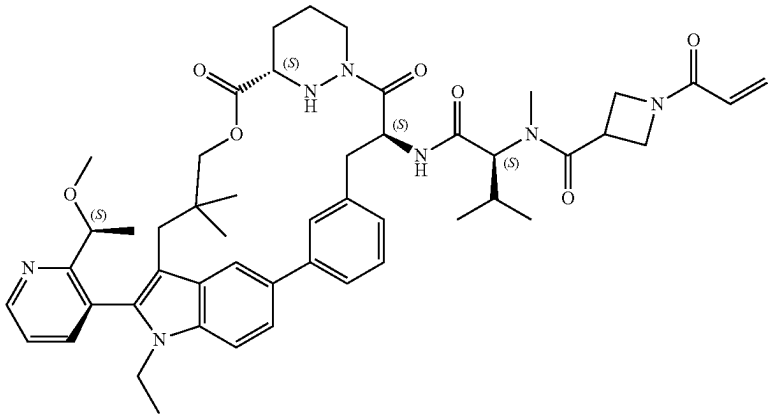
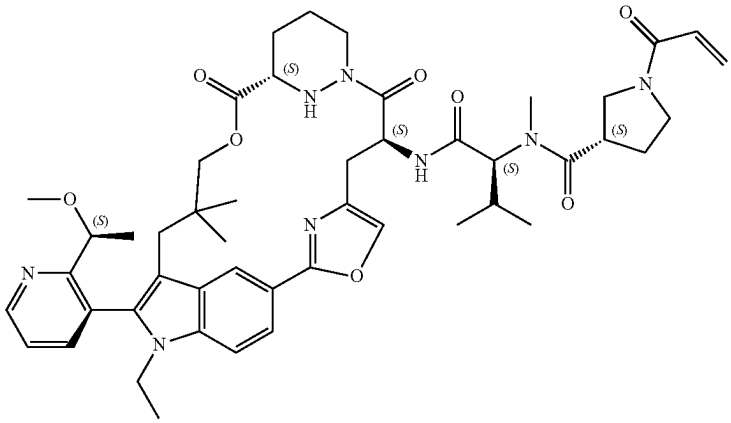
Certain Compounds of the Present Invention	
Ex#	Structure
A466	
A467	
A468	

TABLE 1-continued

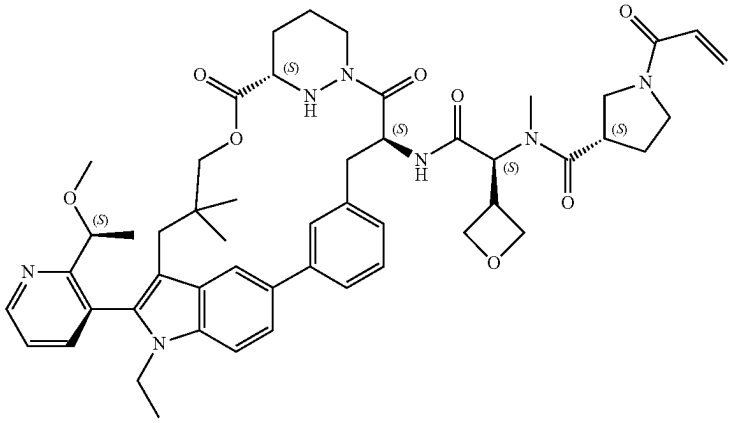
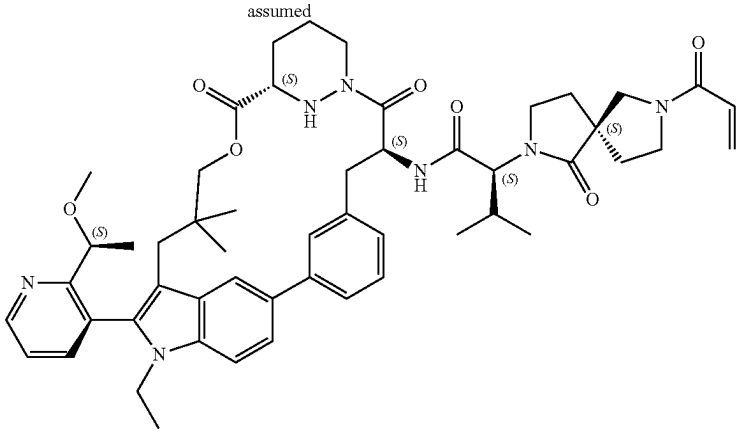
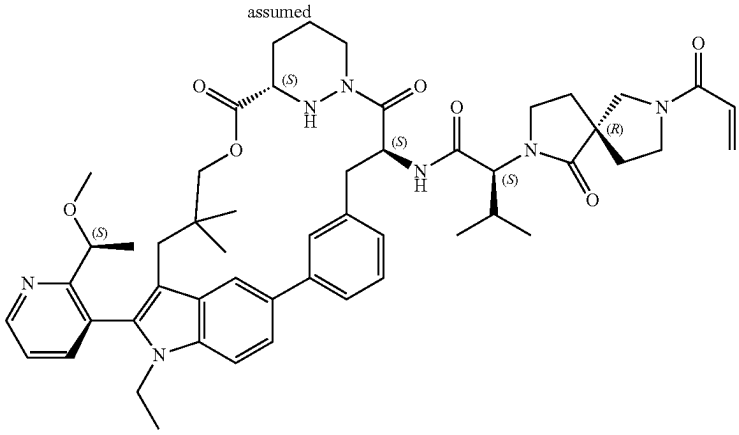
Certain Compounds of the Present Invention	
Ex#	Structure
A469	
A470	assumed 
A471	assumed 

TABLE 1-continued

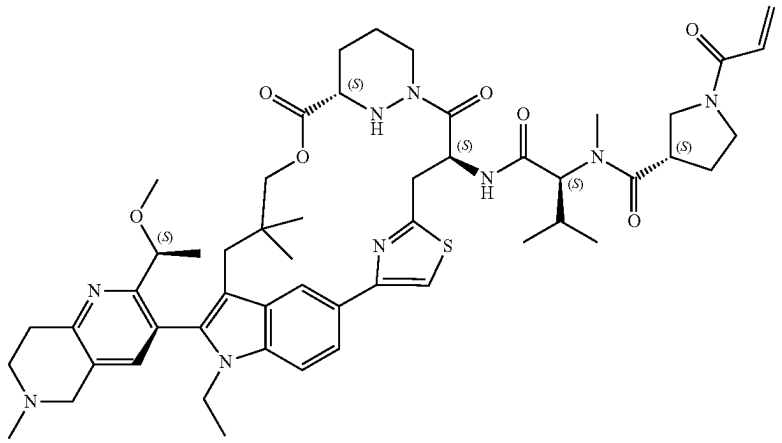
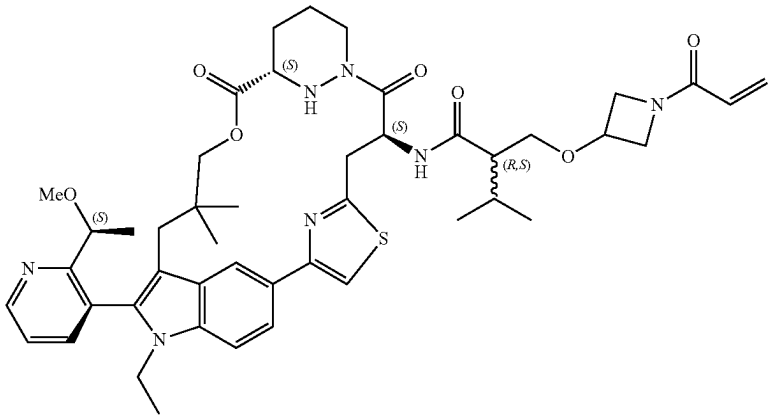
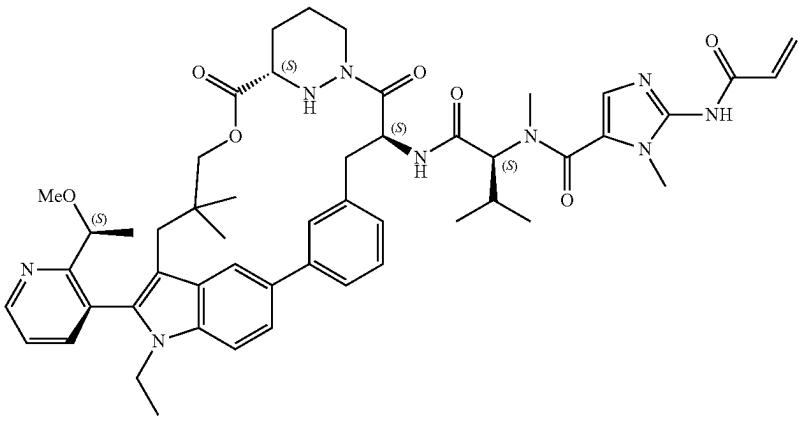
Certain Compounds of the Present Invention	
Ex#	Structure
A472	
A473	
A474	

TABLE 1-continued

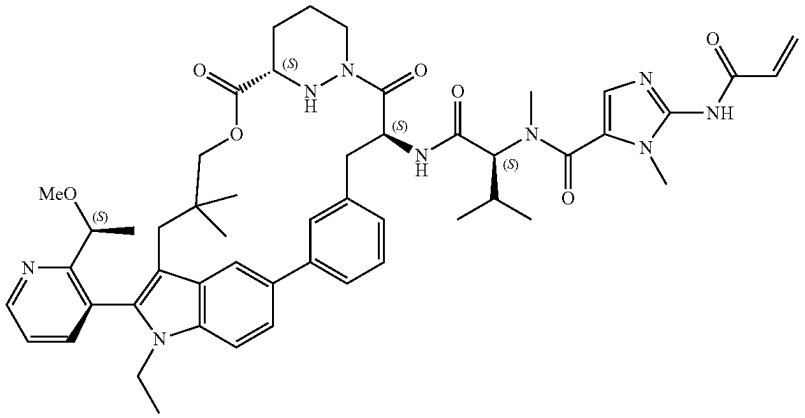
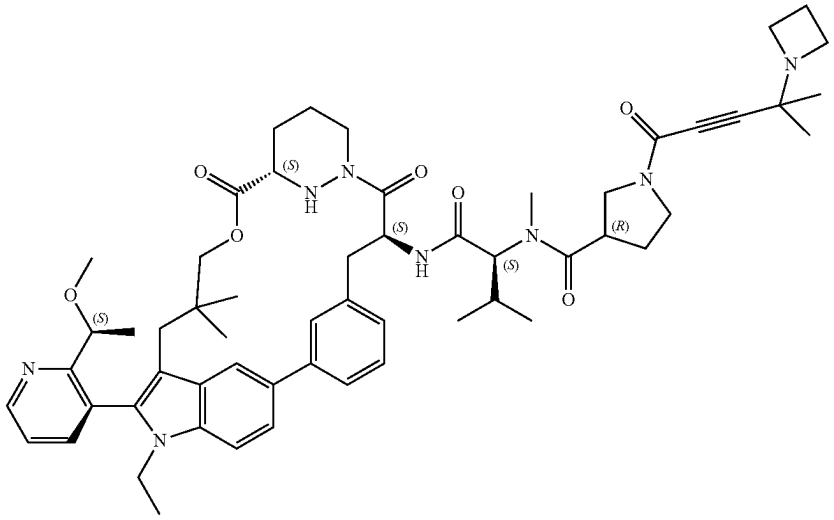
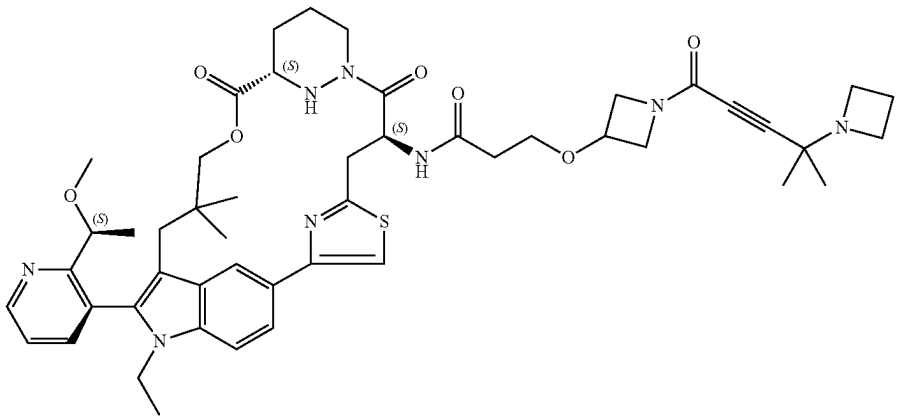
Certain Compounds of the Present Invention	
Ex#	Structure
A475	
A476	
A477	

TABLE 1-continued

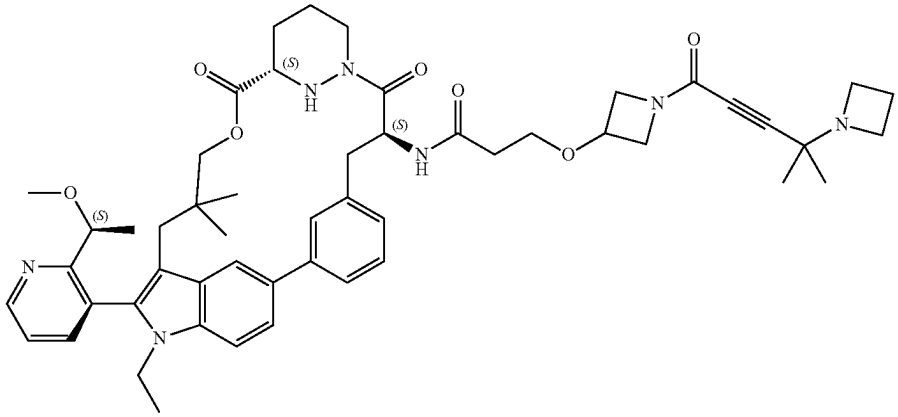
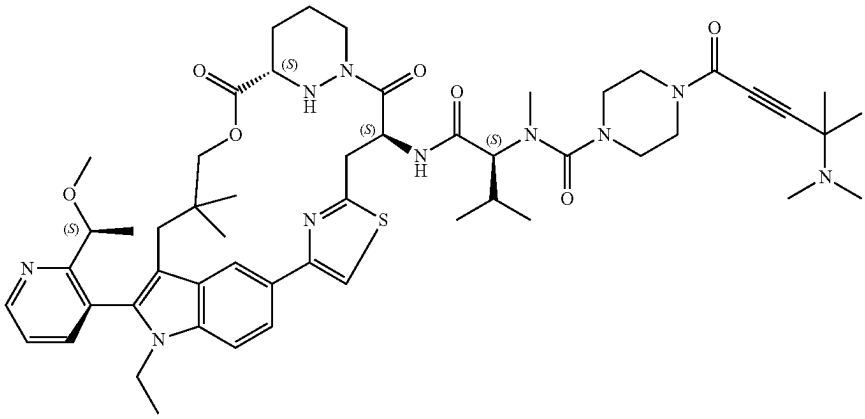
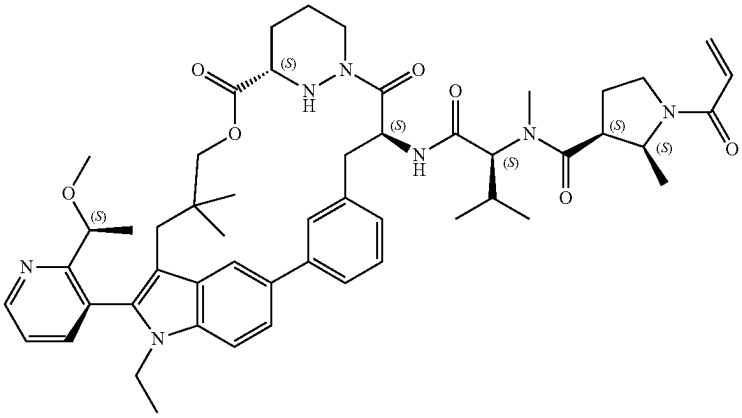
Certain Compounds of the Present Invention	
Ex#	Structure
A478	
A479	
A480	

TABLE 1-continued

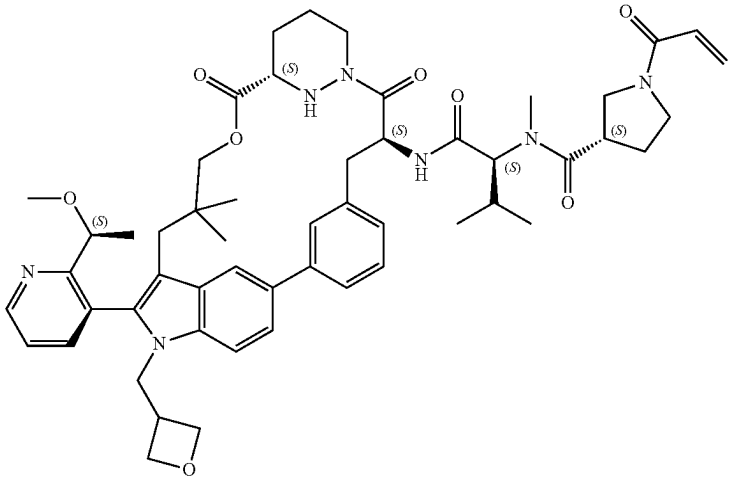
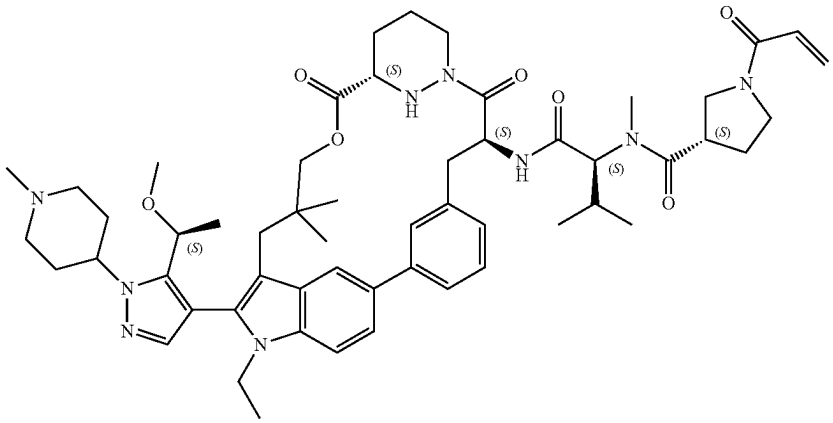
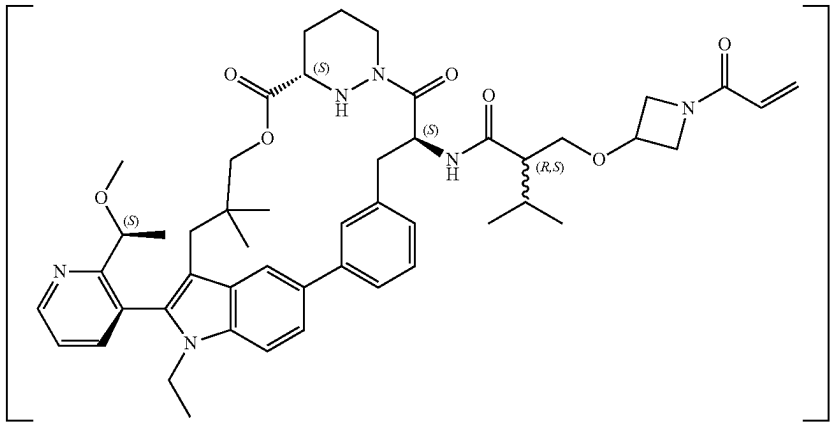
Certain Compounds of the Present Invention	
Ex#	Structure
A481	
A482	
A483	

TABLE 1-continued

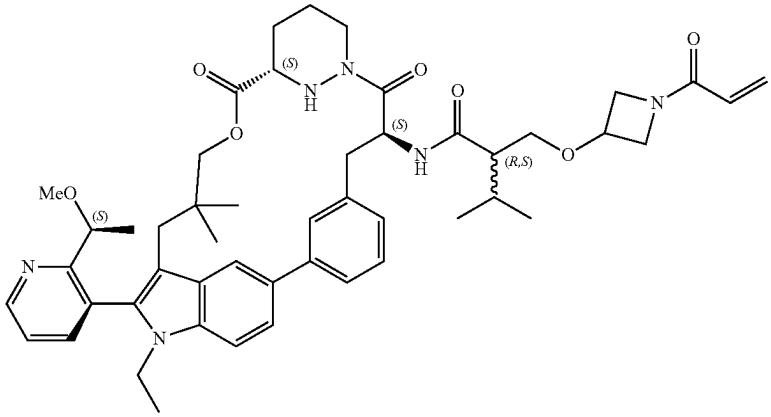
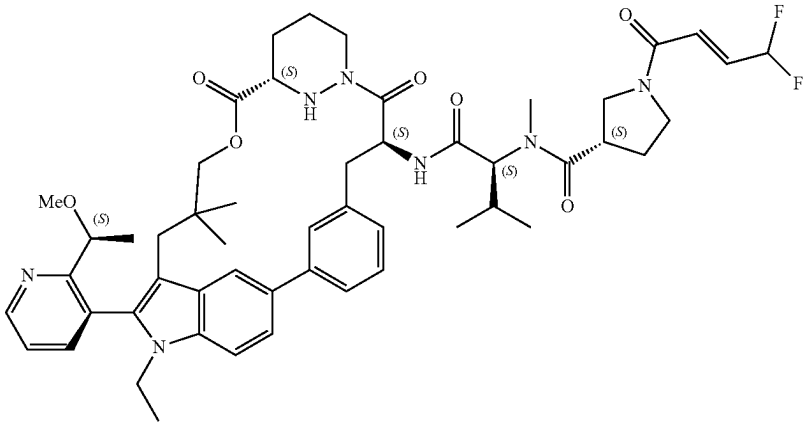
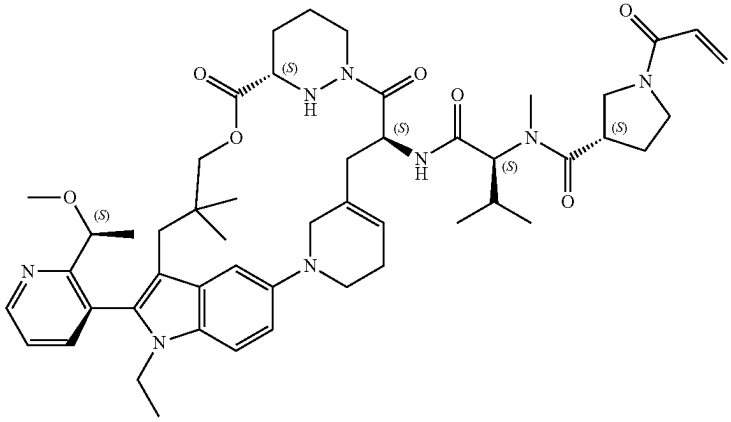
Certain Compounds of the Present Invention	
Ex#	Structure
A484	
A485	
A486	

TABLE 1-continued

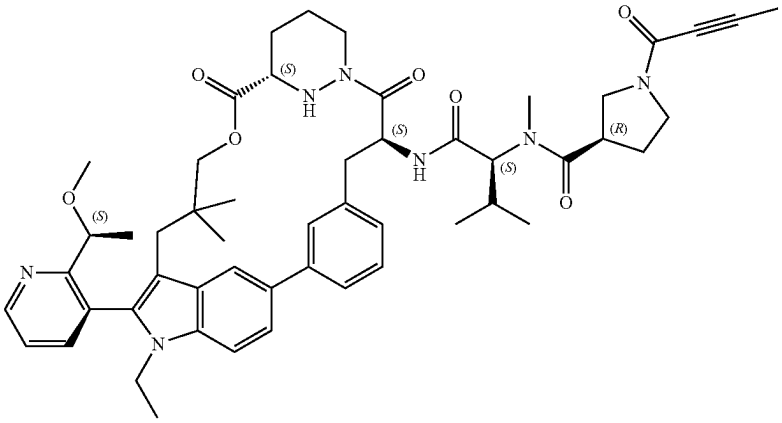
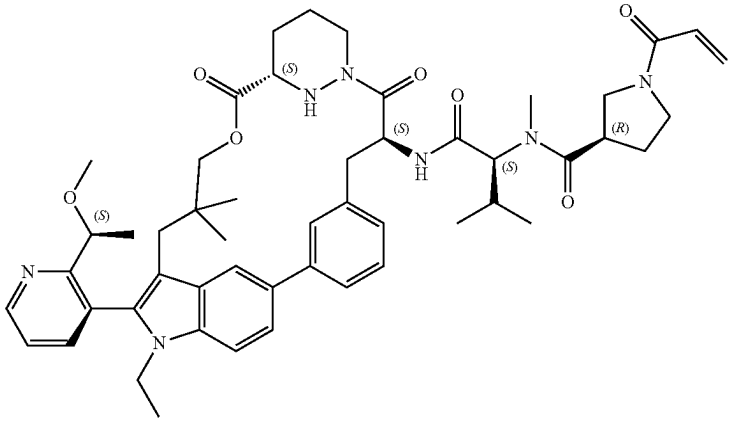
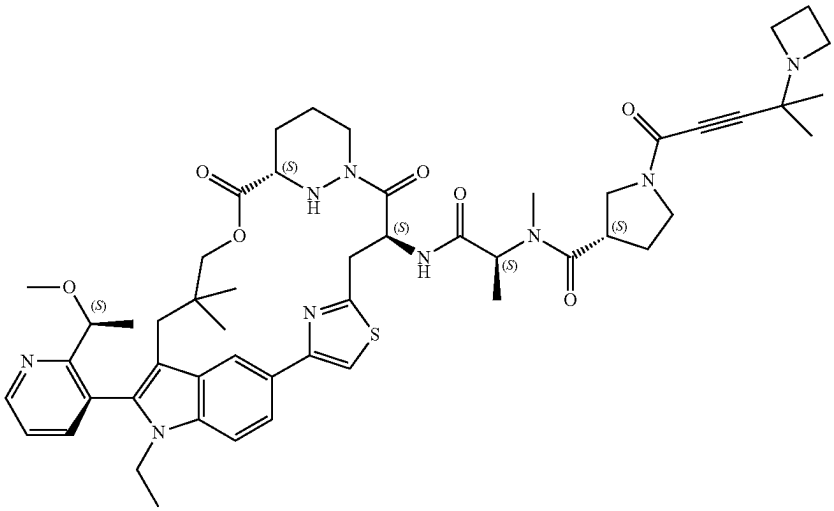
Certain Compounds of the Present Invention	
Ex#	Structure
A487	
A488	
A489	

TABLE 1-continued

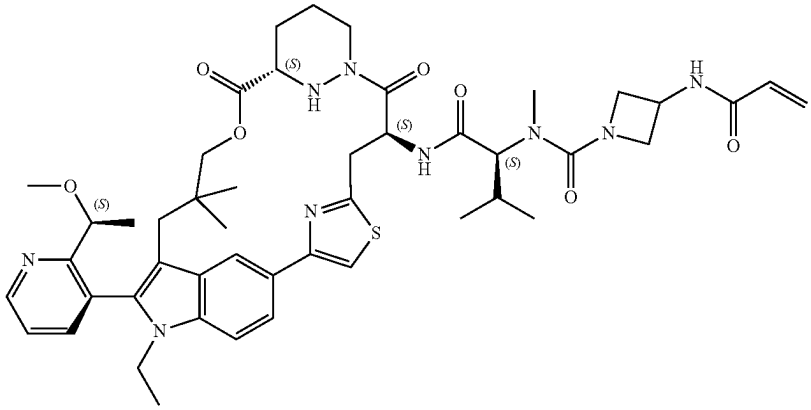
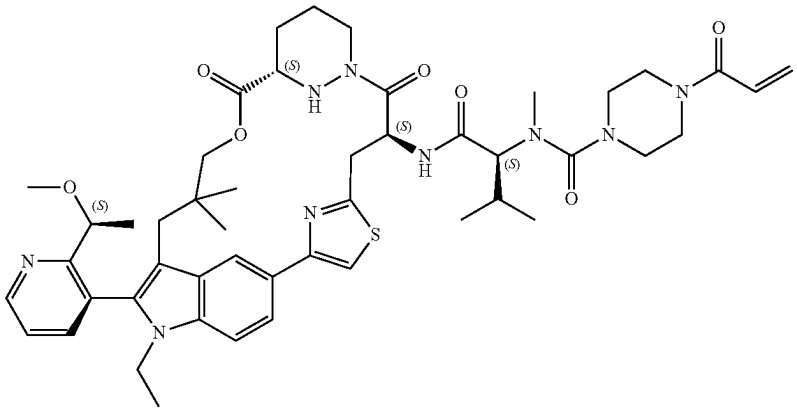
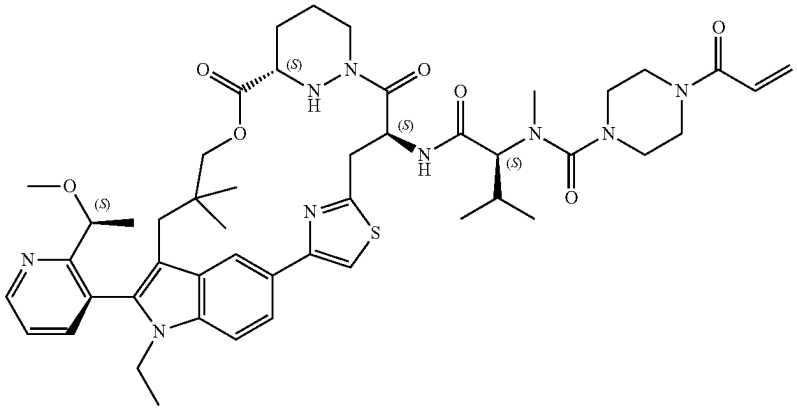
Certain Compounds of the Present Invention	
Ex#	Structure
A490	
A491	
A492	

TABLE 1-continued

Certain Compounds of the Present Invention	
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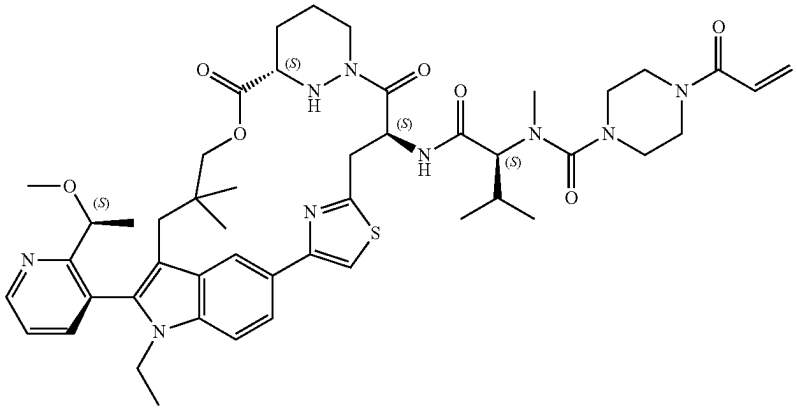
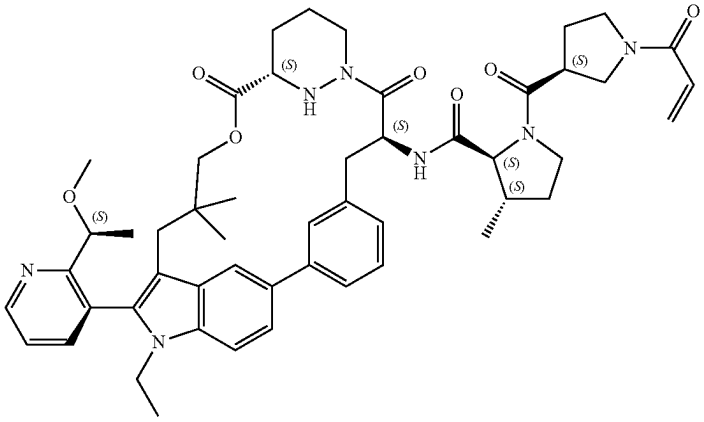
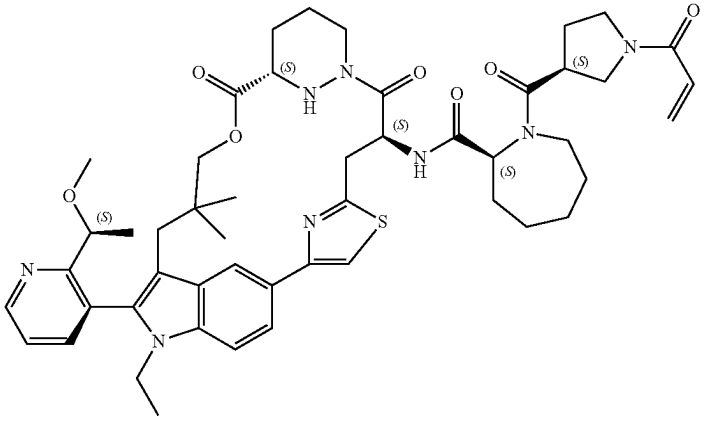
A493	
A494	
A495	

TABLE 1-continued

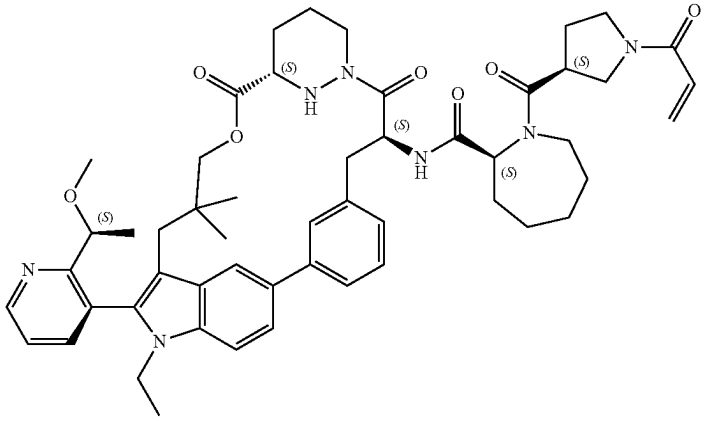
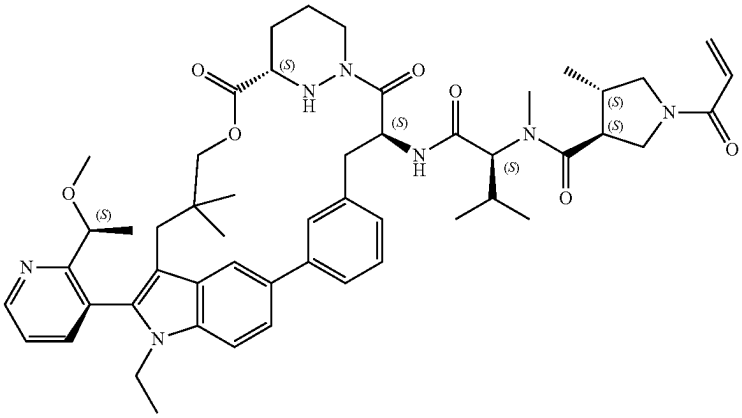
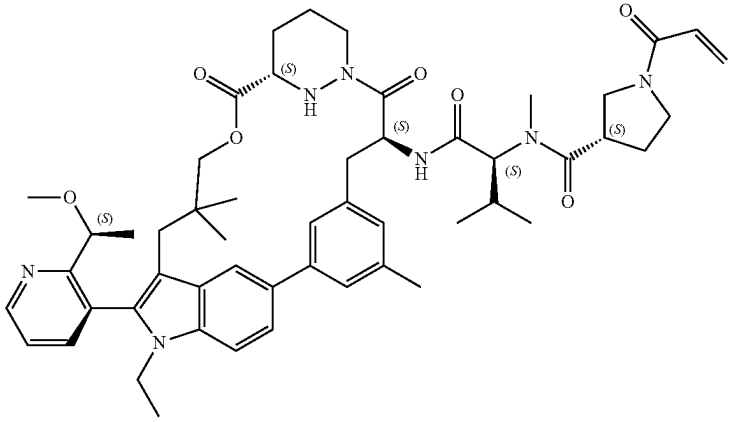
Certain Compounds of the Present Invention	
Ex#	Structure
A496	
A497	
A498	

TABLE 1-continued

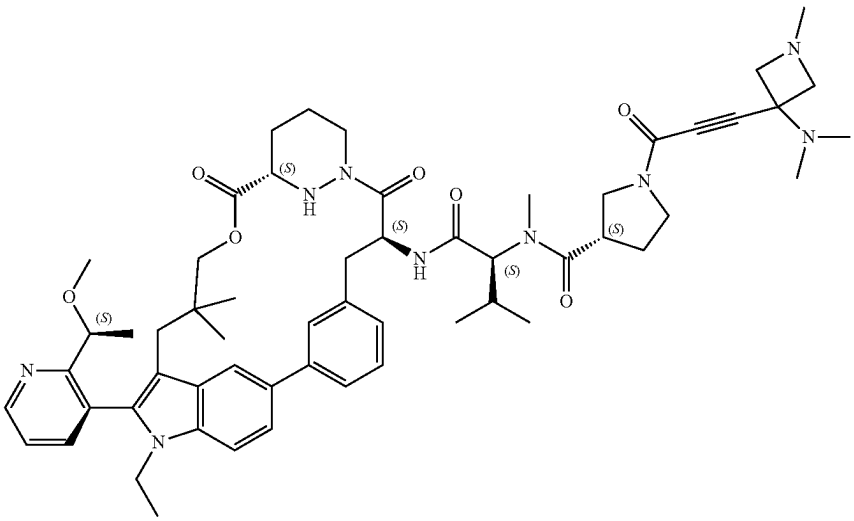
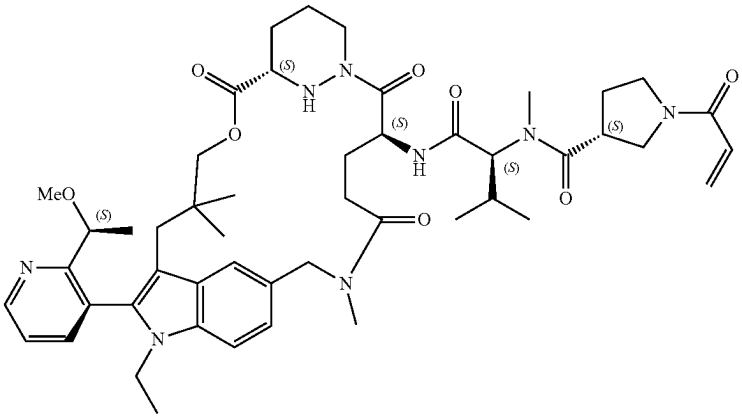
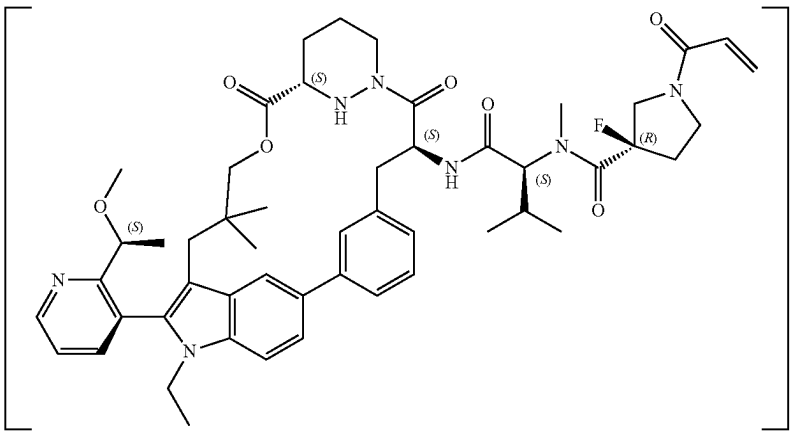
Certain Compounds of the Present Invention	
Ex#	Structure
A499	
A500	
A501	

TABLE 1-continued

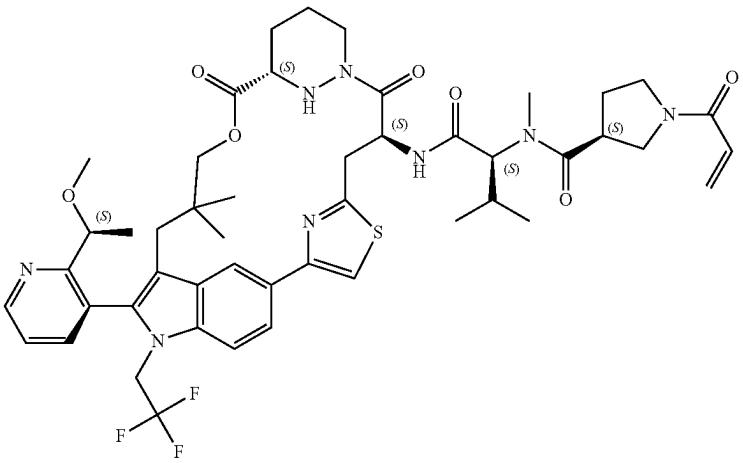
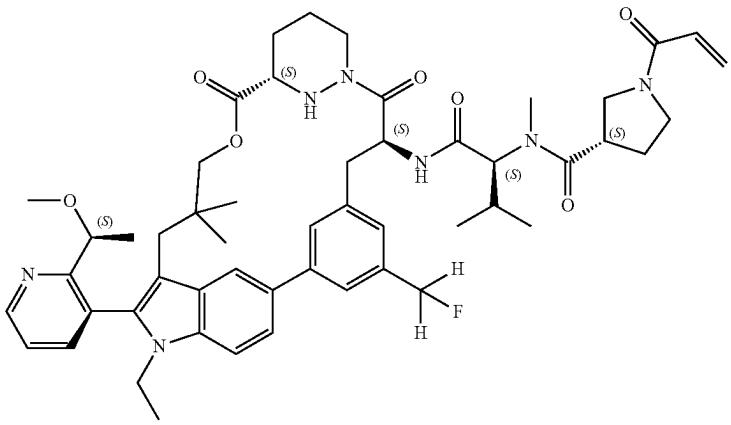
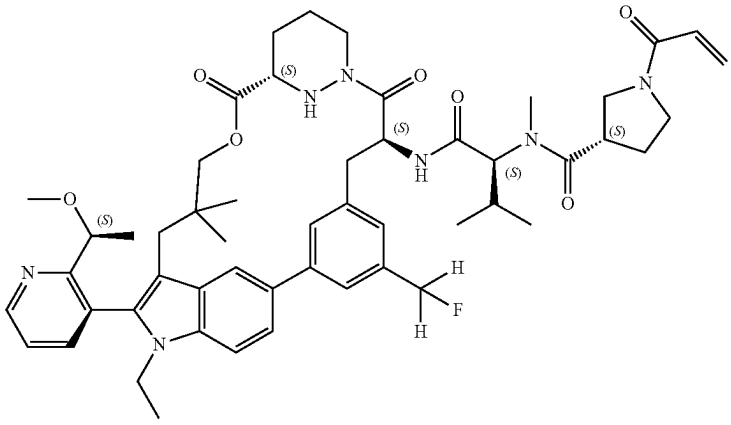
Certain Compounds of the Present Invention	
Ex#	Structure
A502	
A503	
A504	

TABLE 1-continued

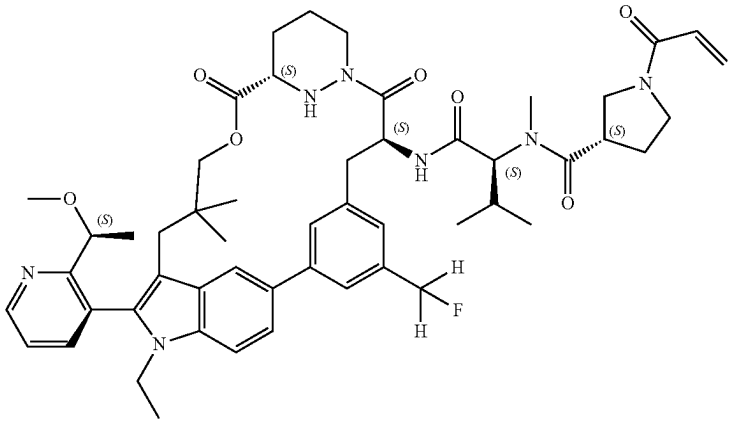
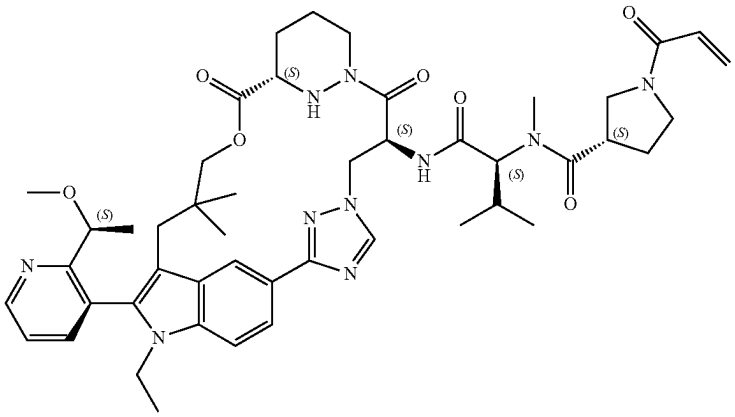
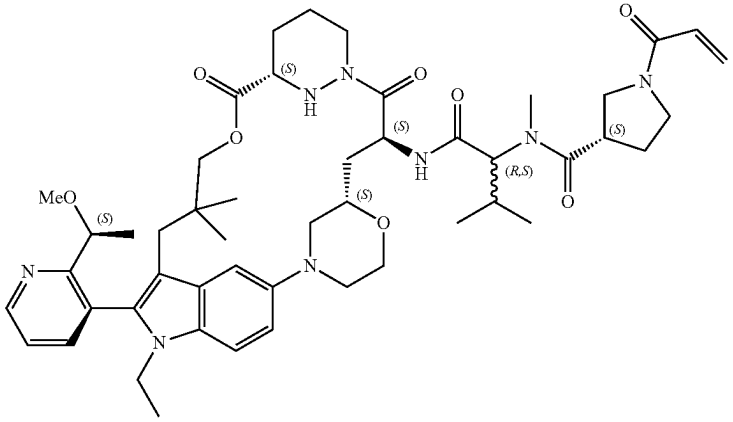
Certain Compounds of the Present Invention	
Ex#	Structure
A505	
A506	
A507	

TABLE 1-continued

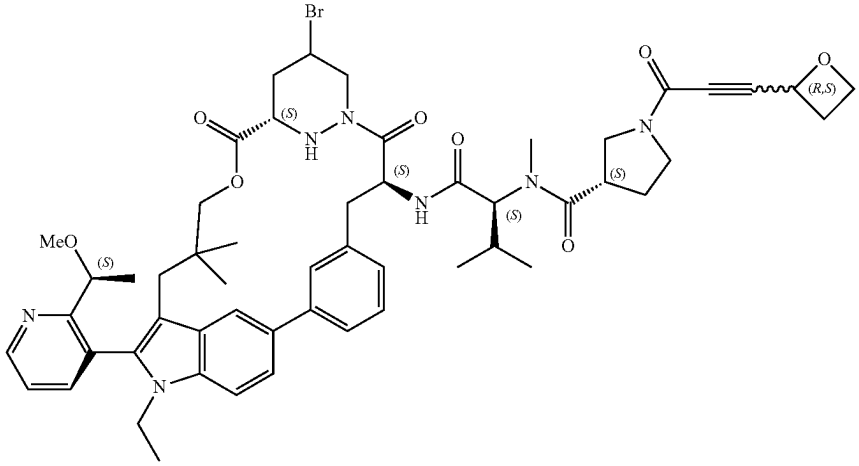
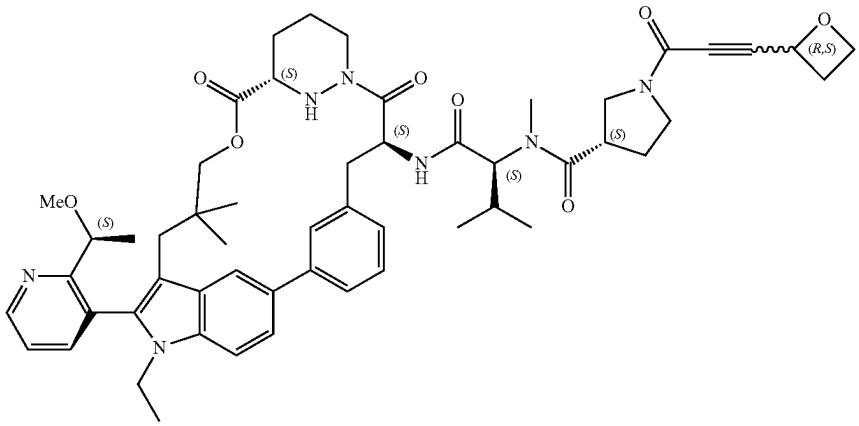
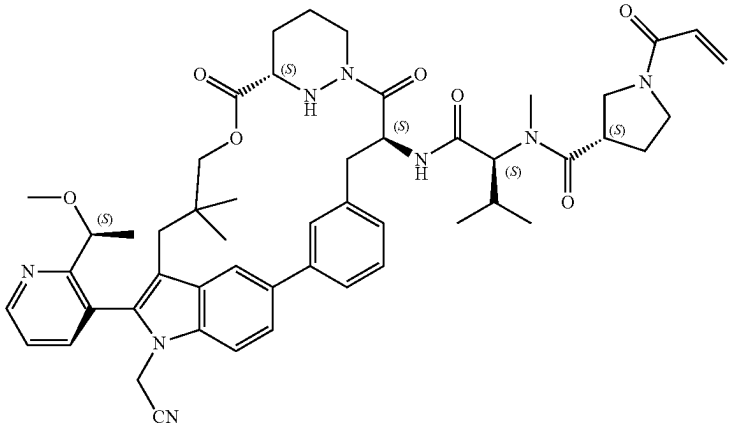
Certain Compounds of the Present Invention	
Ex#	Structure
A508	
A509	
A510	

TABLE 1-continued

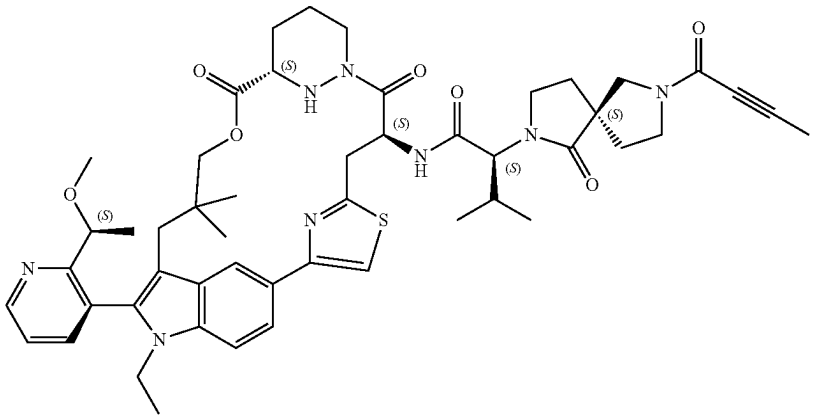
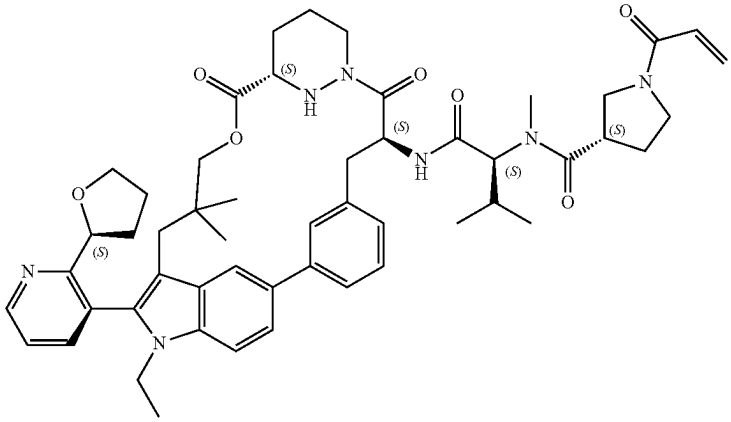
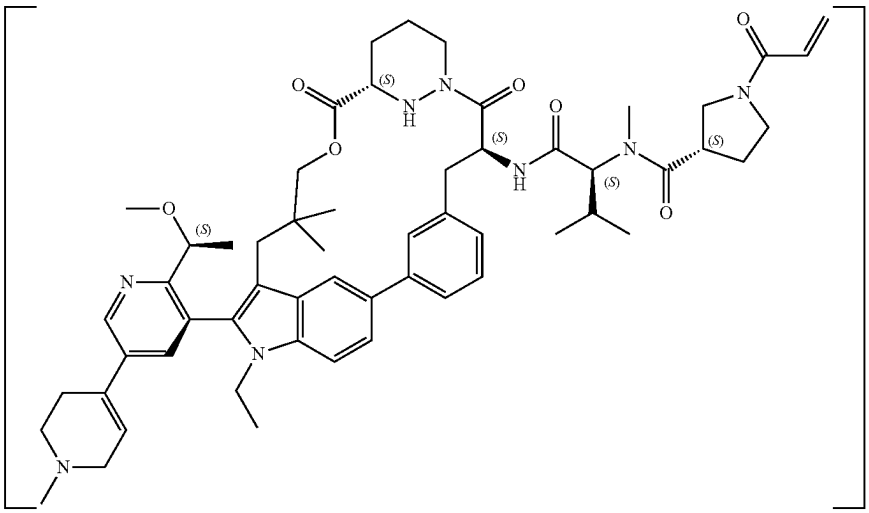
Certain Compounds of the Present Invention	
Ex#	Structure
A511	
A512	
A513	

TABLE 1-continued

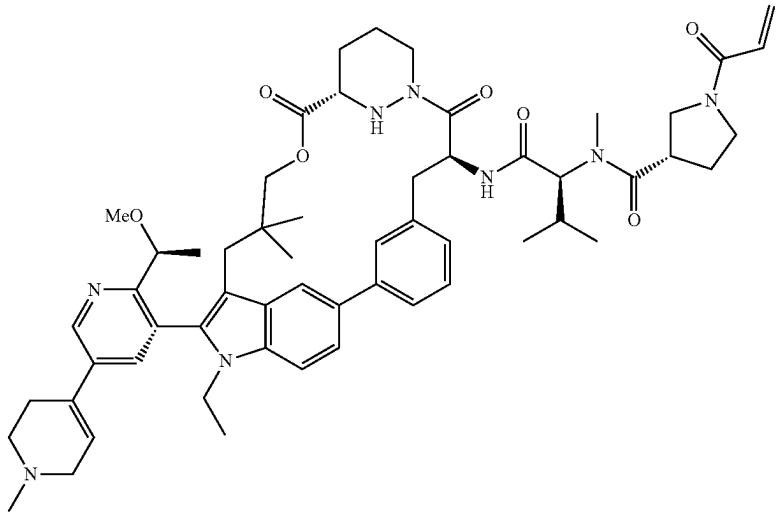
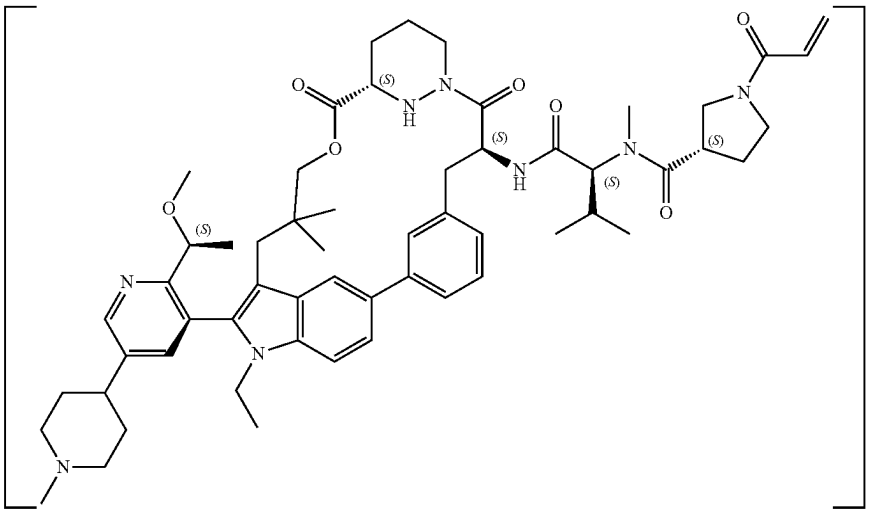
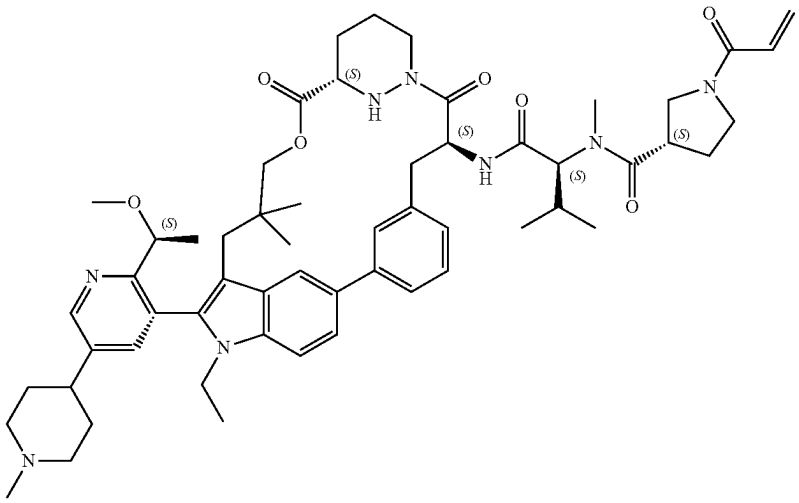
Certain Compounds of the Present Invention	
Ex#	Structure
A514	
A515	
A516	

TABLE 1-continued

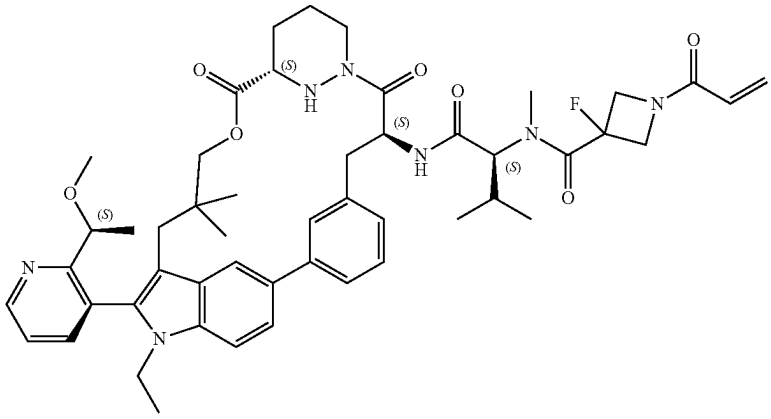
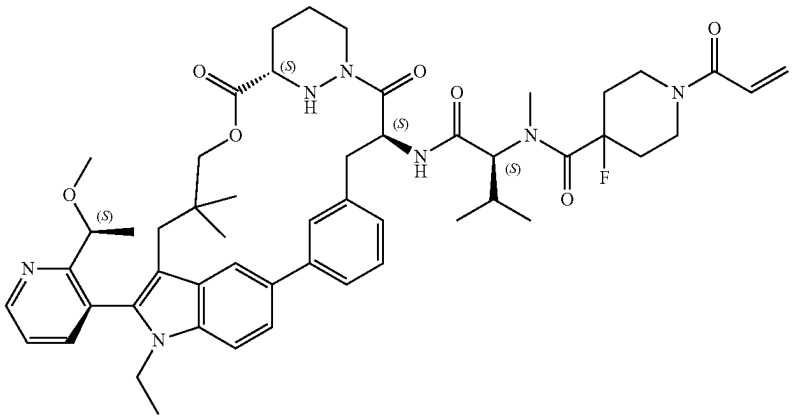
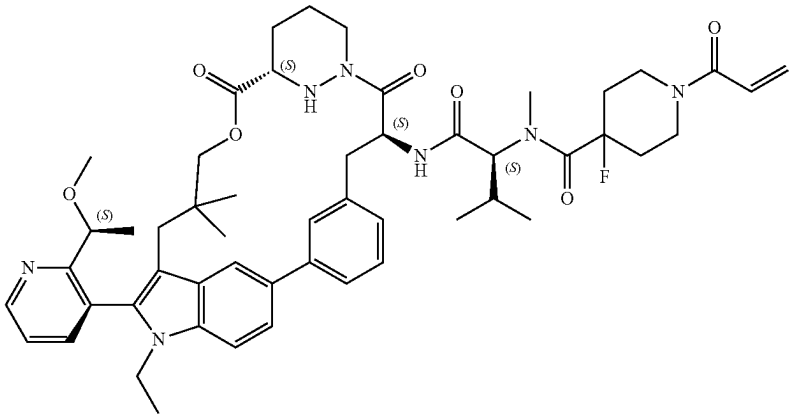
Certain Compounds of the Present Invention	
Ex#	Structure
A517	
A518	
A519	

TABLE 1-continued

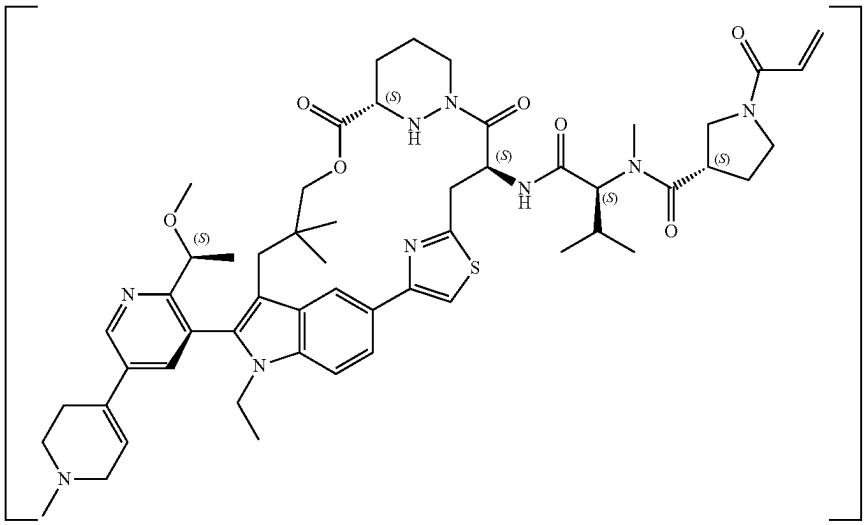
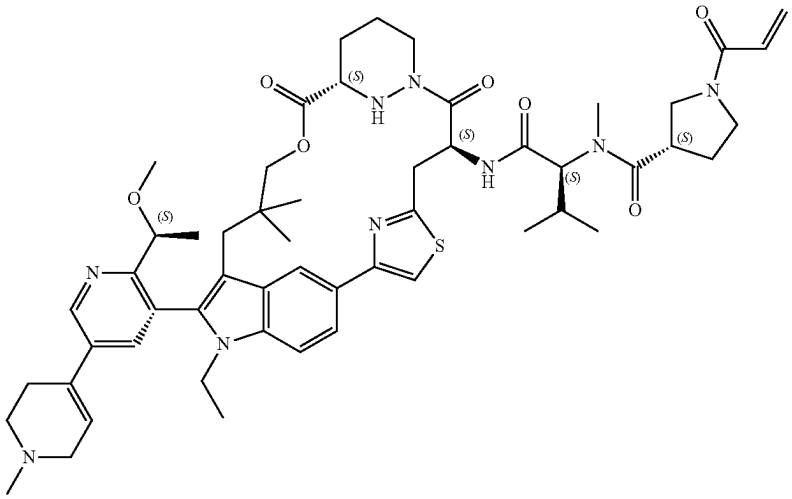
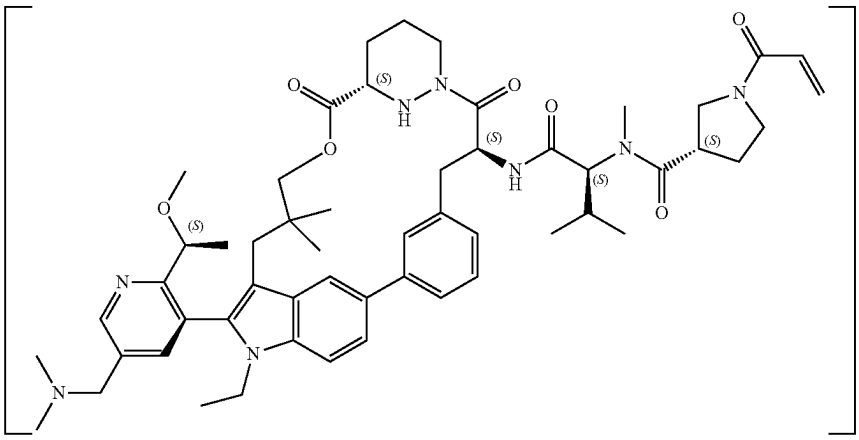
Certain Compounds of the Present Invention	
Ex#	Structure
A520	
A521	
A522	

TABLE 1-continued

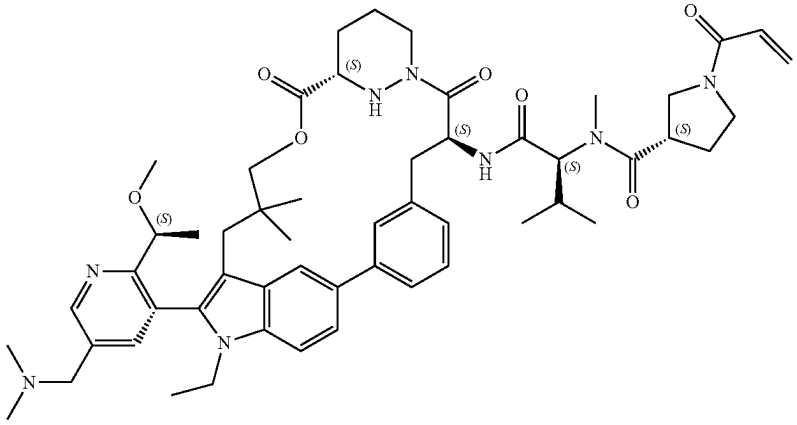
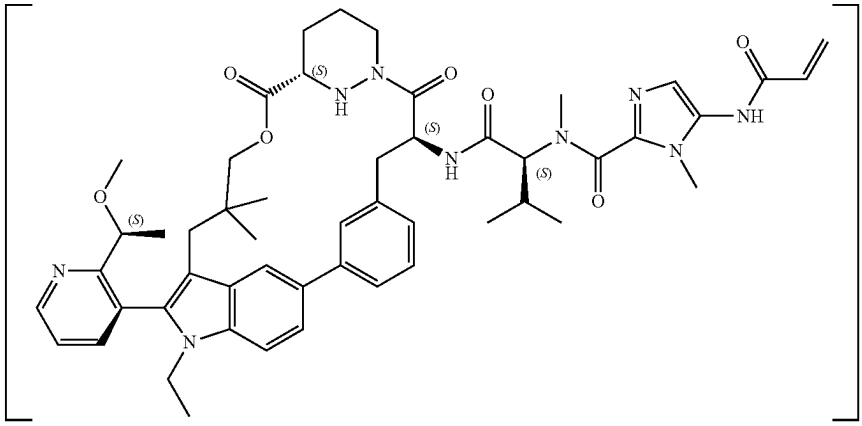
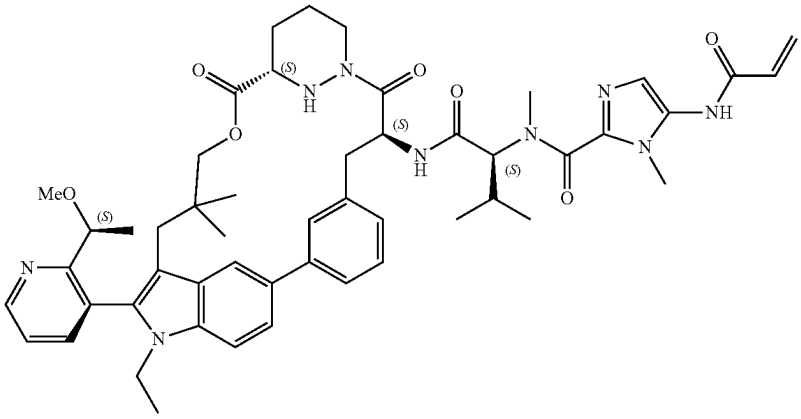
Certain Compounds of the Present Invention	
Ex#	Structure
A523	
A524	
A525	

TABLE 1-continued

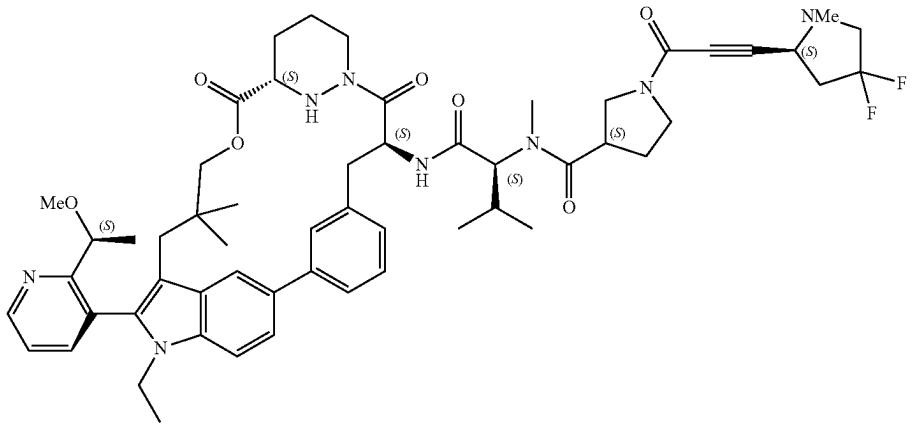
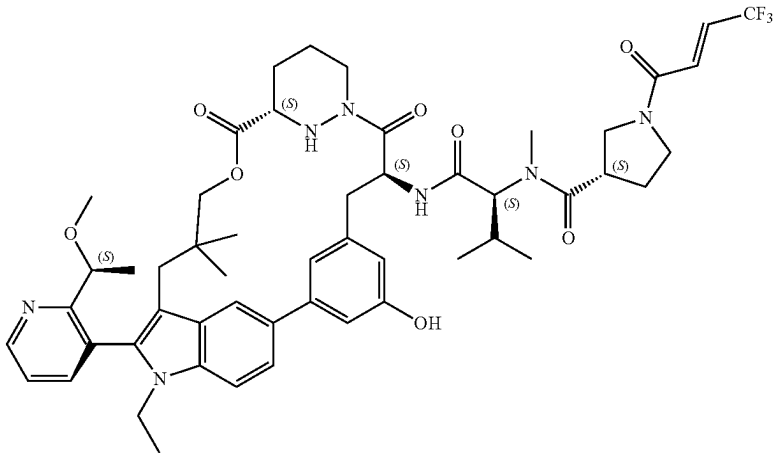
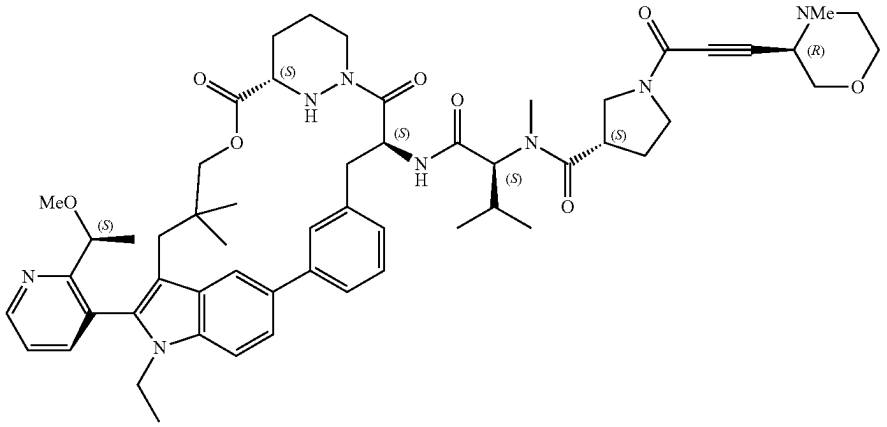
Certain Compounds of the Present Invention	
Ex#	Structure
A526	
A527	
A528	

TABLE 1-continued

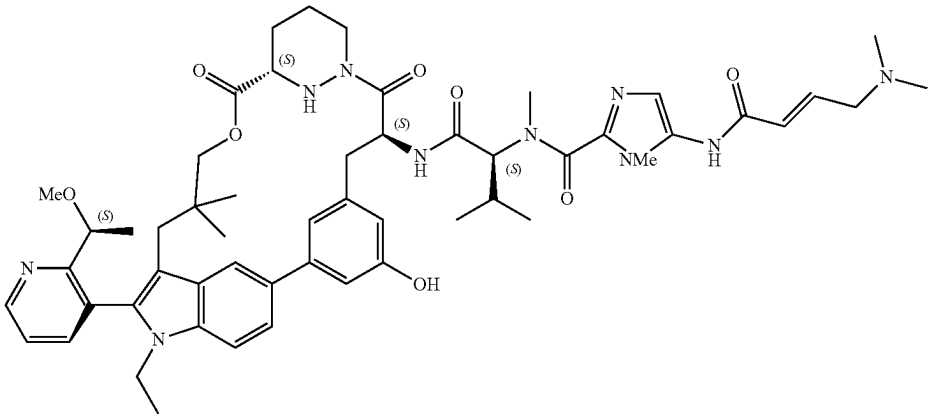
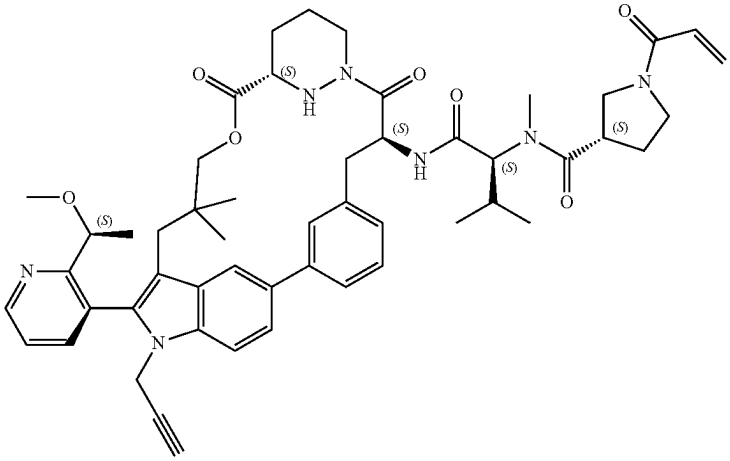
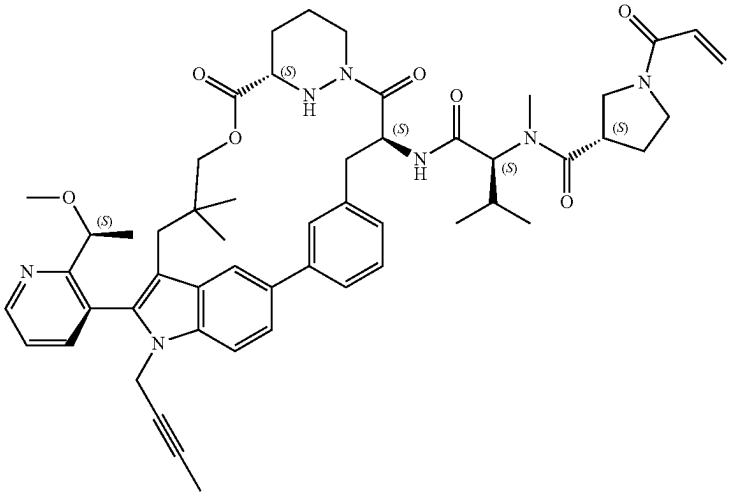
Certain Compounds of the Present Invention	
Ex#	Structure
A529	
A530	
A531	

TABLE 1-continued

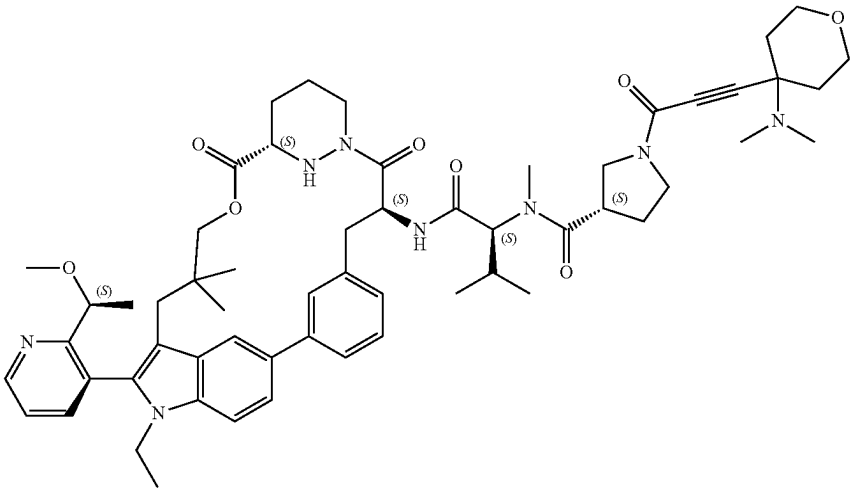
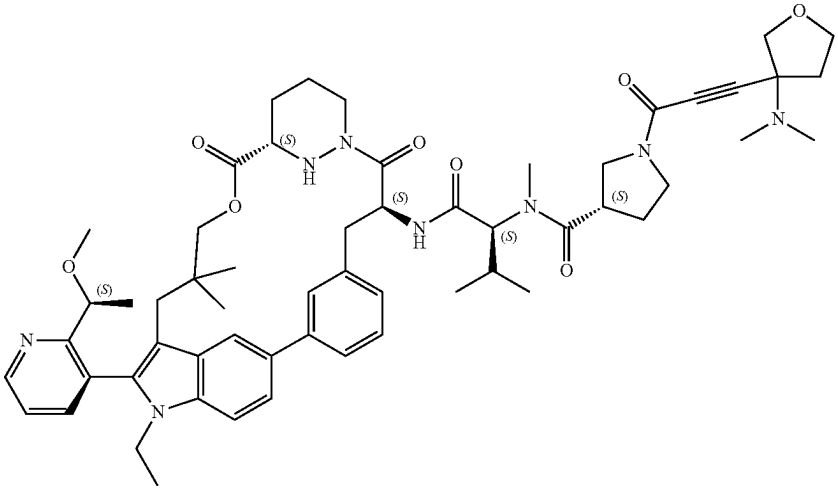
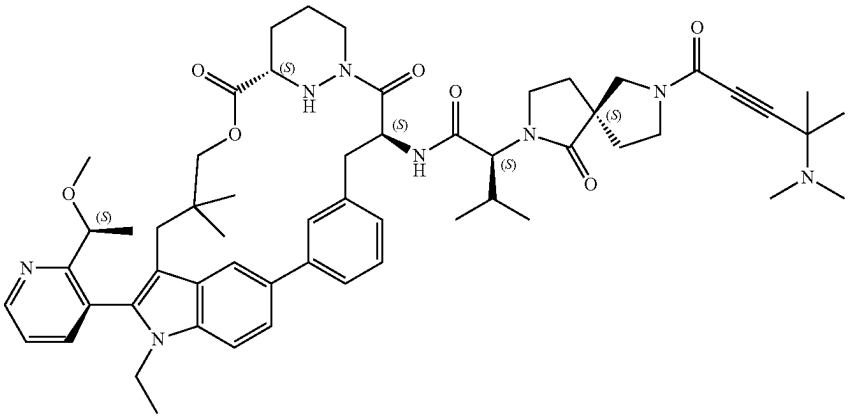
Certain Compounds of the Present Invention	
Ex#	Structure
A532	
A533	
A534	

TABLE 1-continued

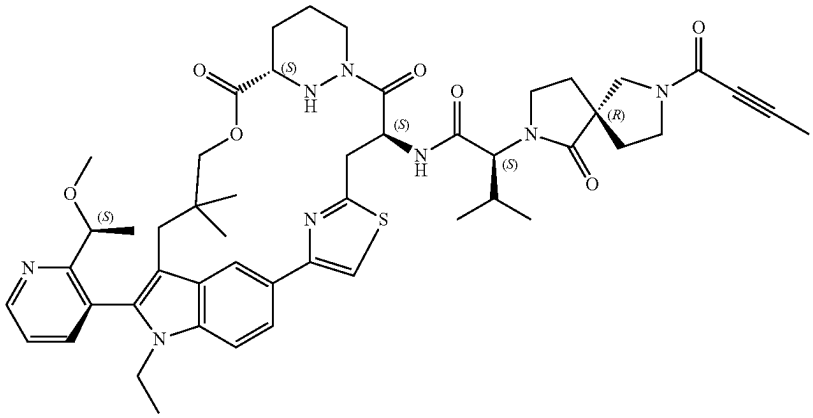
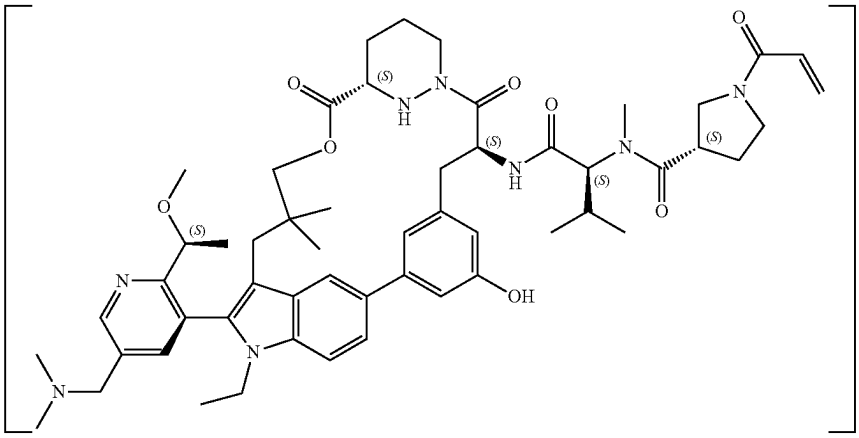
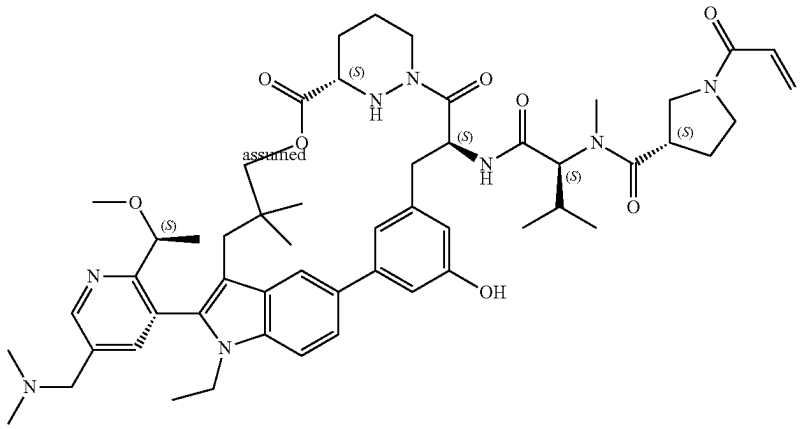
Certain Compounds of the Present Invention	
Ex#	Structure
A535	
A536	
A537	

TABLE 1-continued

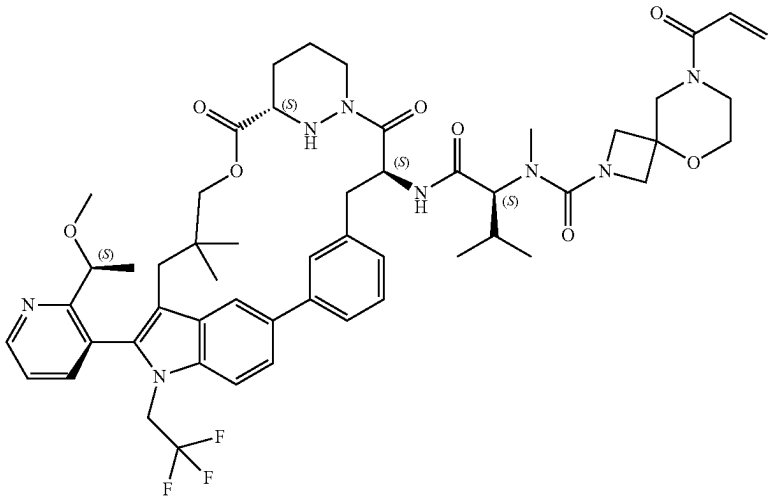
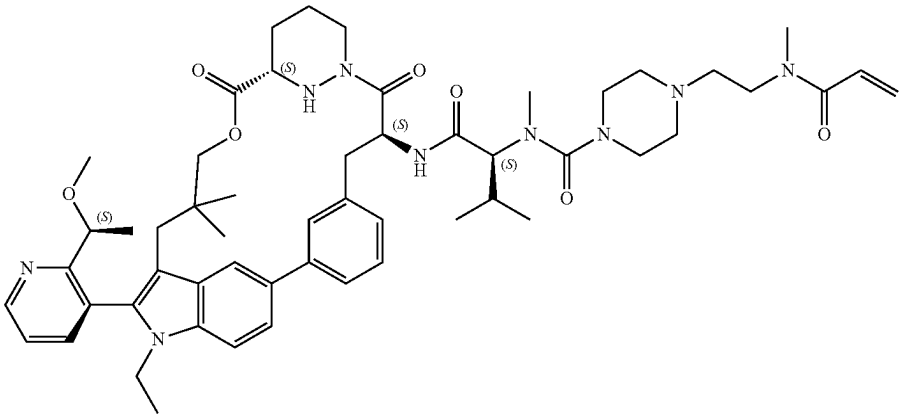
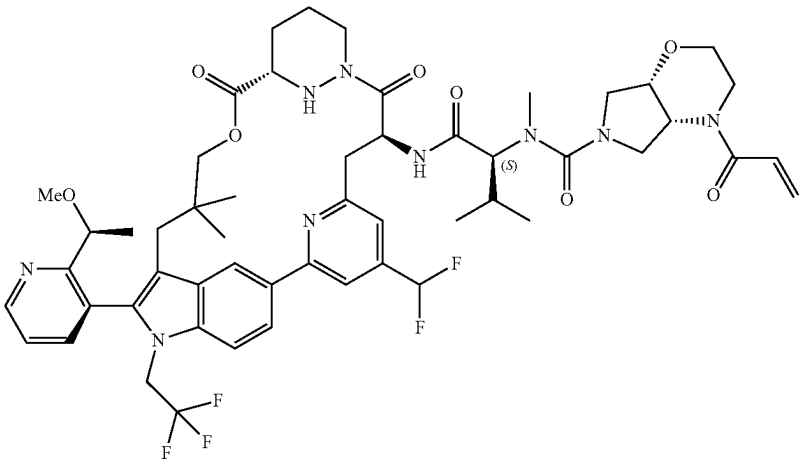
Certain Compounds of the Present Invention	
Ex#	Structure
A538	
A539	
A540	

TABLE 1-continued

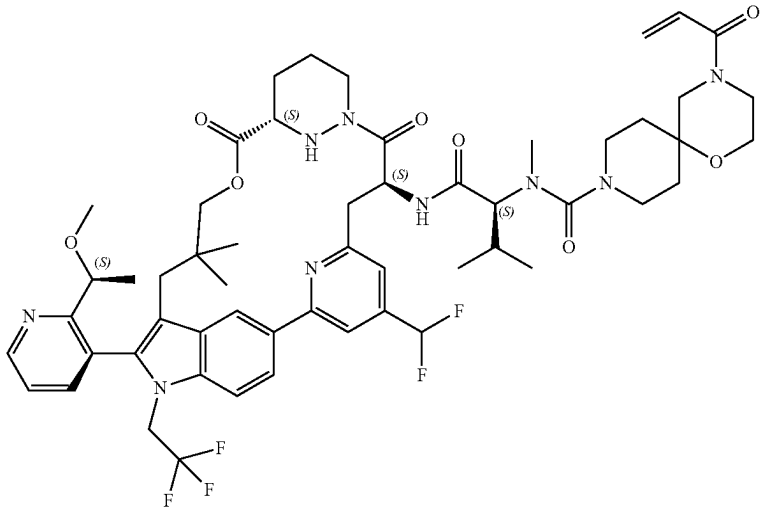
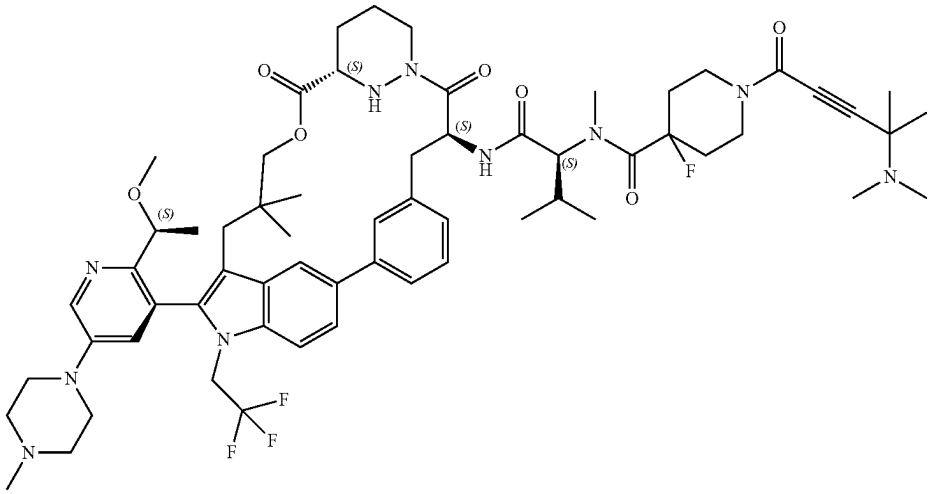
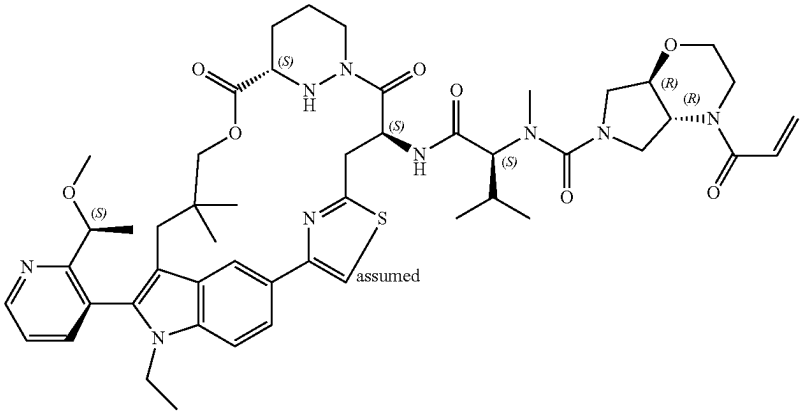
Certain Compounds of the Present Invention	
Ex#	Structure
A541	
A542	
A543	

TABLE 1-continued

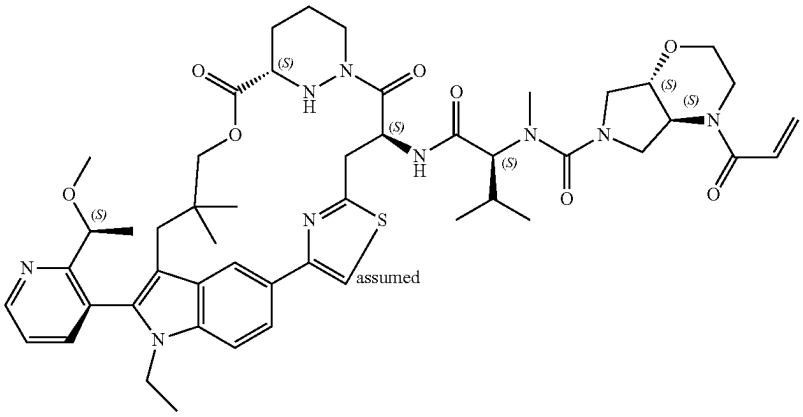
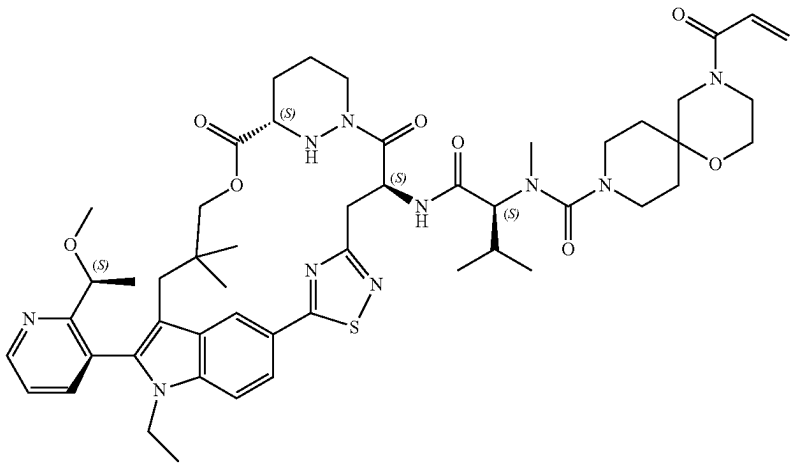
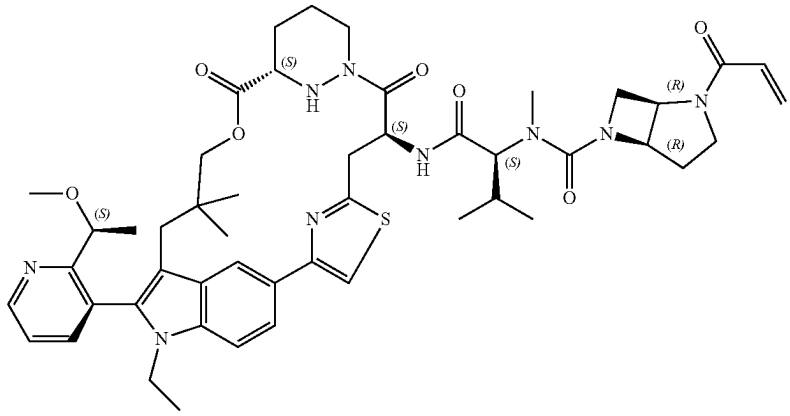
Certain Compounds of the Present Invention	
Ex#	Structure
A544	
A545	
A546	

TABLE 1-continued

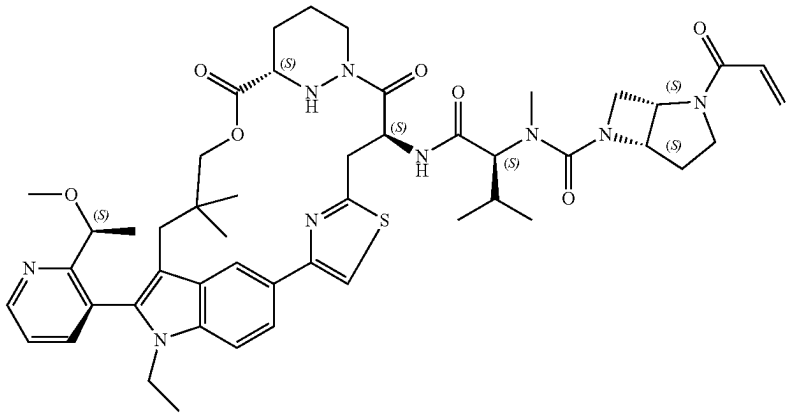
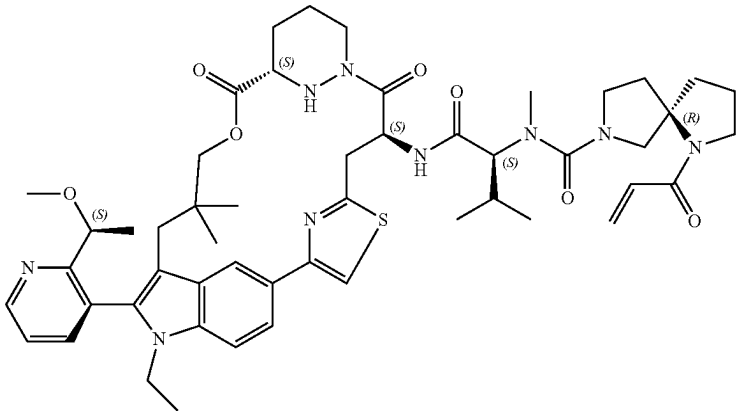
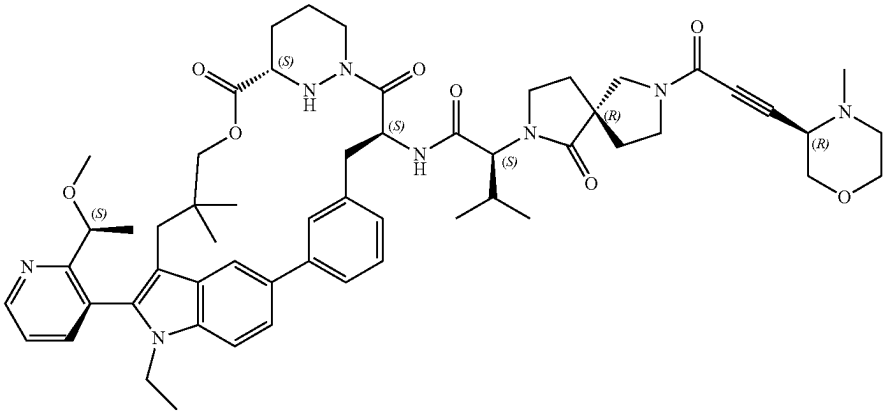
Certain Compounds of the Present Invention	
Ex#	Structure
A547	
A548	
A549	

TABLE 1-continued

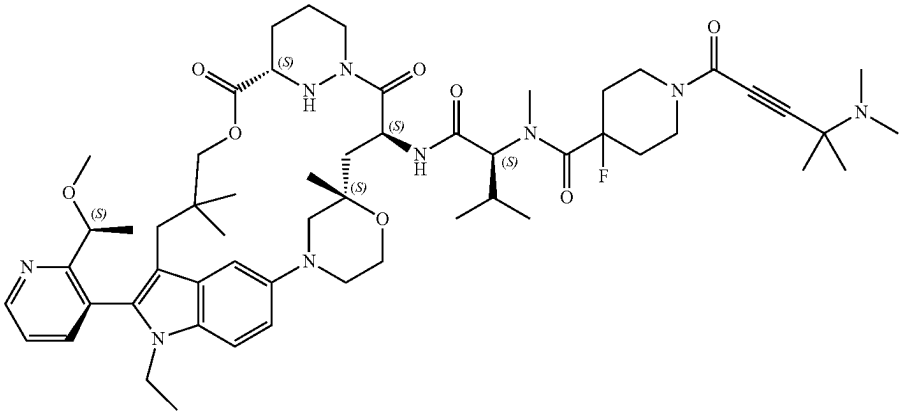
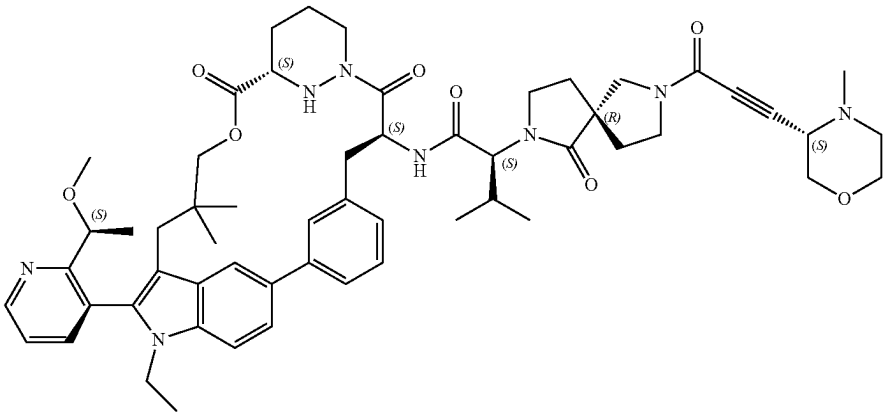
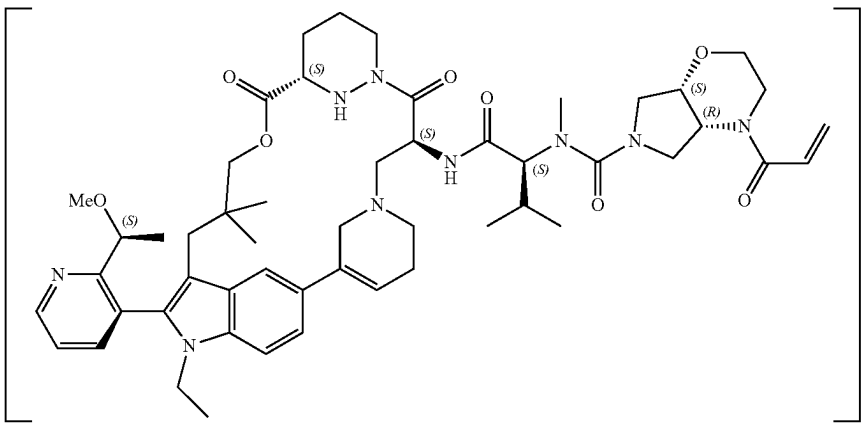
Certain Compounds of the Present Invention	
Ex#	Structure
A550	
A551	
A552	

TABLE 1-continued

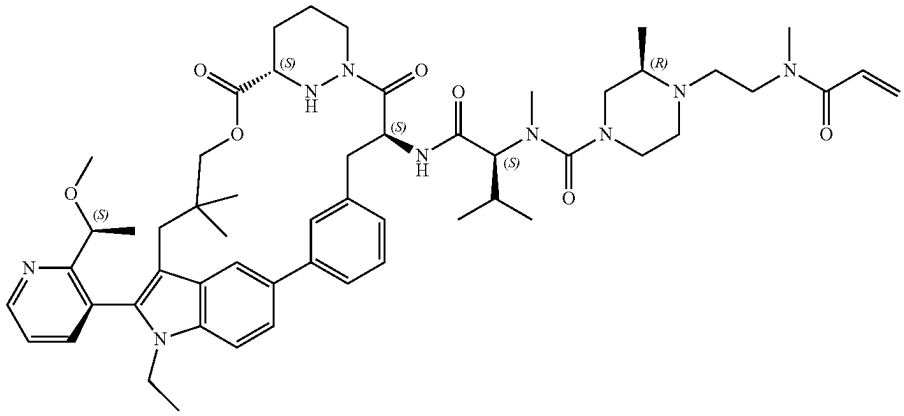
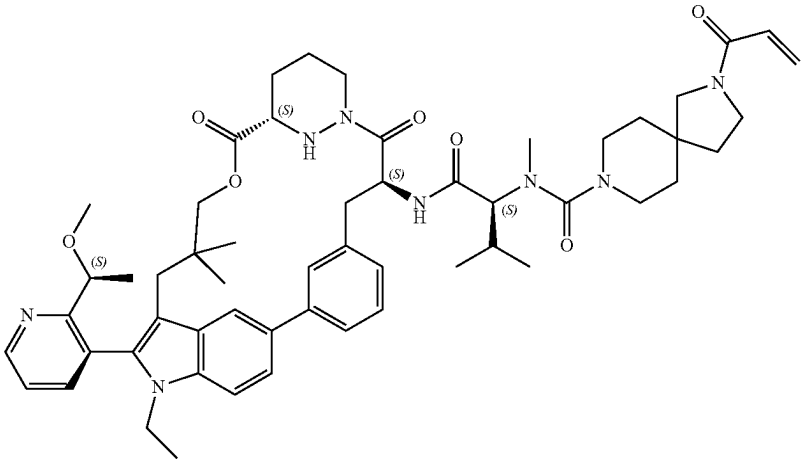
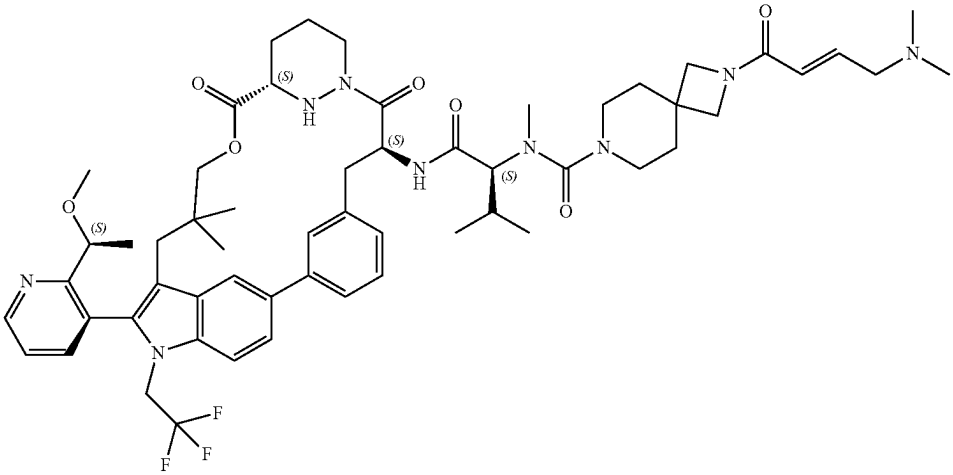
Certain Compounds of the Present Invention	
Ex#	Structure
A553	
A554	
A555	

TABLE 1-continued

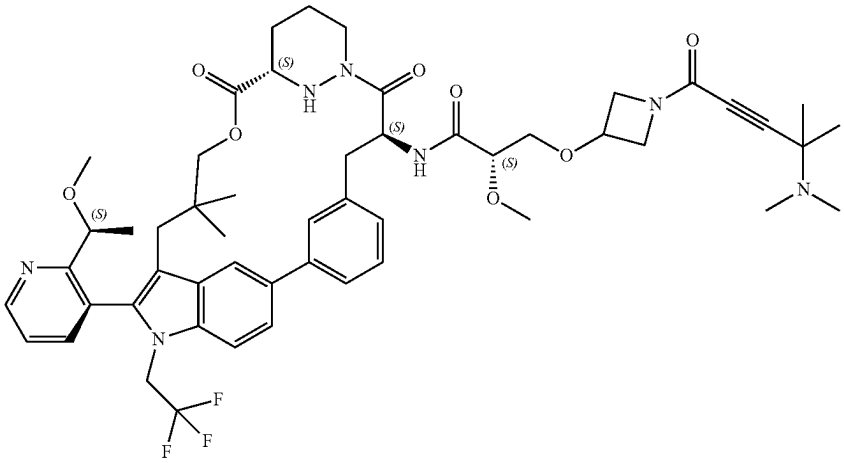
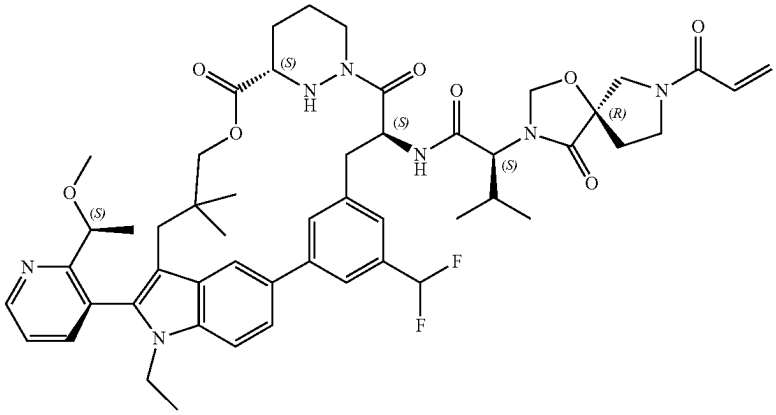
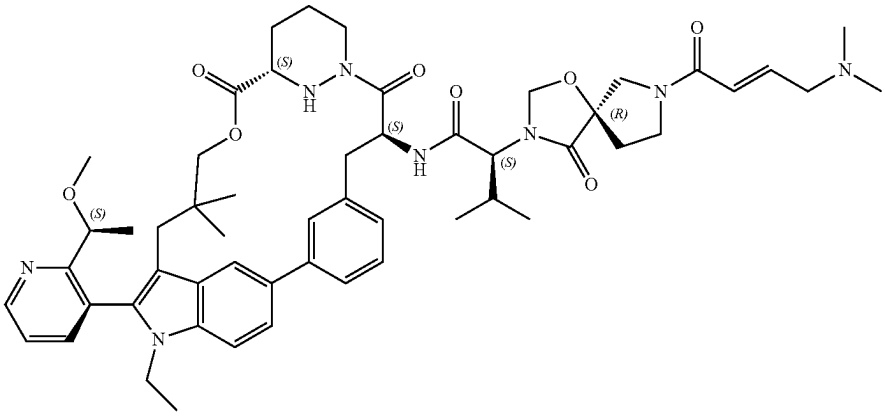
Certain Compounds of the Present Invention	
Ex#	Structure
A556	
A557	
A558	

TABLE 1-continued

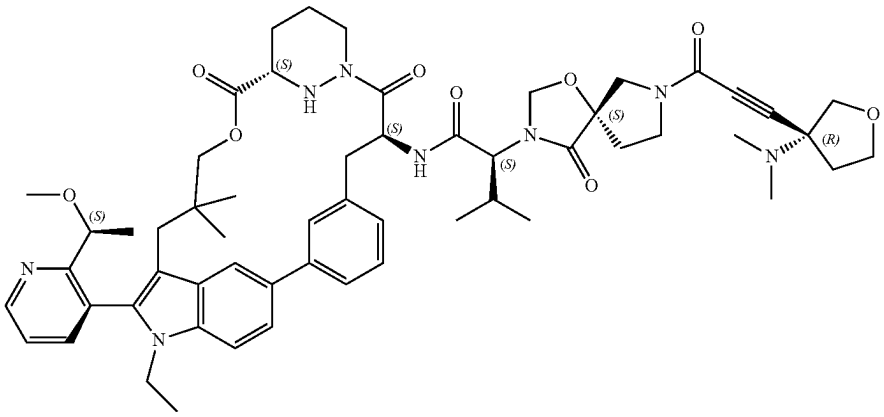
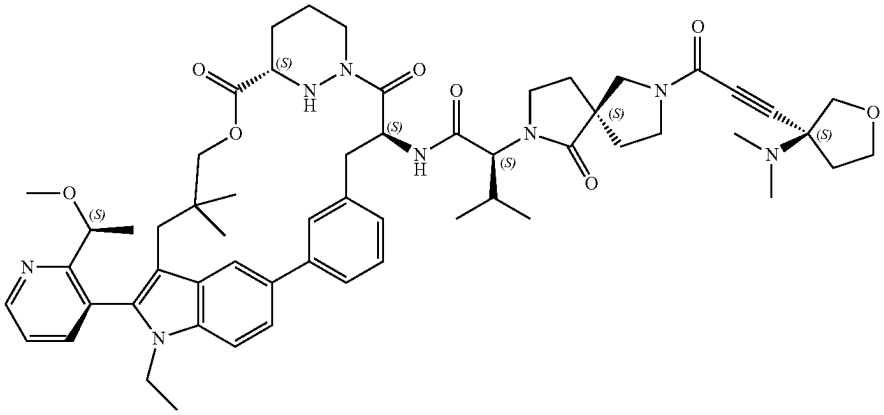
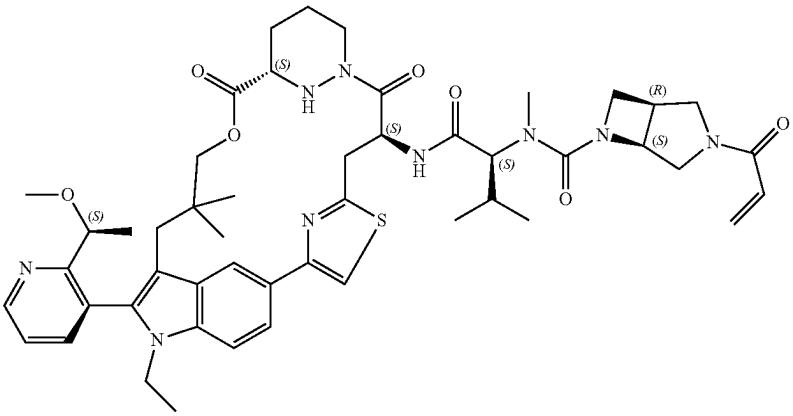
Certain Compounds of the Present Invention	
Ex#	Structure
A559	
A560	
A561	

TABLE 1-continued

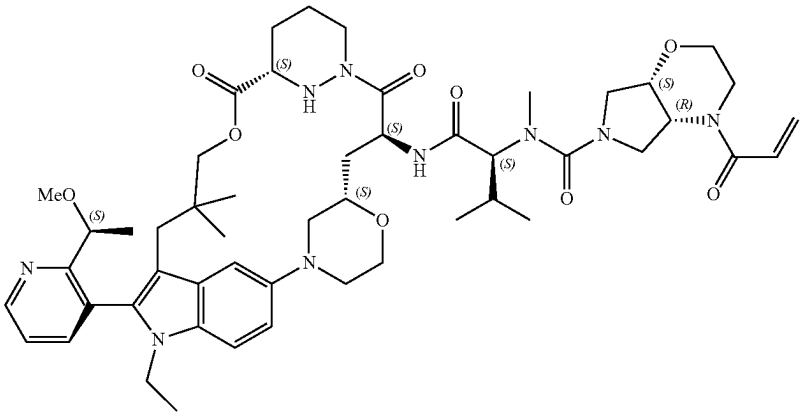
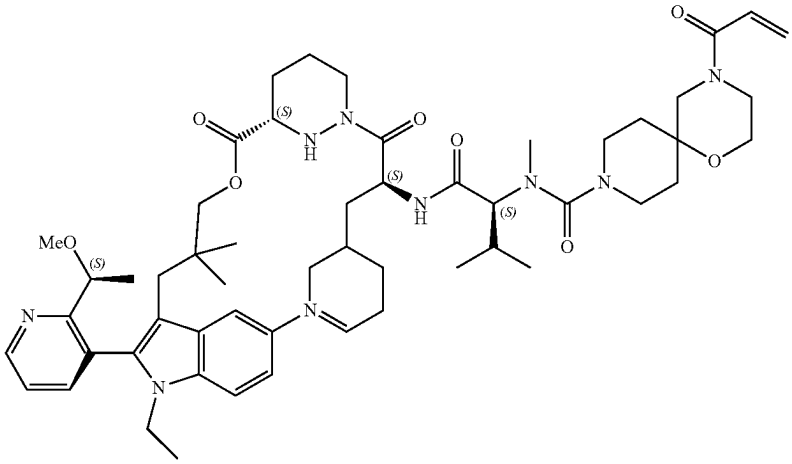
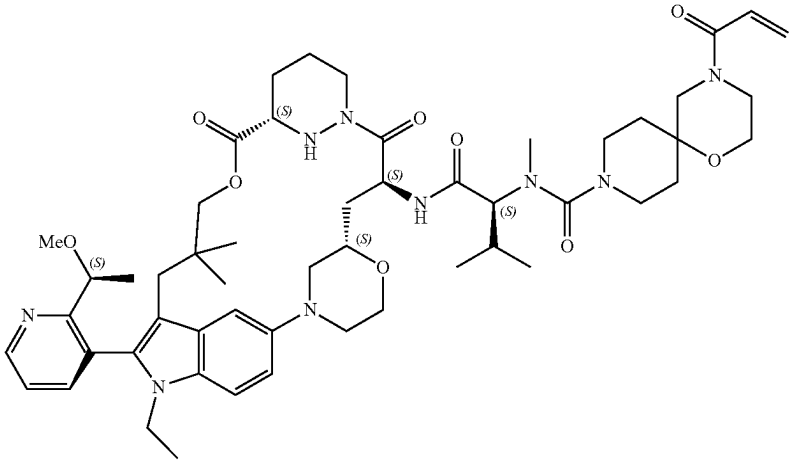
Certain Compounds of the Present Invention	
Ex#	Structure
A562	
A563	
A564	

TABLE 1-continued

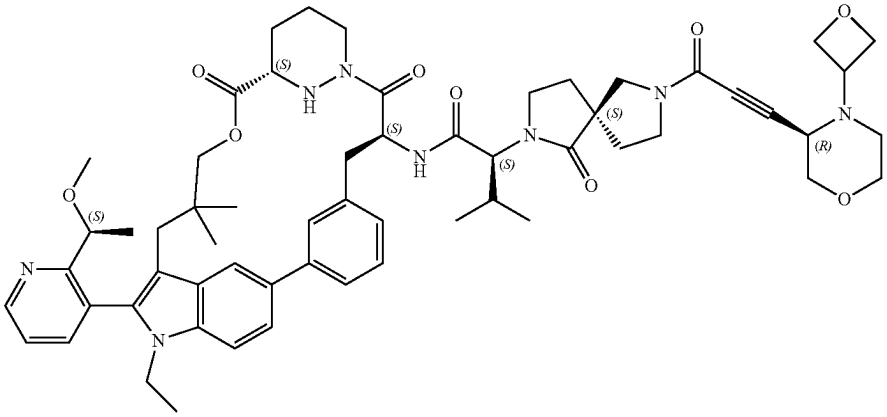
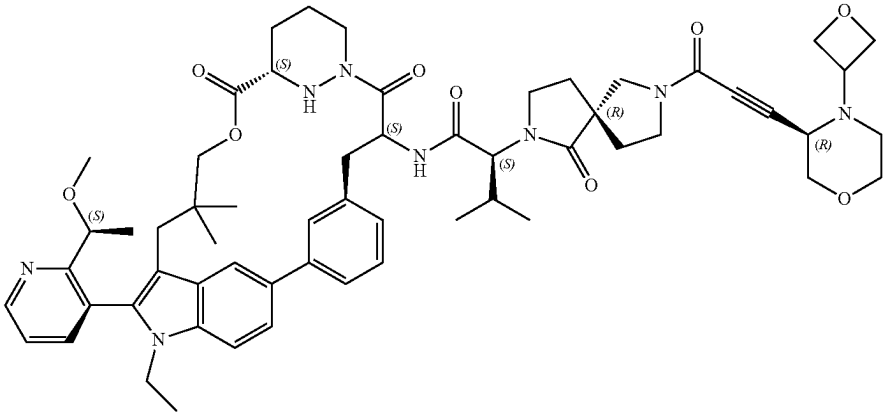
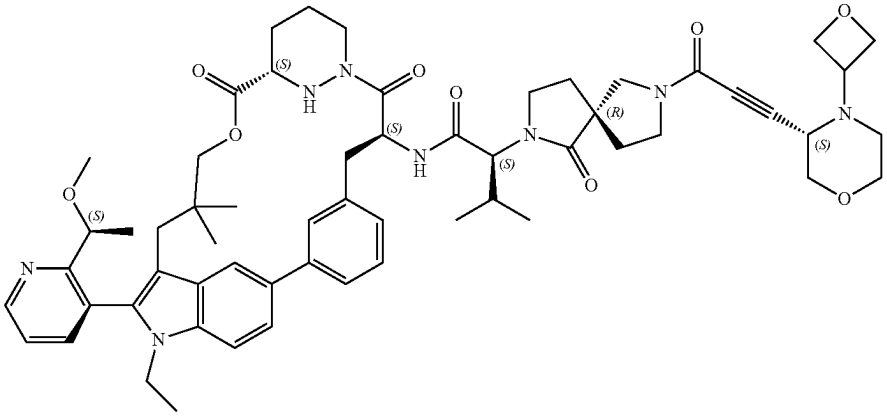
Certain Compounds of the Present Invention	
Ex#	Structure
A565	
A566	
A567	

TABLE 1-continued

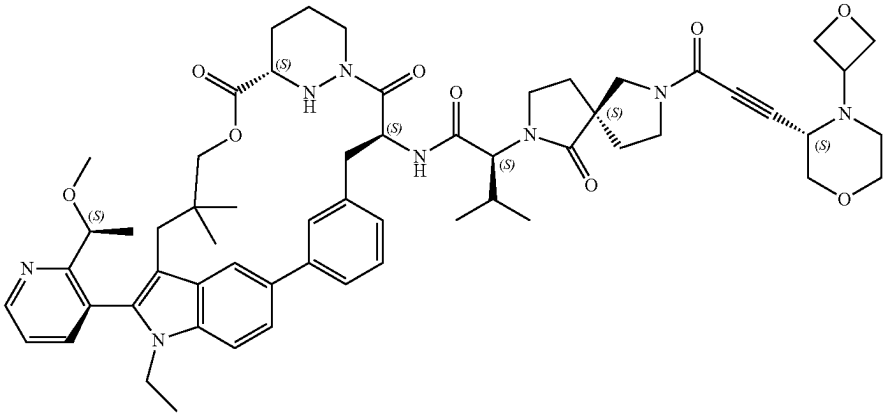
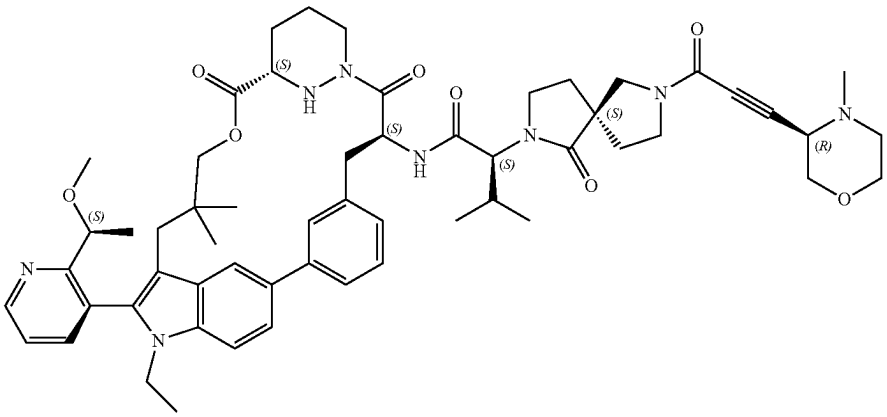
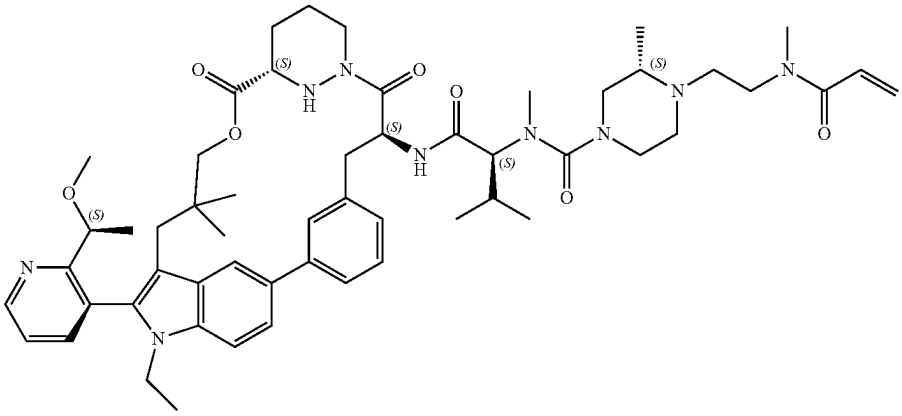
Certain Compounds of the Present Invention	
Ex#	Structure
A568	
A569	
A570	

TABLE 1-continued

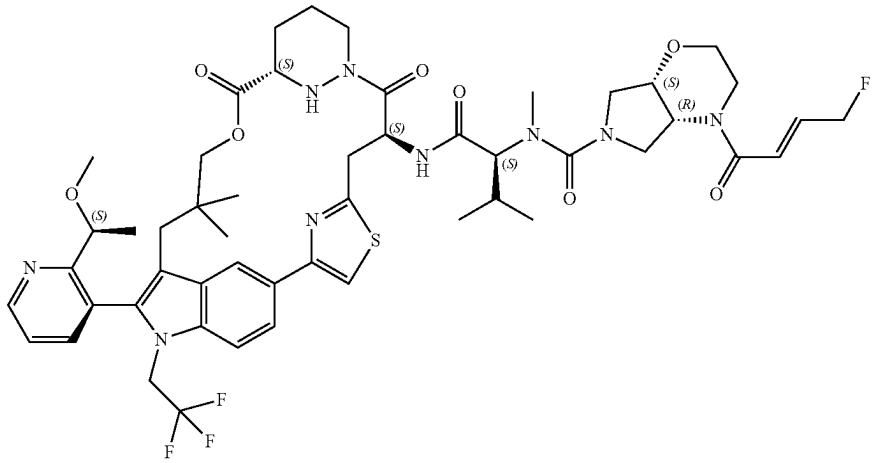
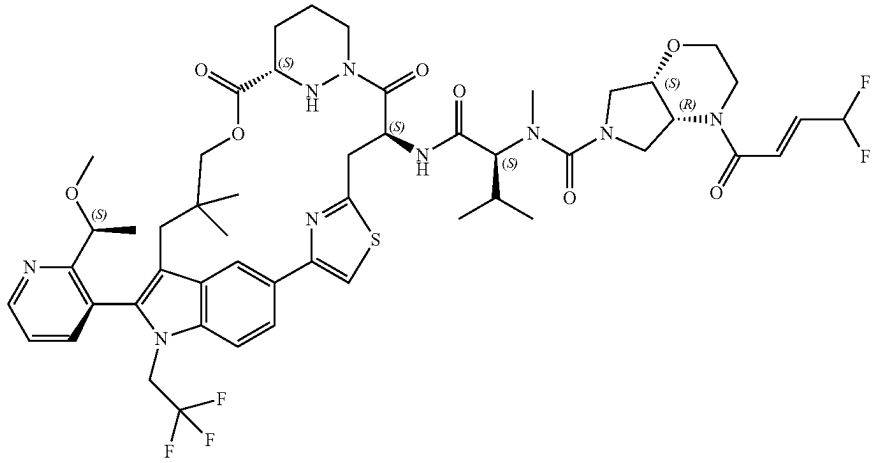
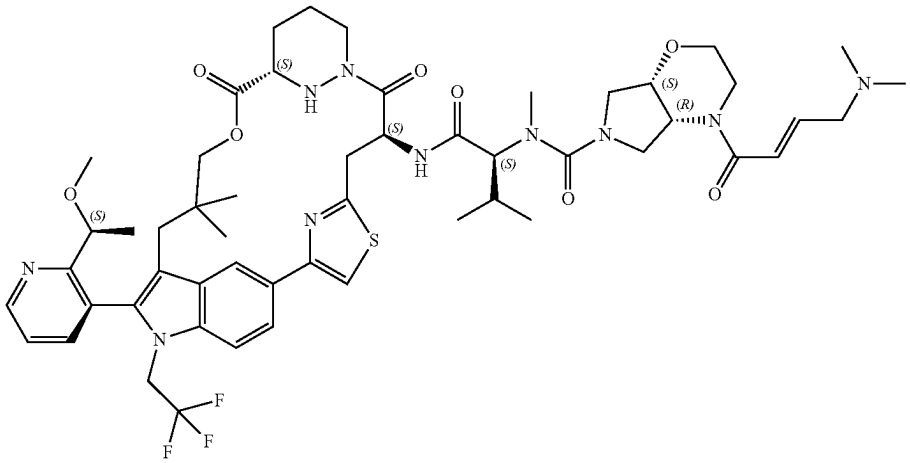
Certain Compounds of the Present Invention	
Ex#	Structure
A571	
A572	
A573	

TABLE 1-continued

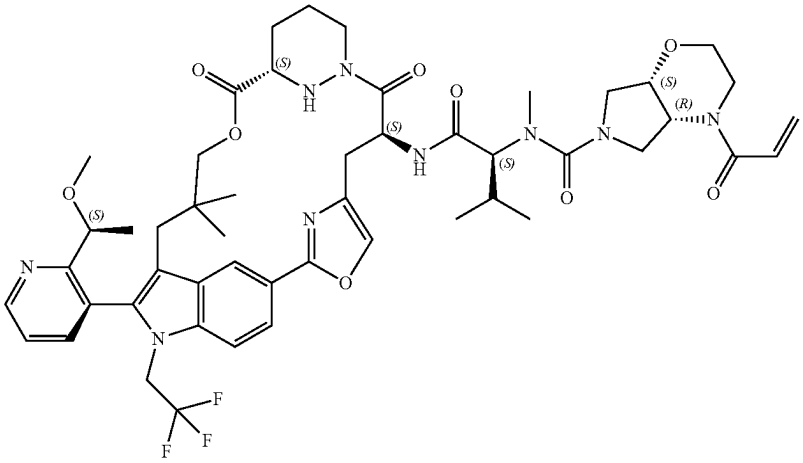
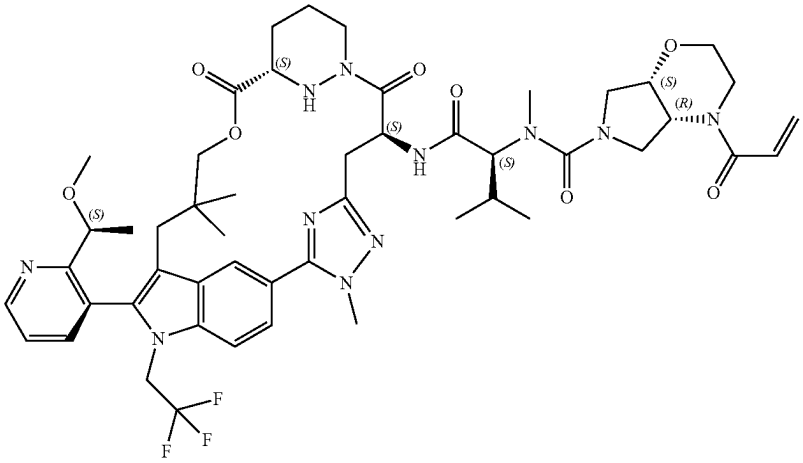
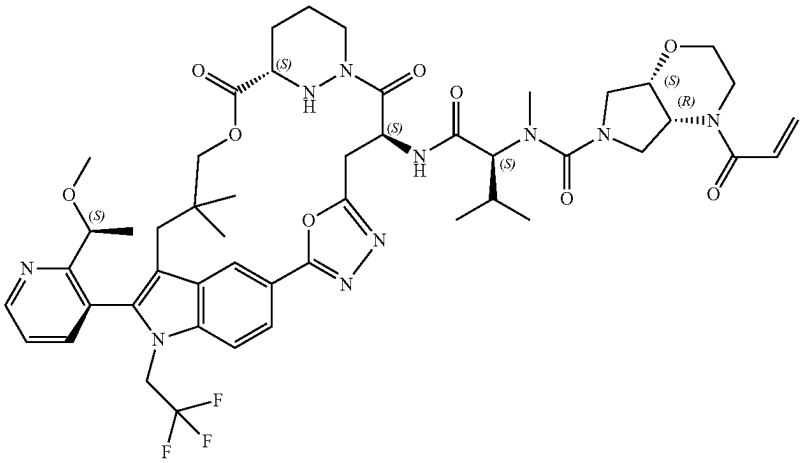
Certain Compounds of the Present Invention	
Ex#	Structure
A574	
A575	
A576	

TABLE 1-continued

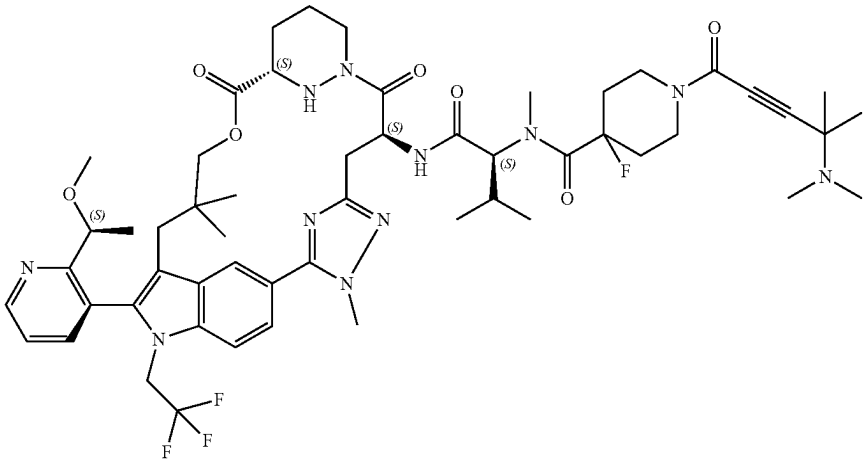
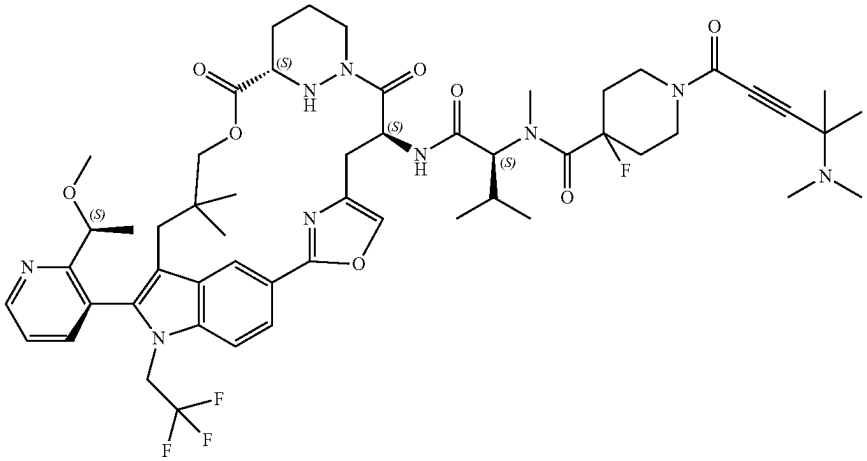
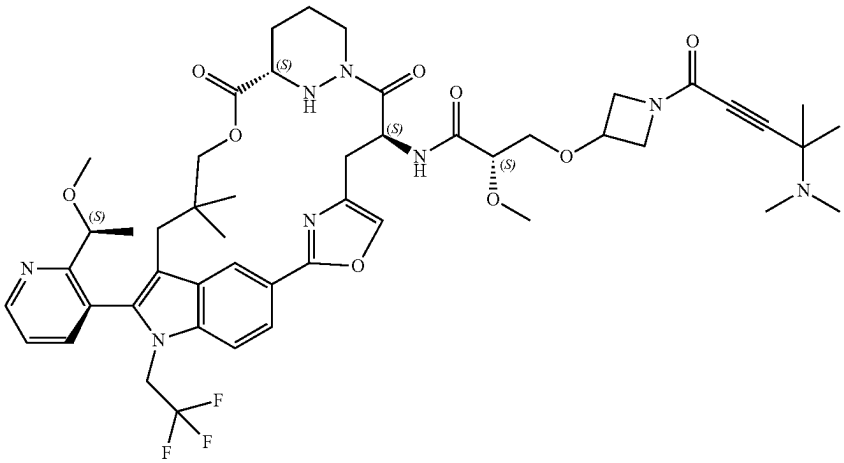
Certain Compounds of the Present Invention	
Ex#	Structure
A577	
A578	
A579	

TABLE 1-continued

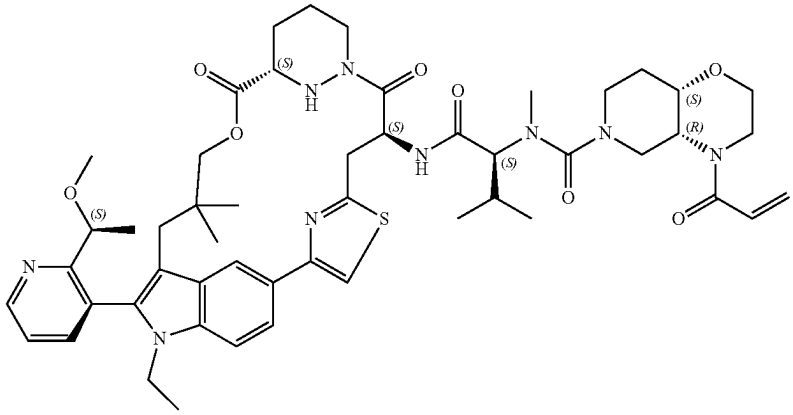
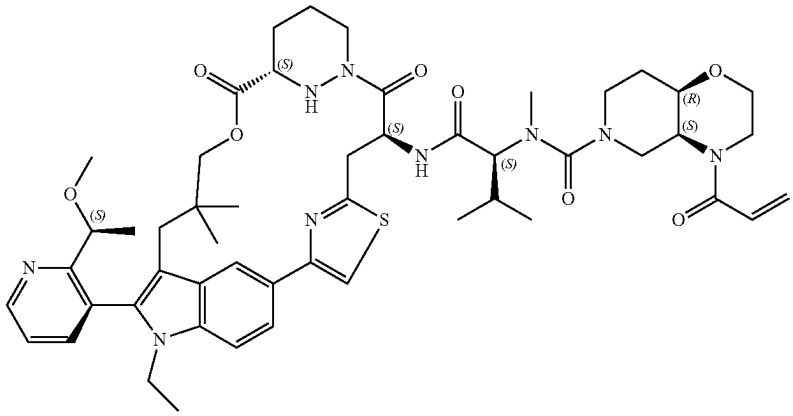
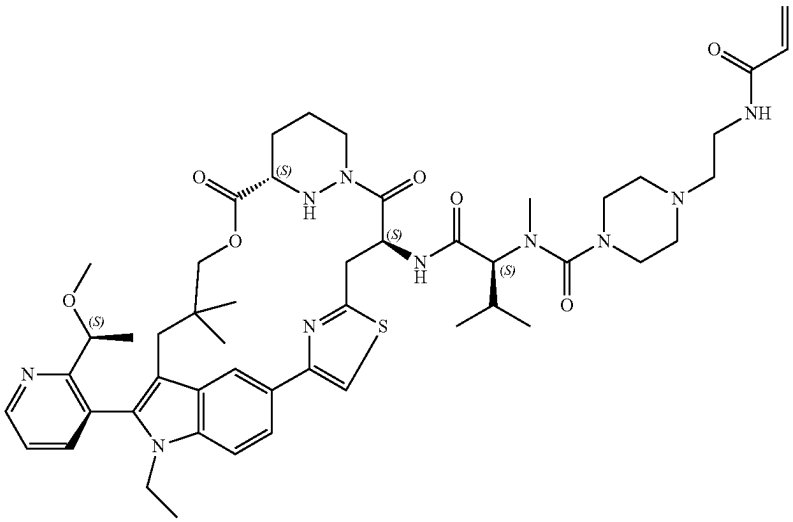
Certain Compounds of the Present Invention	
Ex#	Structure
A580	
A581	
A582	

TABLE 1-continued

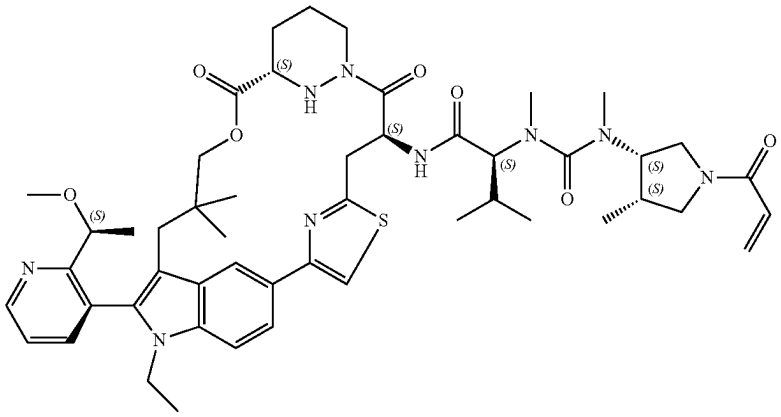
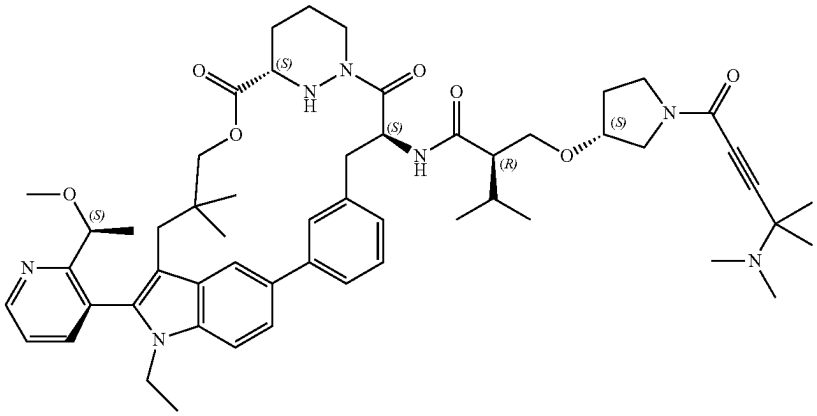
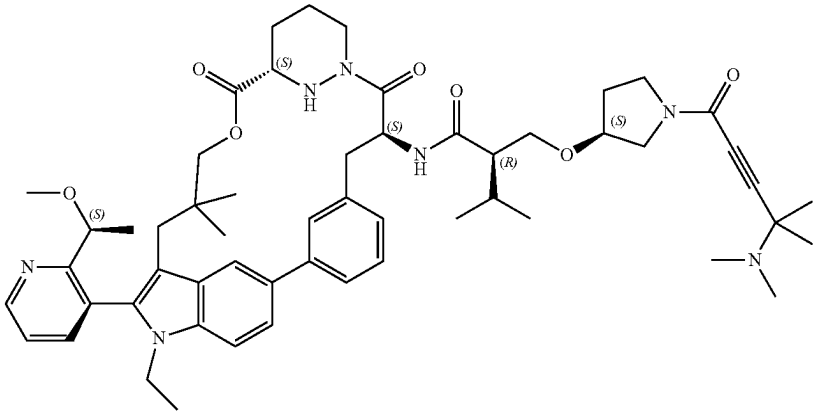
Certain Compounds of the Present Invention	
Ex#	Structure
A583	
A584	
A585	

TABLE 1-continued

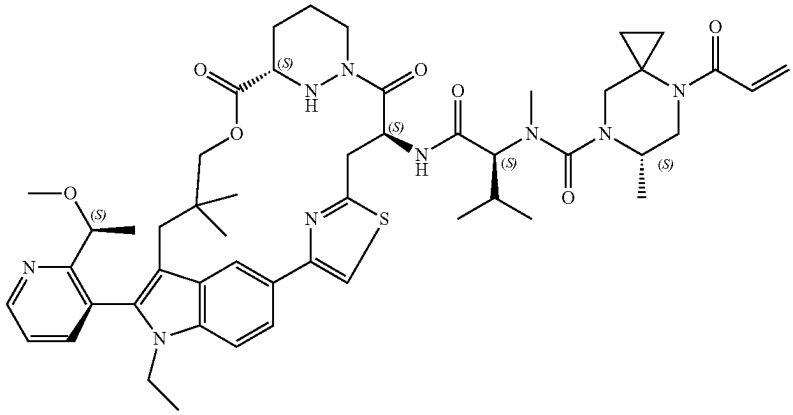
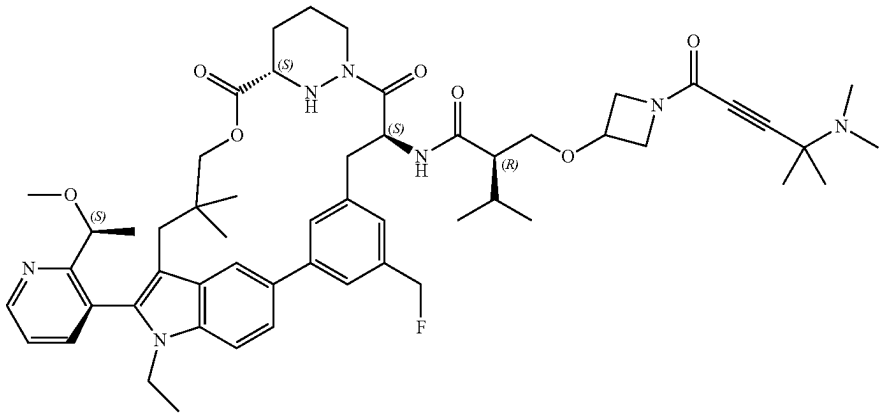
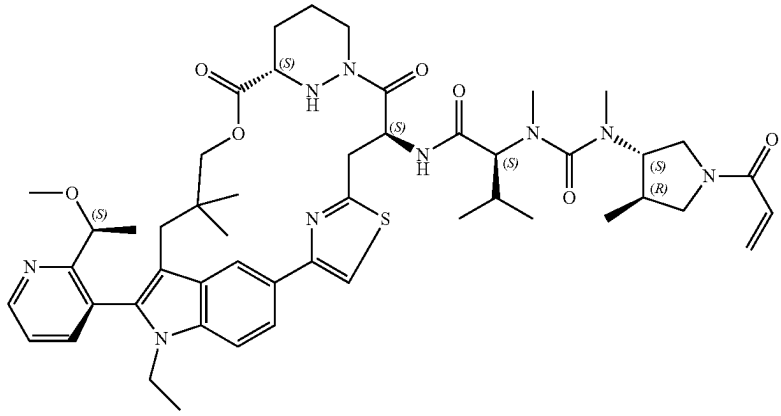
Certain Compounds of the Present Invention	
Ex#	Structure
A586	
A587	
A588	

TABLE 1-continued

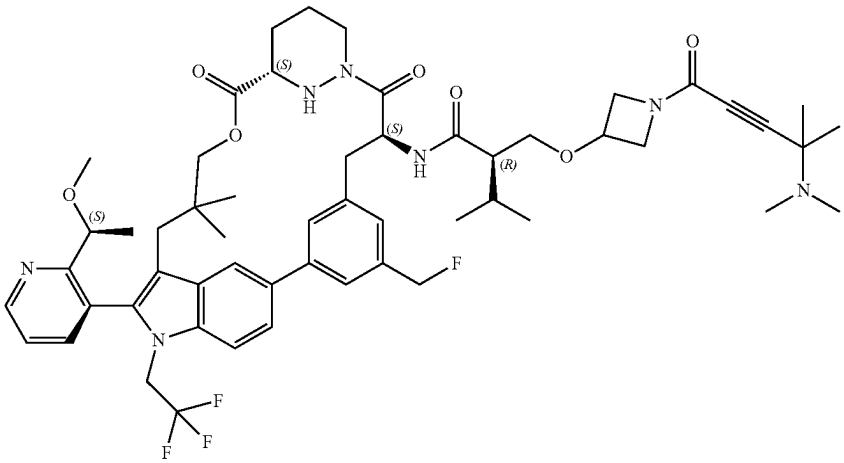
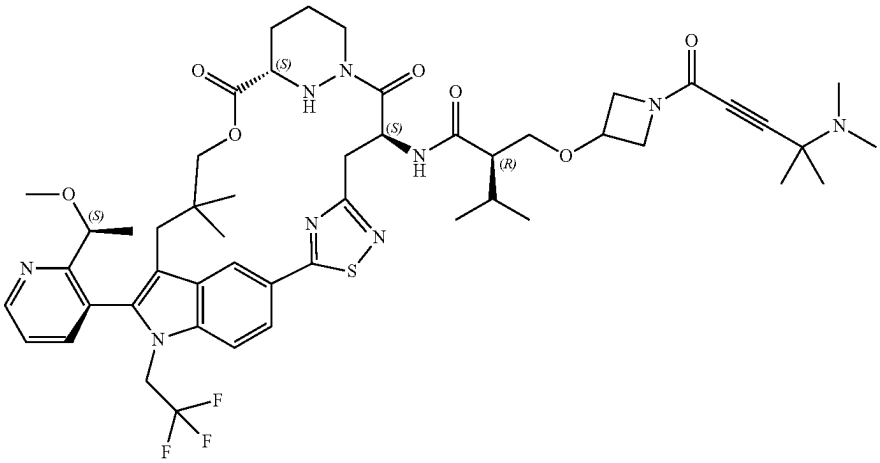
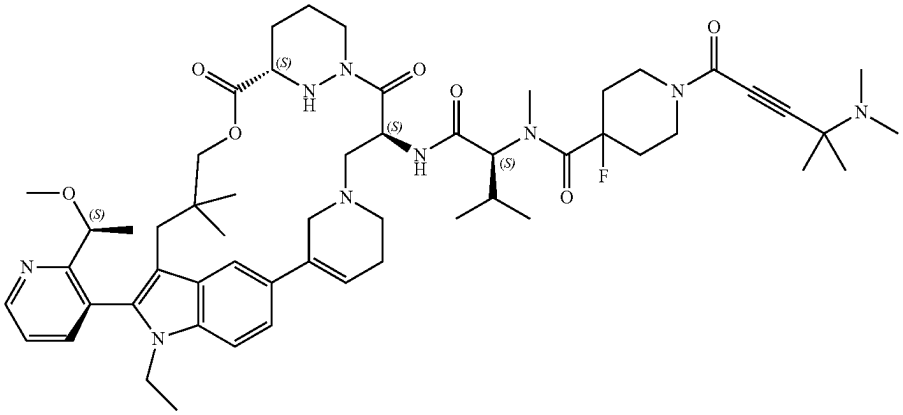
Certain Compounds of the Present Invention	
Ex#	Structure
A589	
A590	
A591	

TABLE 1-continued

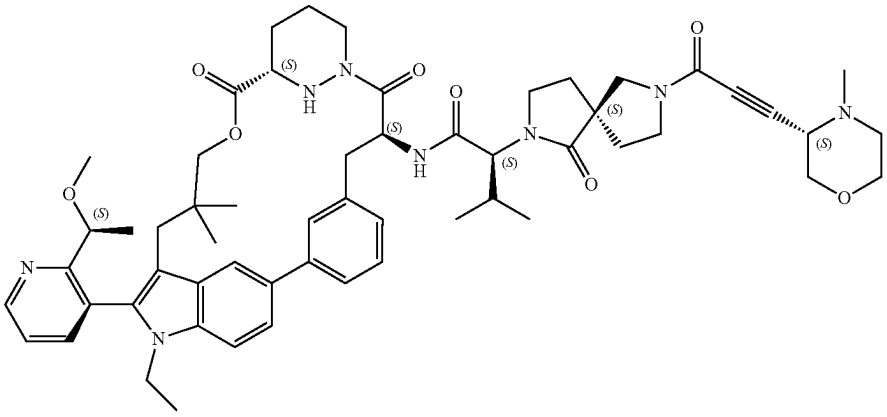
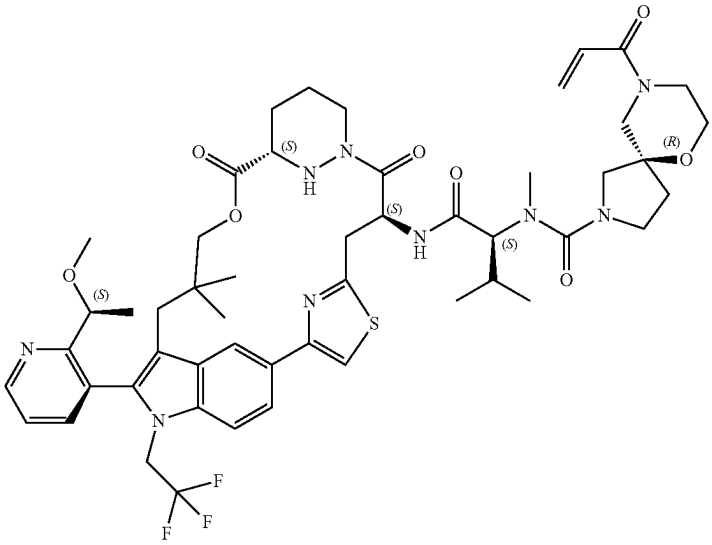
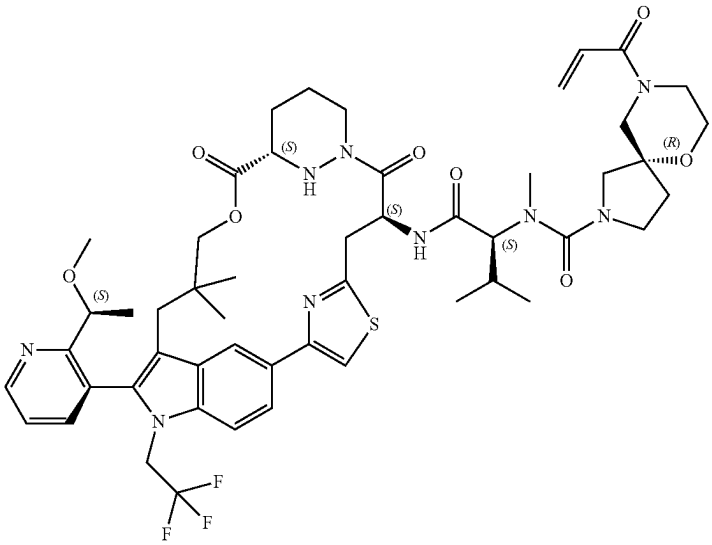
Certain Compounds of the Present Invention	
Ex#	Structure
A592	
A593	
A594	

TABLE 1-continued

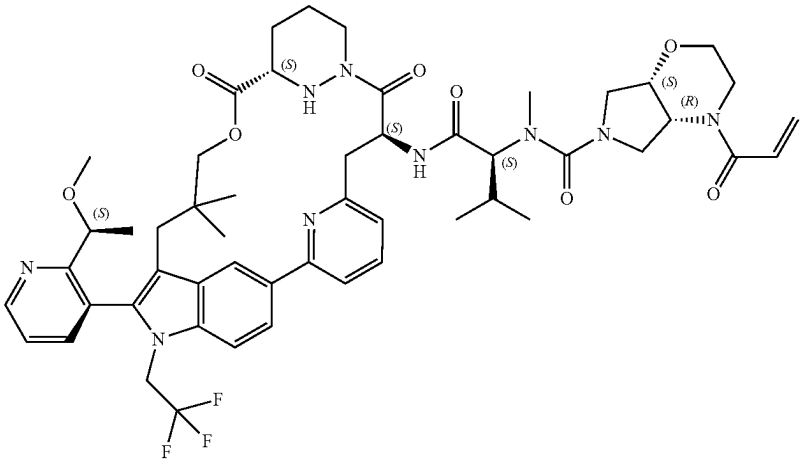
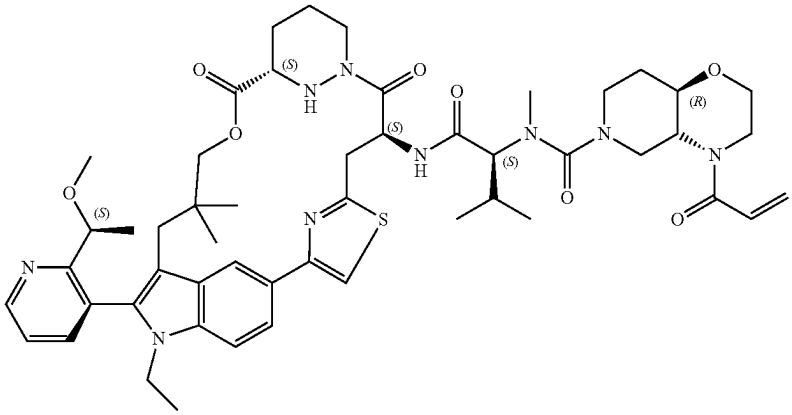
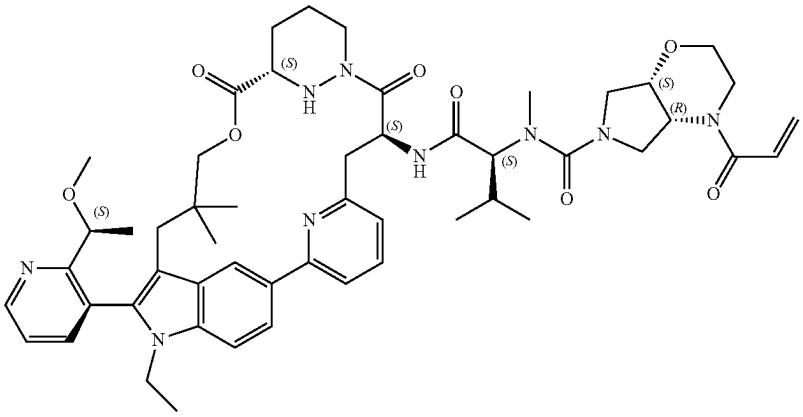
Certain Compounds of the Present Invention	
Ex#	Structure
A595	
A596	
A597	

TABLE 1-continued

Certain Compounds of the Present Invention	
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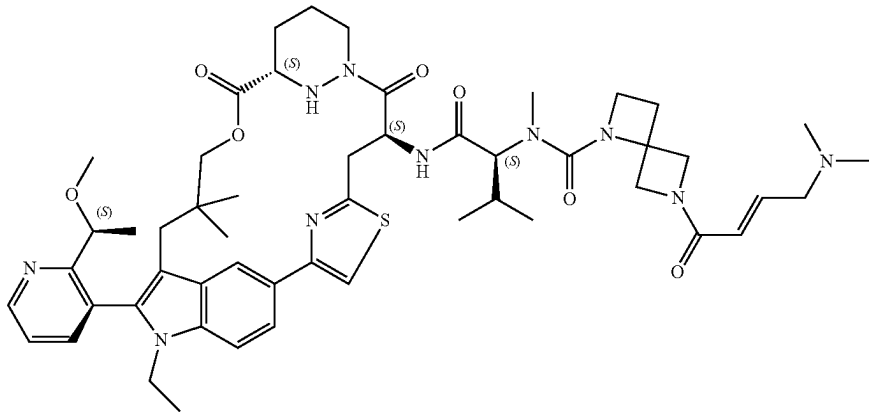
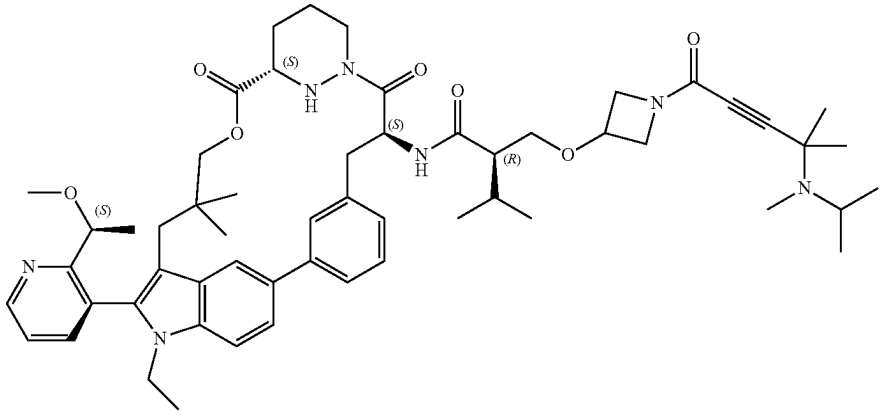
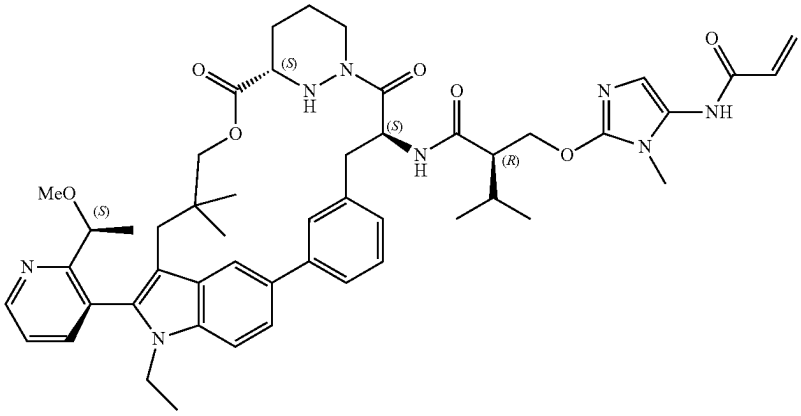
A598	
A599	
A600	

TABLE 1-continued

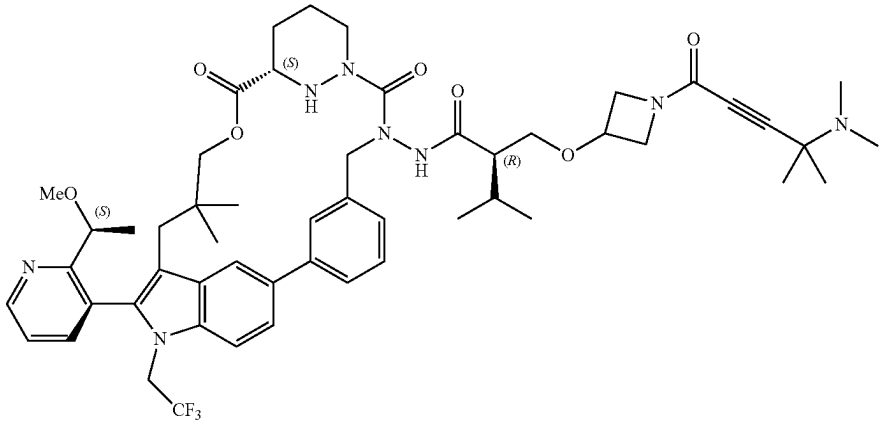
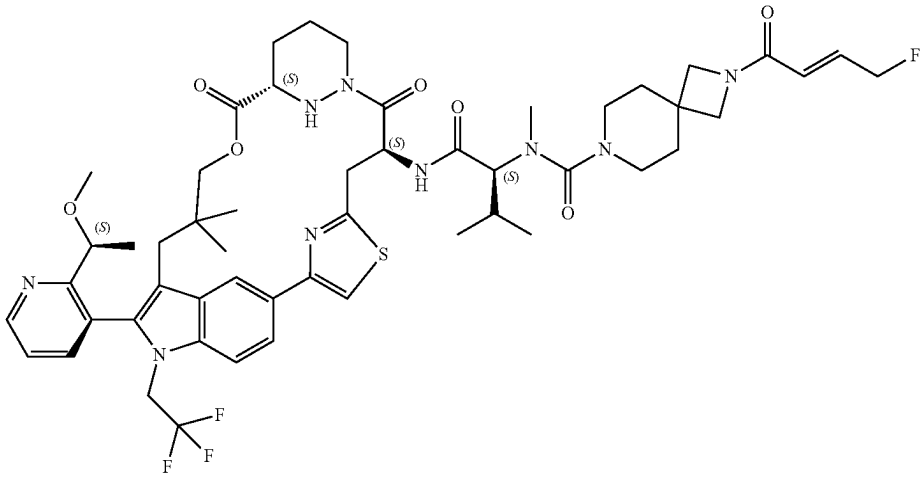
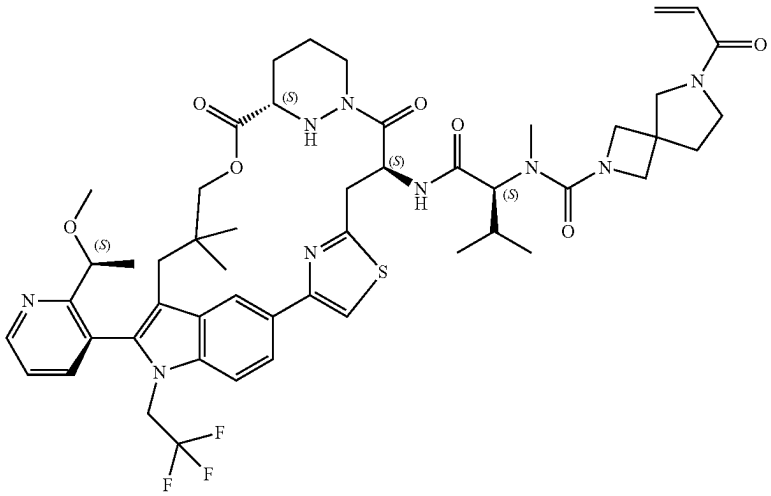
Certain Compounds of the Present Invention	
Ex#	Structure
A601	
A602	
A603	

TABLE 1-continued

Certain Compounds of the Present Invention	
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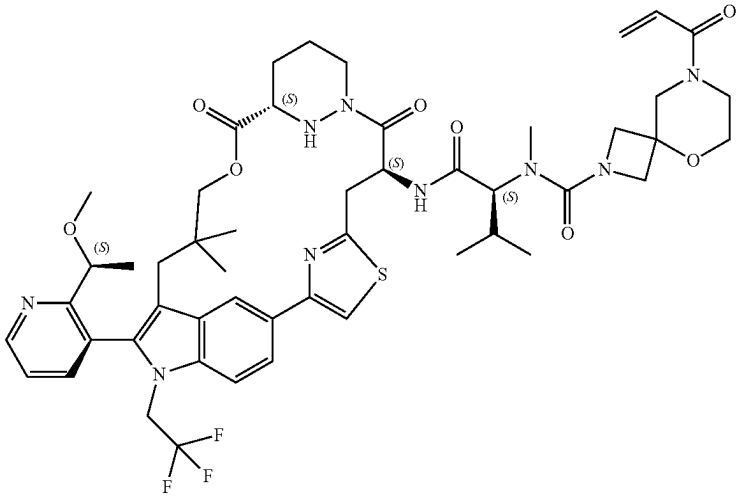
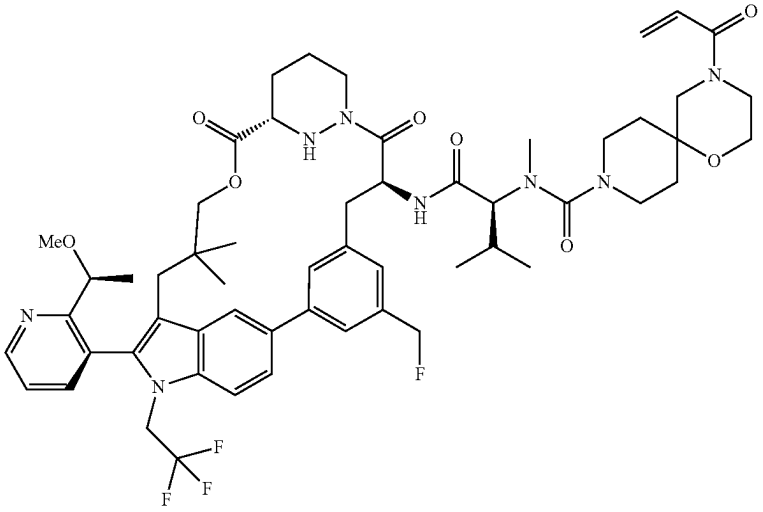
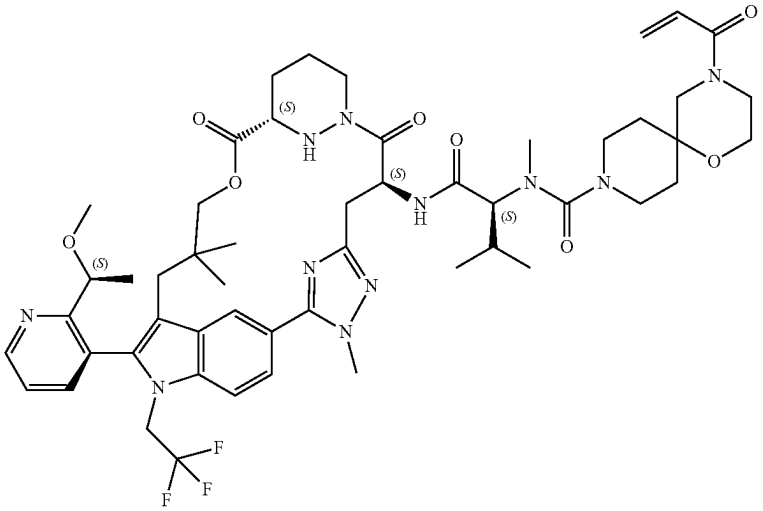
A604	
A605	
A606	

TABLE 1-continued

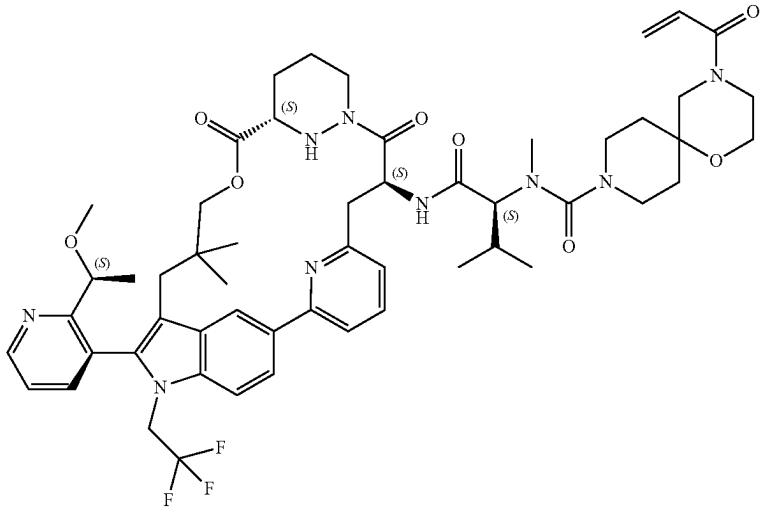
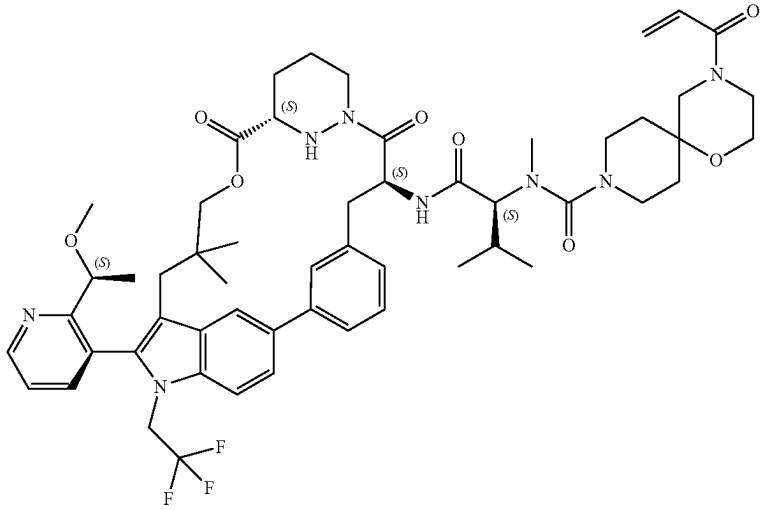
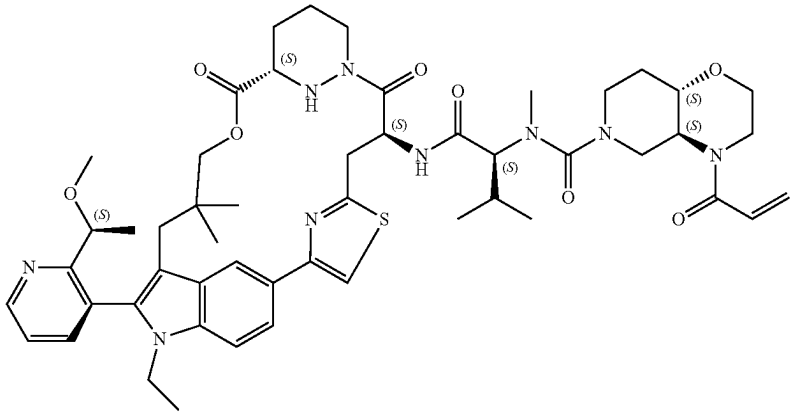
Certain Compounds of the Present Invention	
Ex#	Structure
A607	
A608	
A609	

TABLE 1-continued

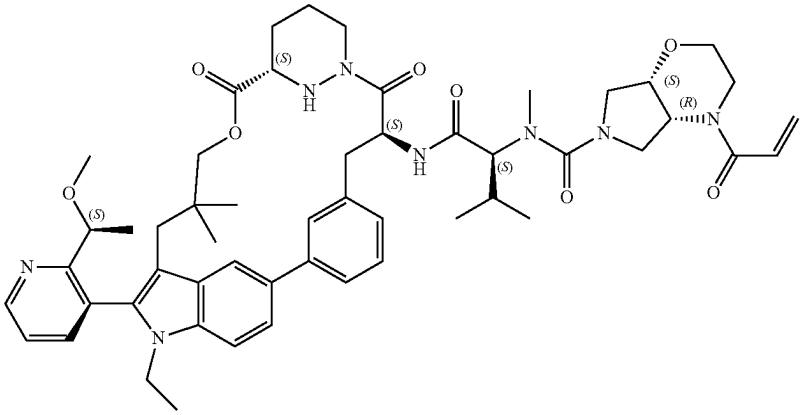
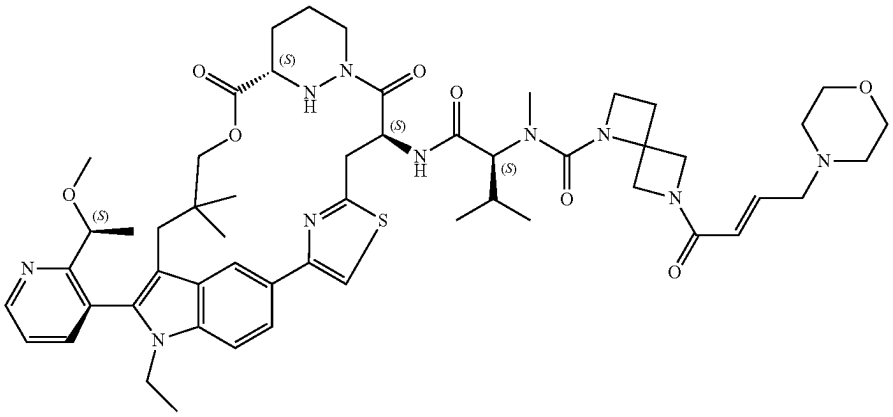
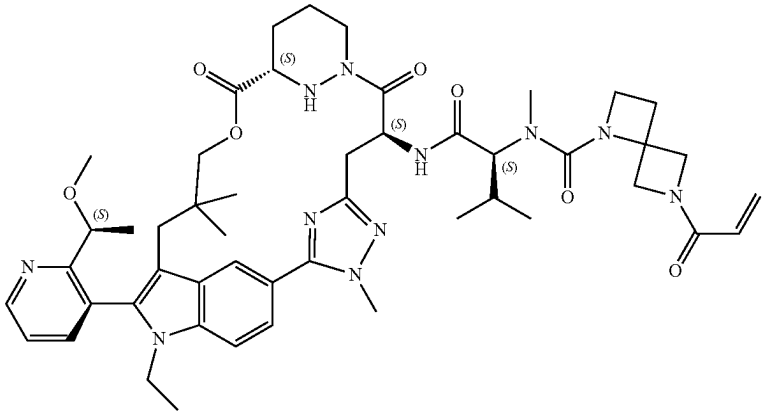
Certain Compounds of the Present Invention	
Ex#	Structure
A610	
A611	
A612	

TABLE 1-continued

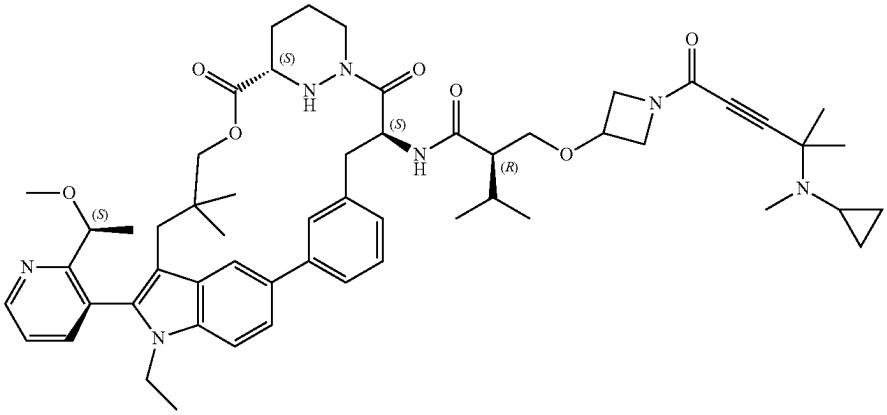
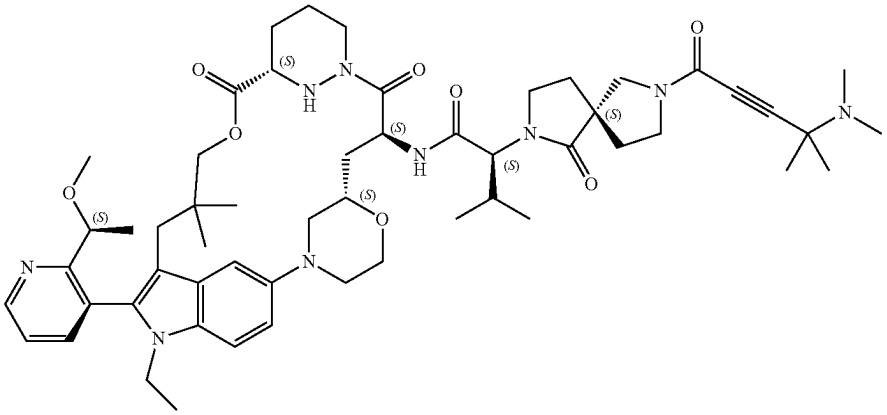
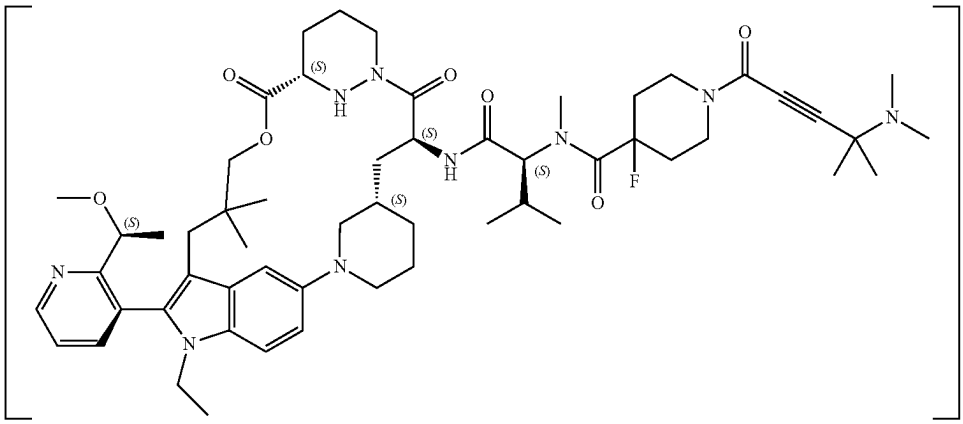
Certain Compounds of the Present Invention	
Ex#	Structure
A613	
A614	
A615	

TABLE 1-continued

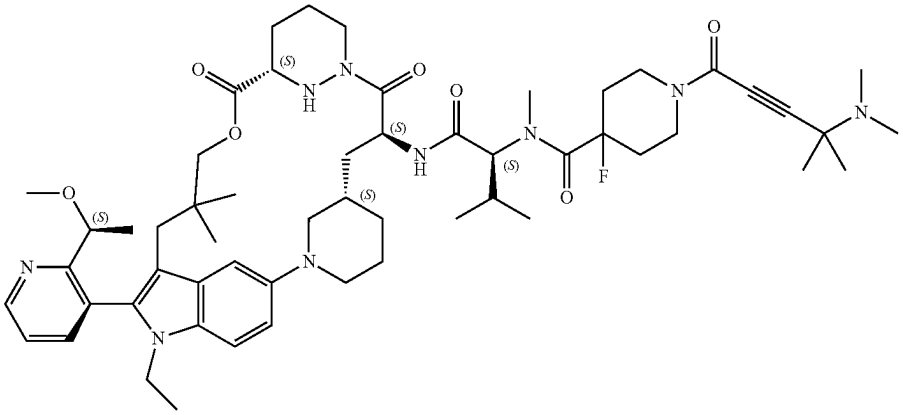
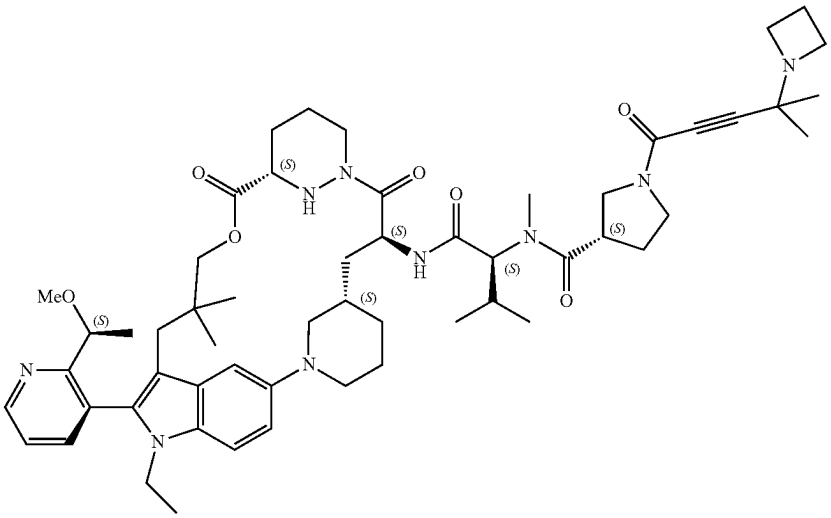
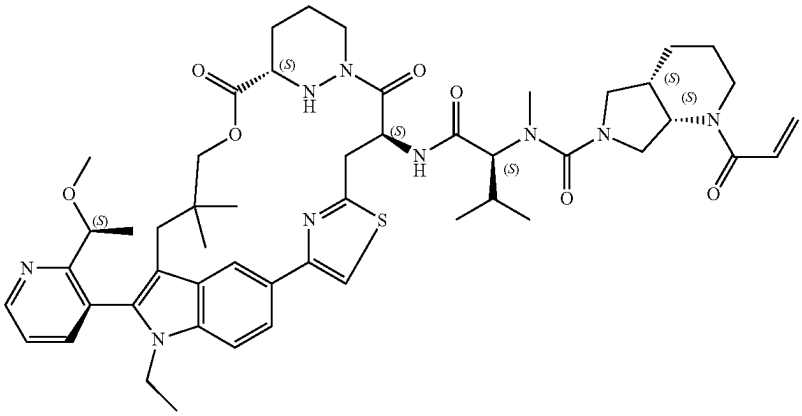
Certain Compounds of the Present Invention	
Ex#	Structure
A616	
A617	
A618	

TABLE 1-continued

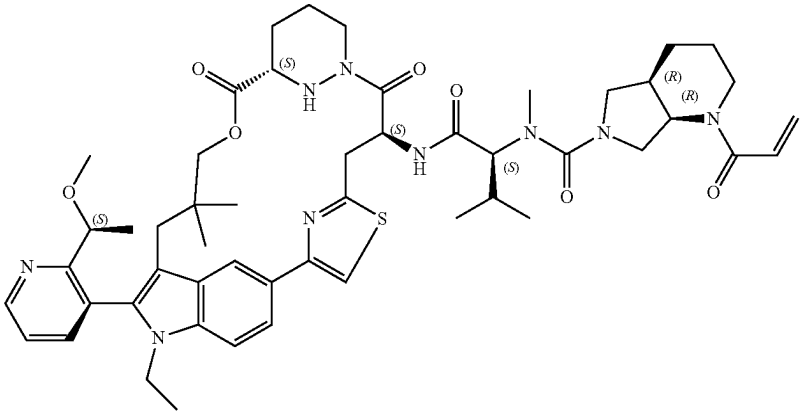
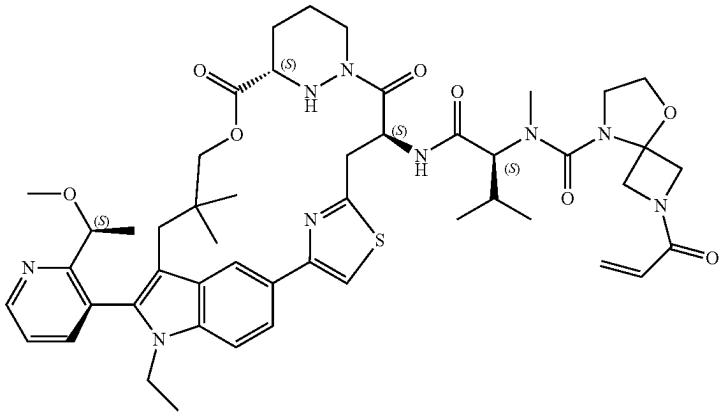
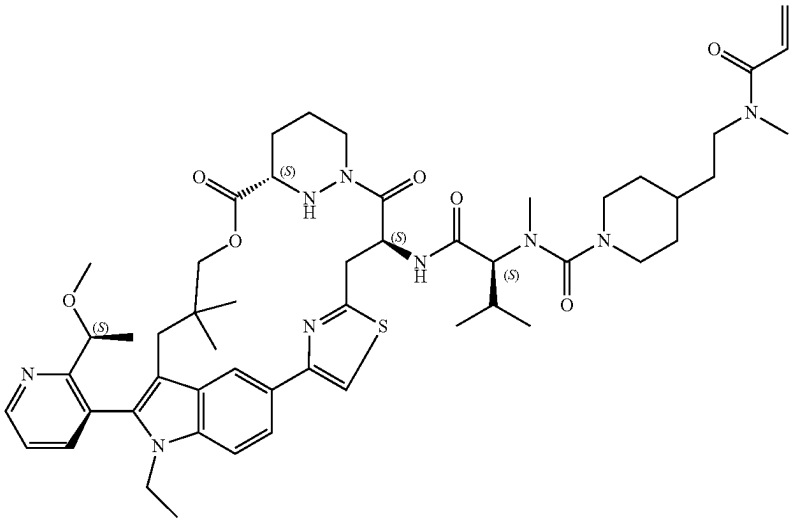
Certain Compounds of the Present Invention	
Ex#	Structure
A619	
A620	
A621	

TABLE 1-continued

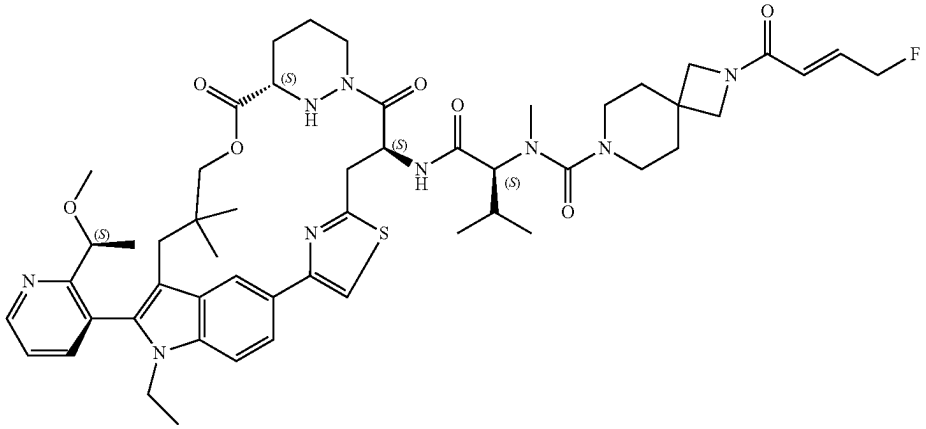
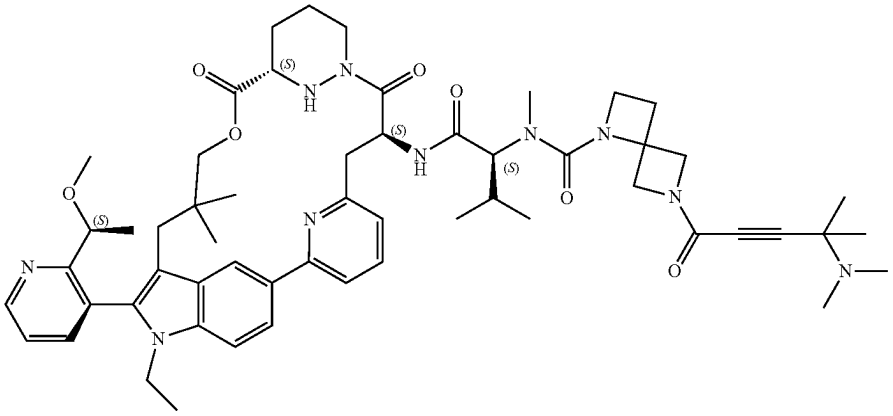
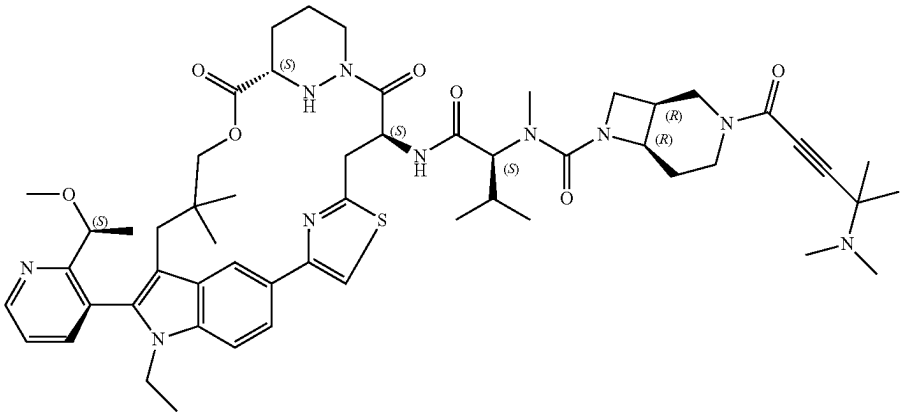
Certain Compounds of the Present Invention	
Ex#	Structure
A622	
A623	
A624	

TABLE 1-continued

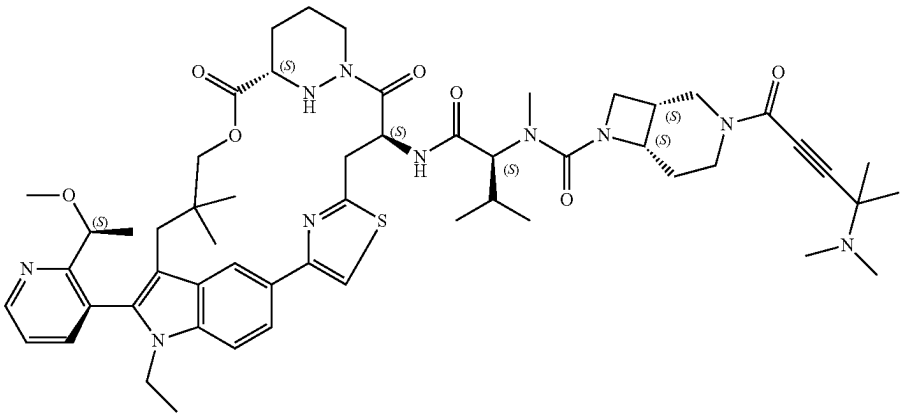
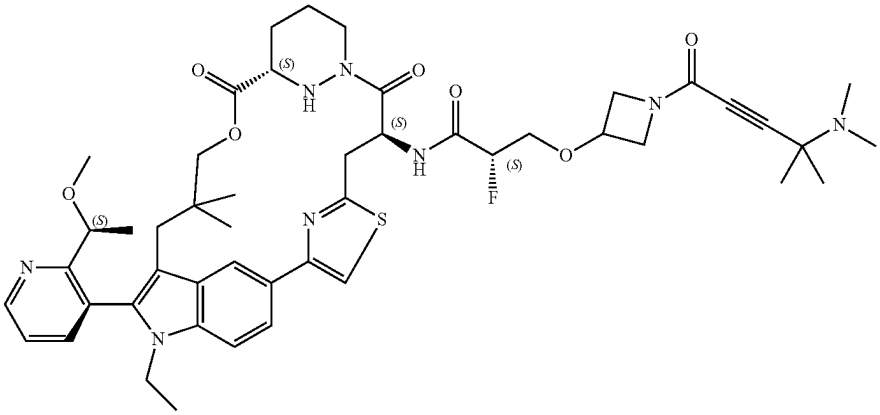
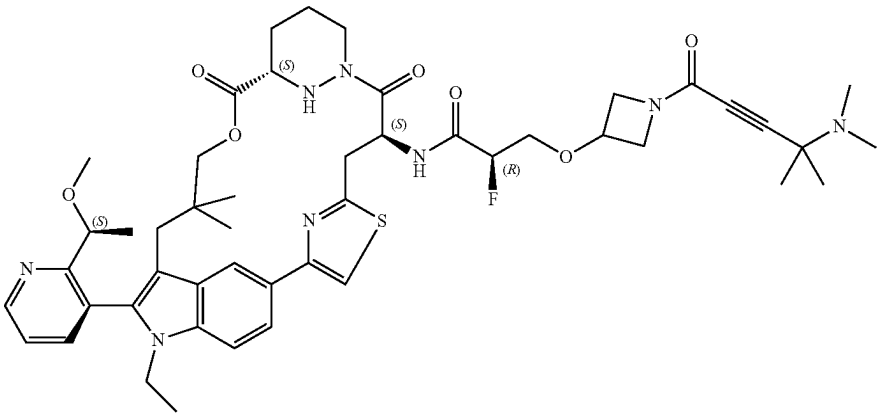
Certain Compounds of the Present Invention	
Ex#	Structure
A625	
A626	
A627	

TABLE 1-continued

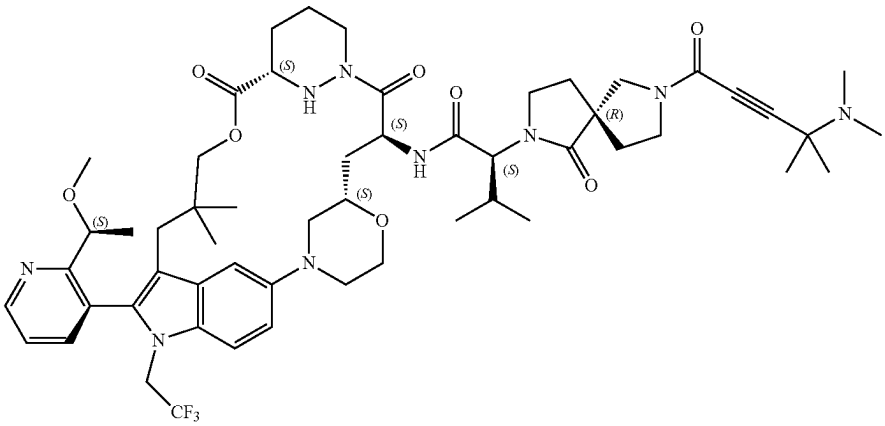
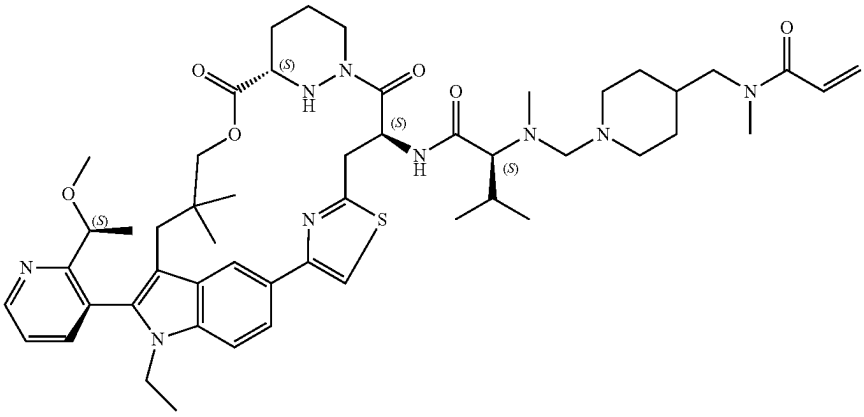
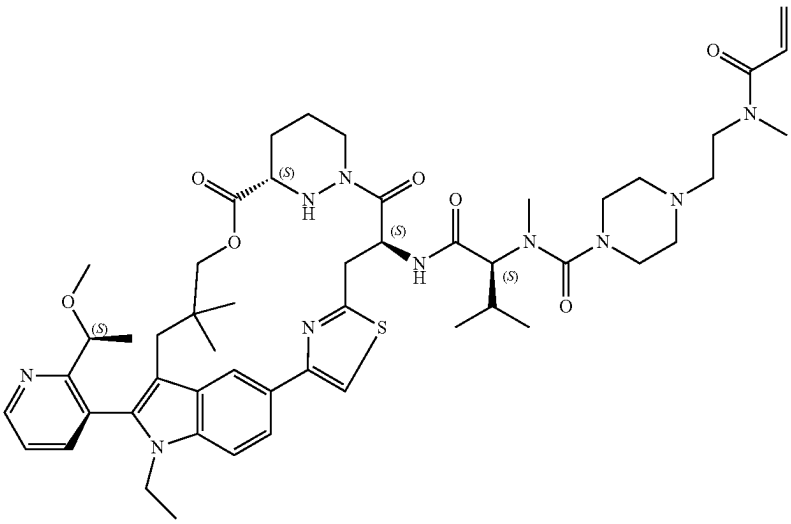
Certain Compounds of the Present Invention	
Ex#	Structure
A628	
A629	
A630	

TABLE 1-continued

Certain Compounds of the Present Invention	
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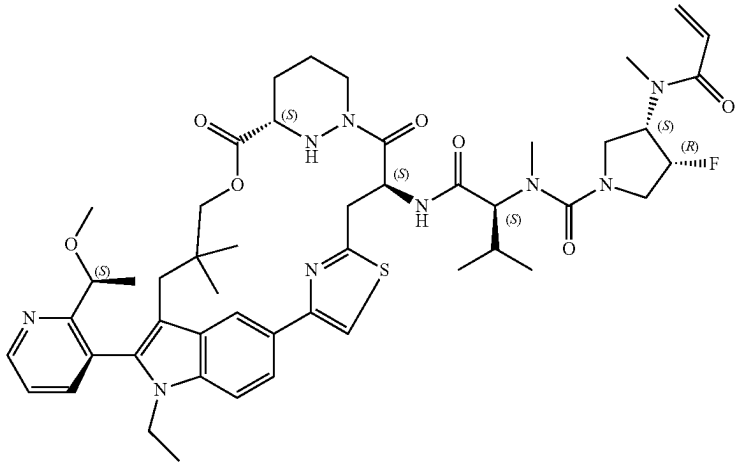
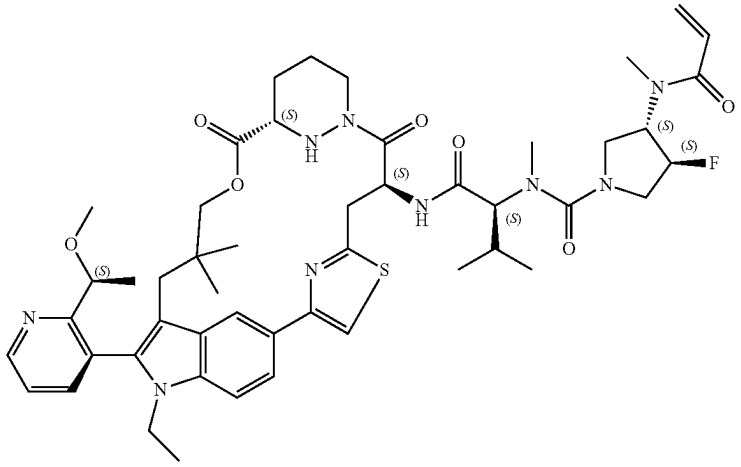
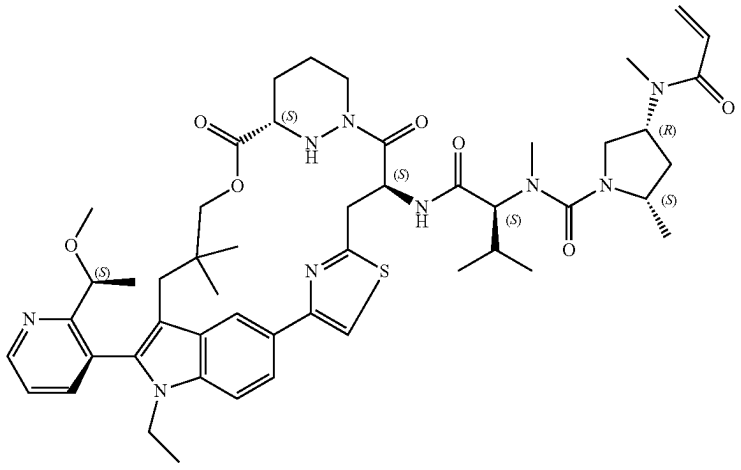
A631	
A632	
A633	

TABLE 1-continued

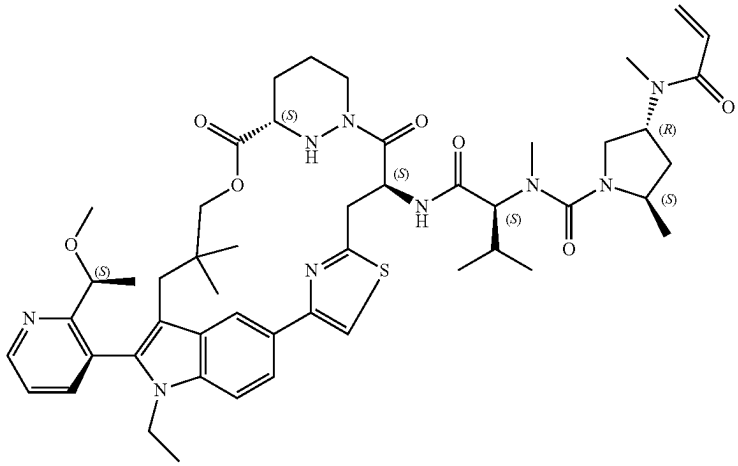
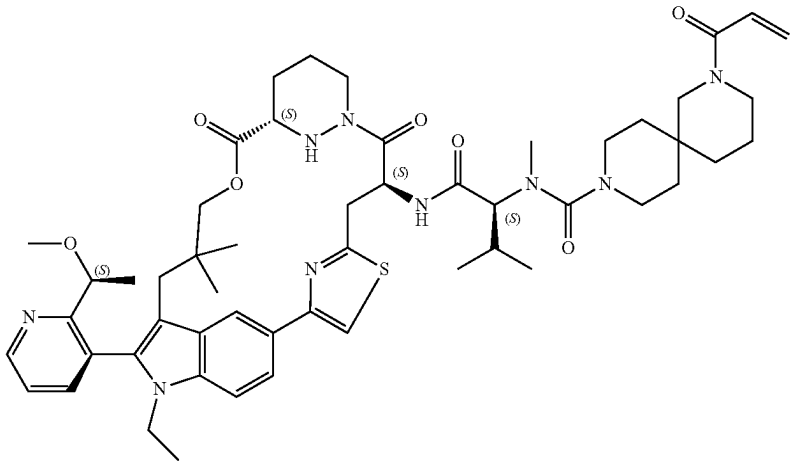
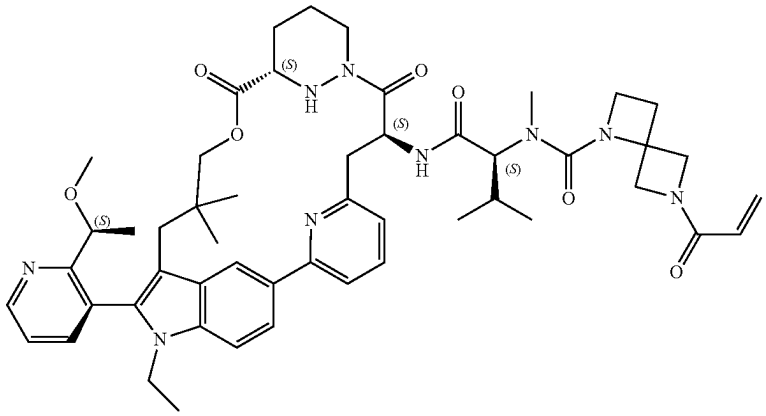
Certain Compounds of the Present Invention	
Ex#	Structure
A634	
A635	
A636	

TABLE 1-continued

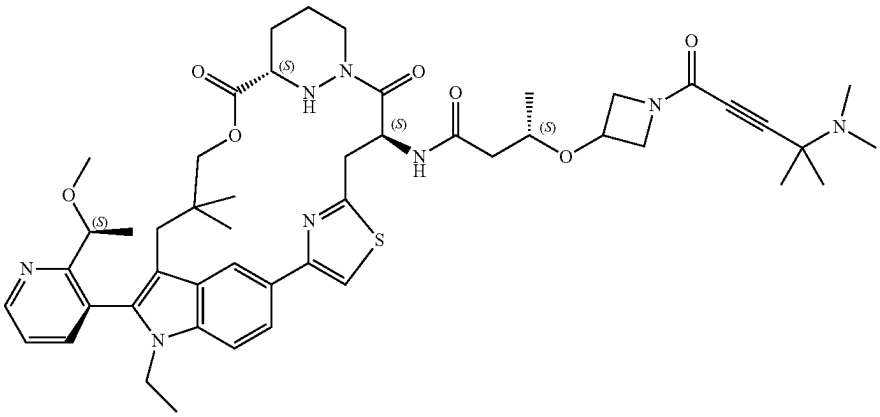
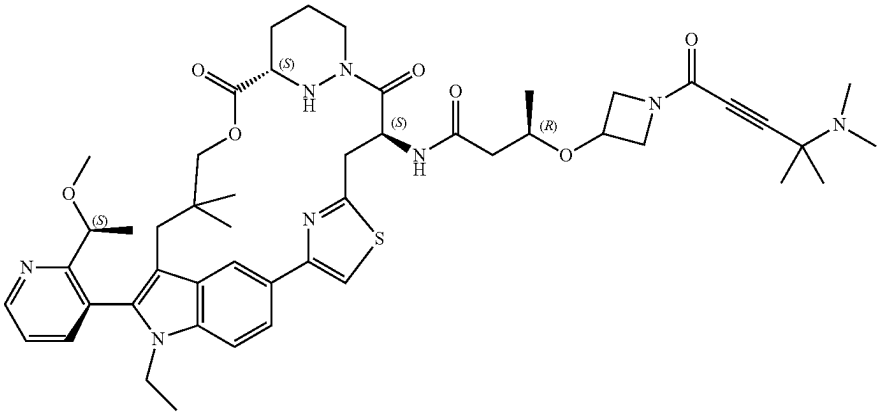
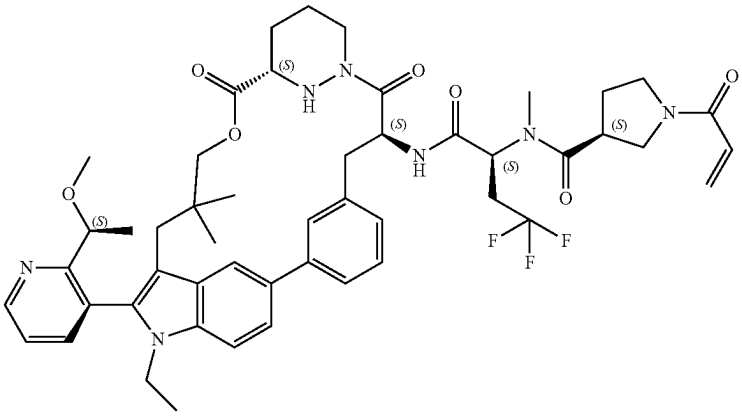
Certain Compounds of the Present Invention	
Ex#	Structure
A637	
A638	
A639	

TABLE 1-continued

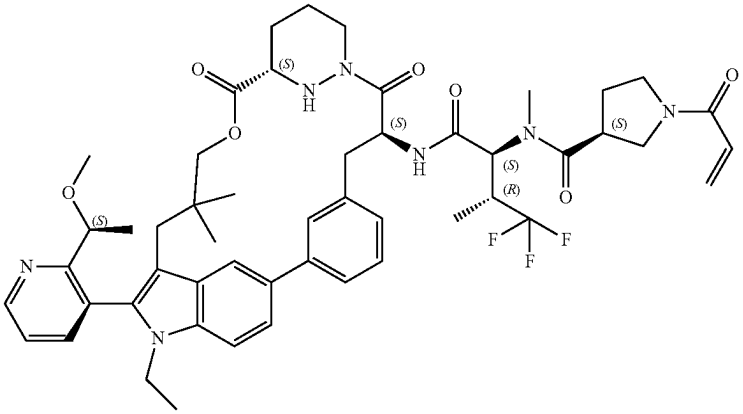
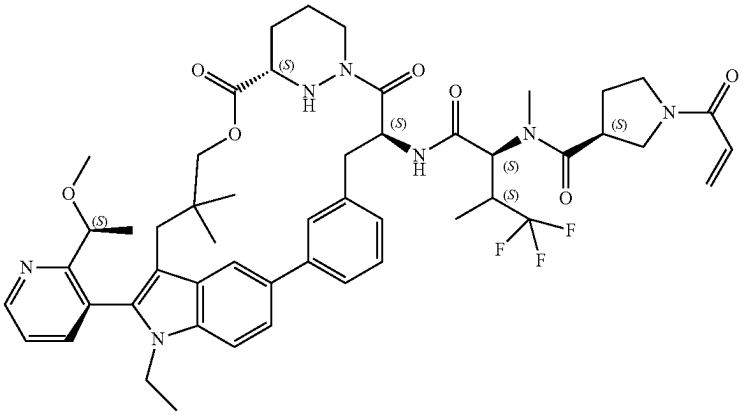
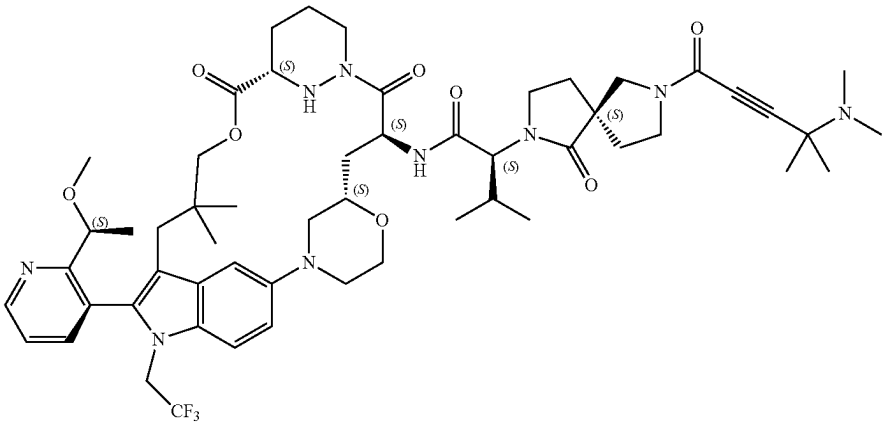
Certain Compounds of the Present Invention	
Ex#	Structure
A640	
A641	
A642	

TABLE 1-continued

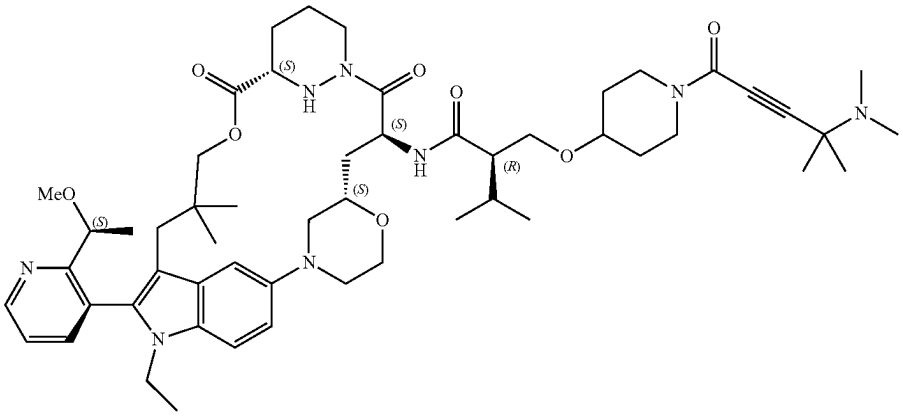
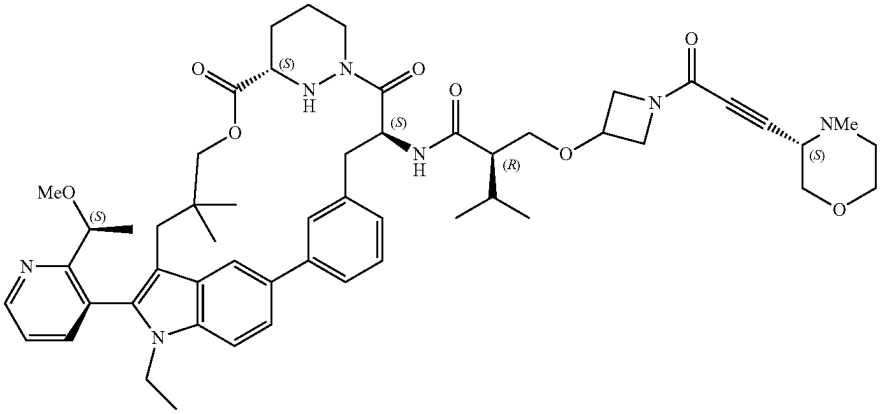
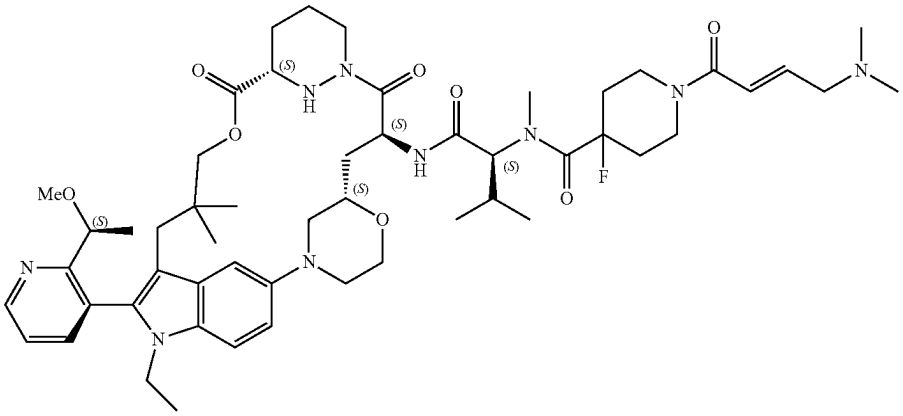
Certain Compounds of the Present Invention	
Ex#	Structure
A643	
A644	
A645	

TABLE 1-continued

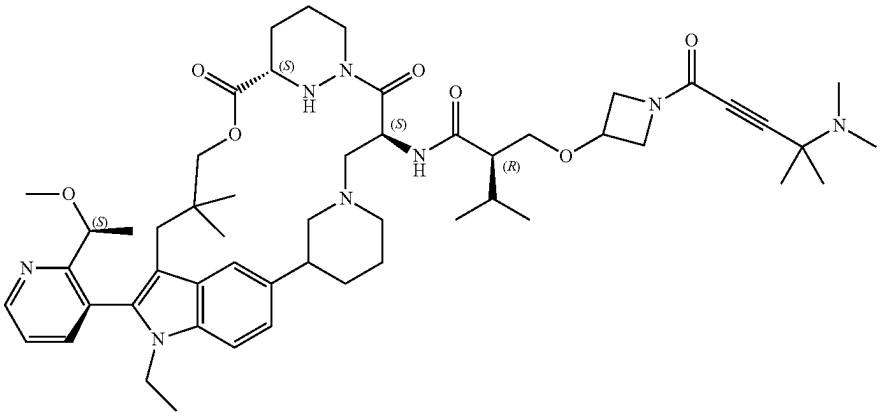
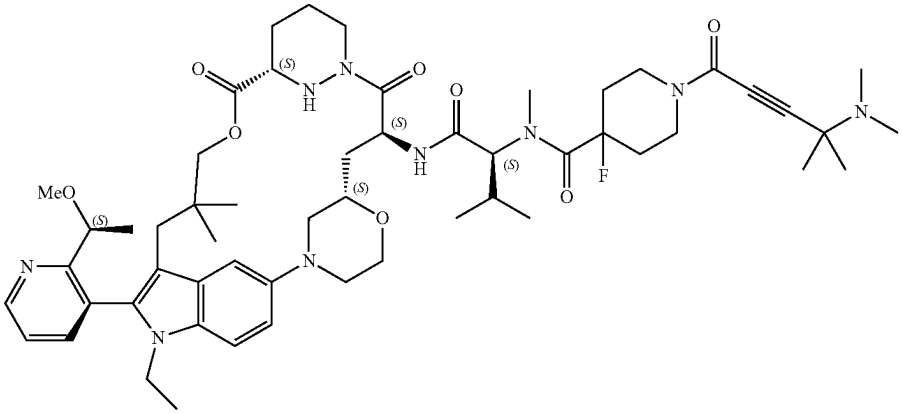
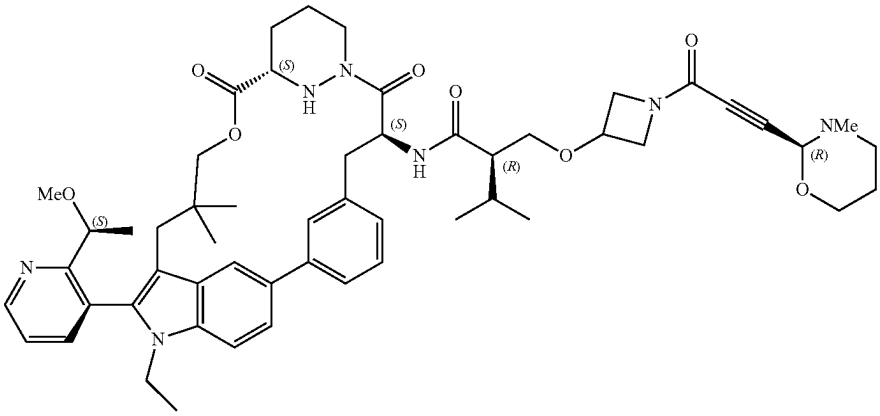
Certain Compounds of the Present Invention	
Ex#	Structure
A646	
A647	
A648	

TABLE 1-continued

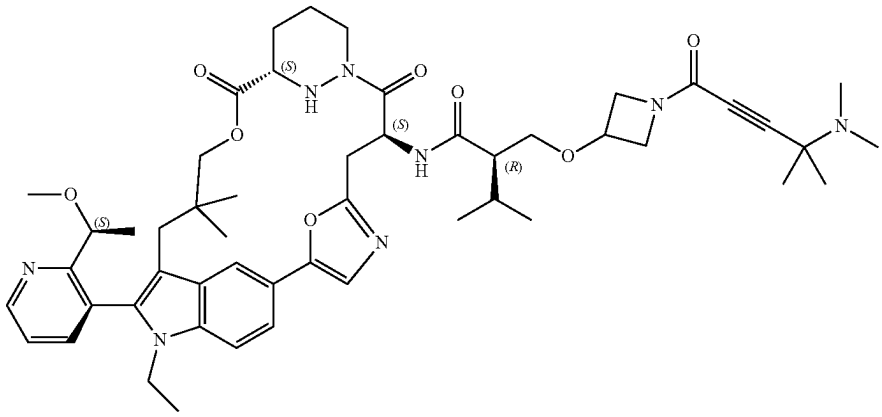
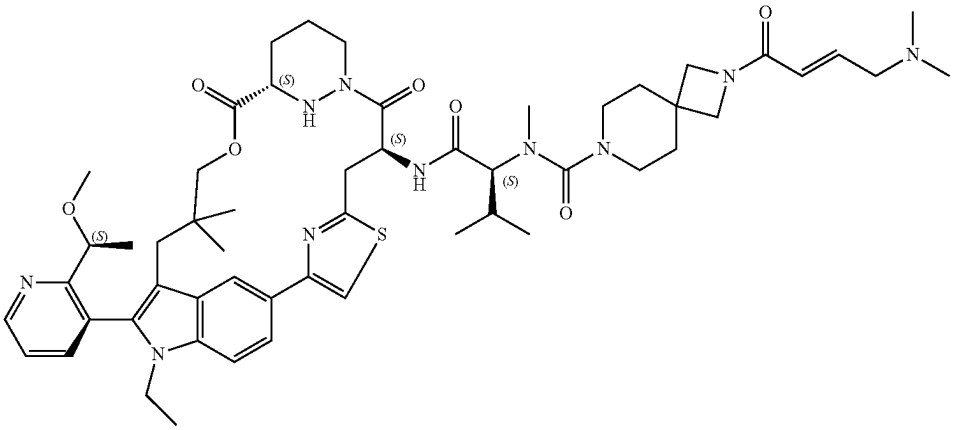
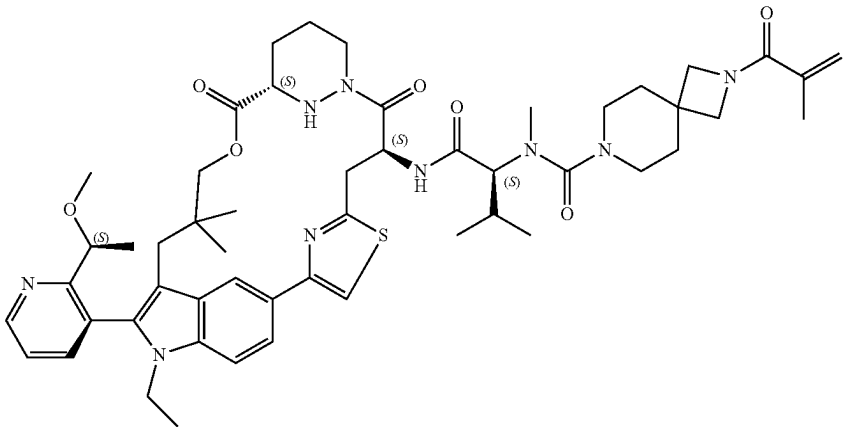
Certain Compounds of the Present Invention	
Ex#	Structure
A649	
A650	
A651	

TABLE 1-continued

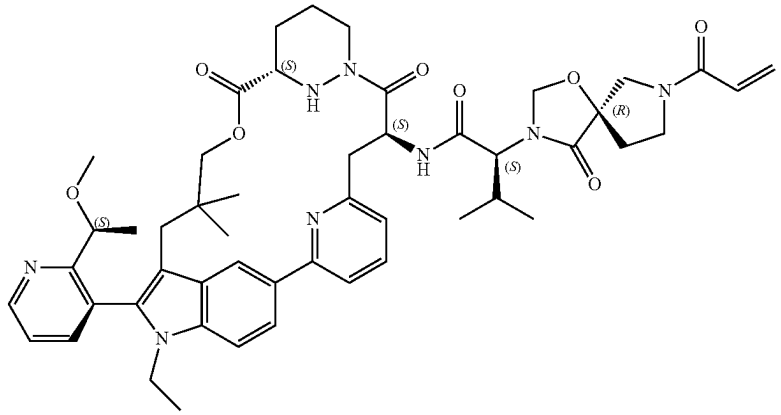
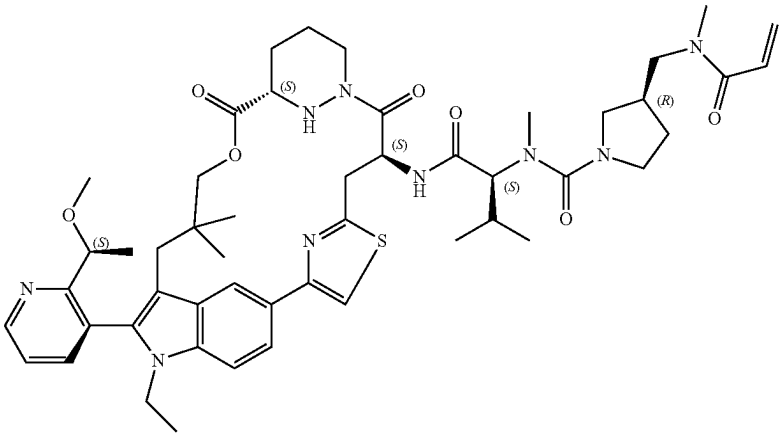
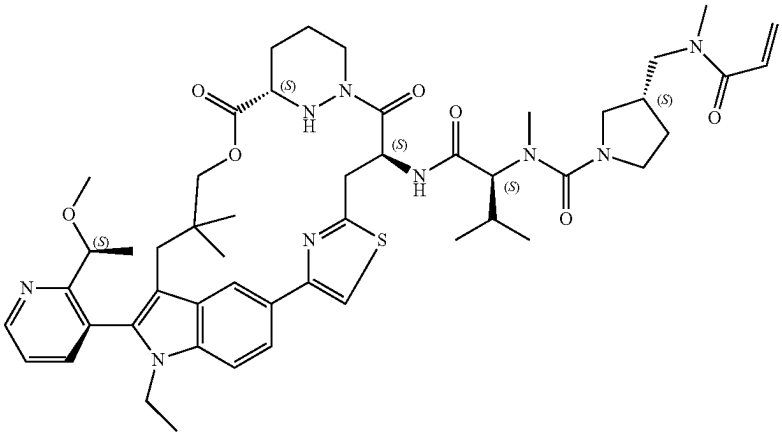
Certain Compounds of the Present Invention	
Ex#	Structure
A652	
A653	
A654	

TABLE 1-continued

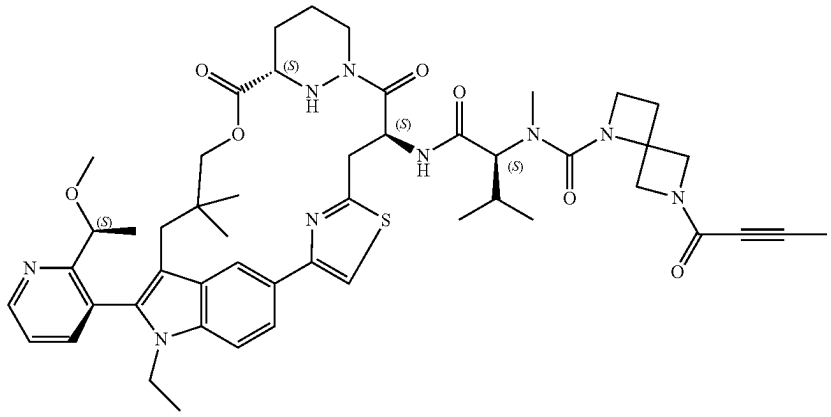
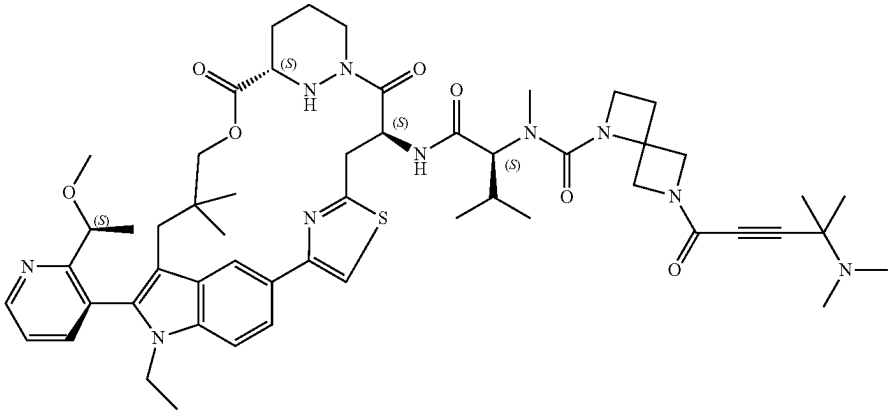
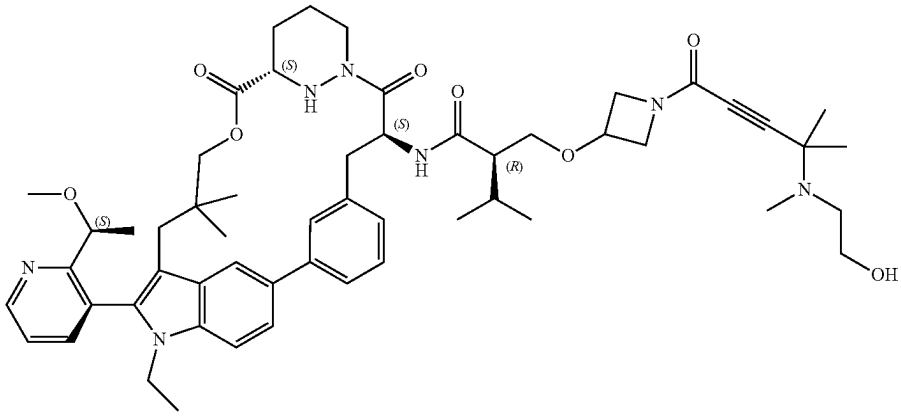
Certain Compounds of the Present Invention	
Ex#	Structure
A655	
A656	
A657	

TABLE 1-continued

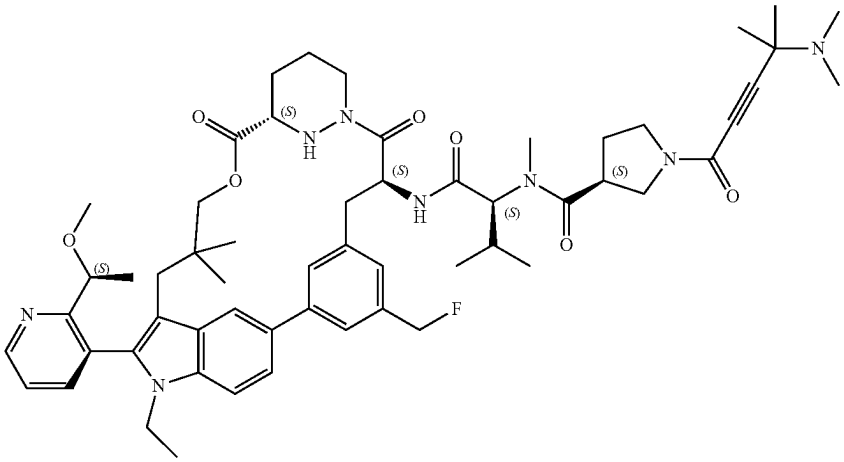
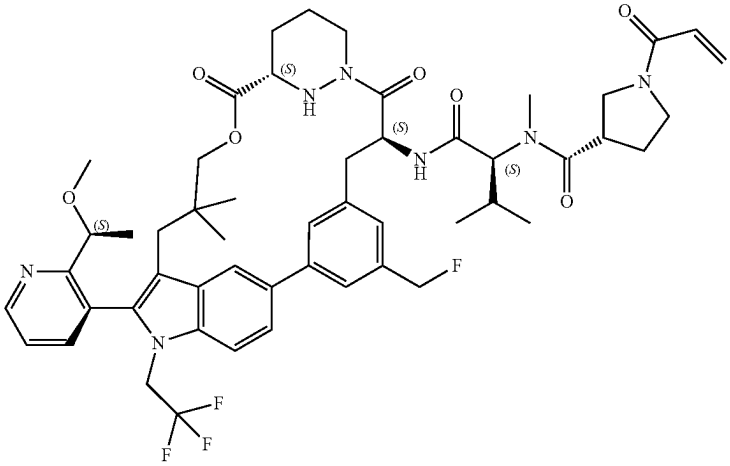
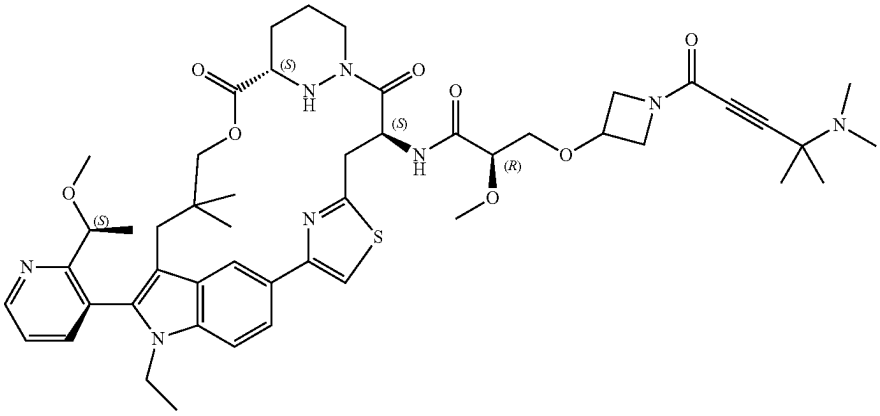
Certain Compounds of the Present Invention	
Ex#	Structure
A658	
A659	
A660	

TABLE 1-continued

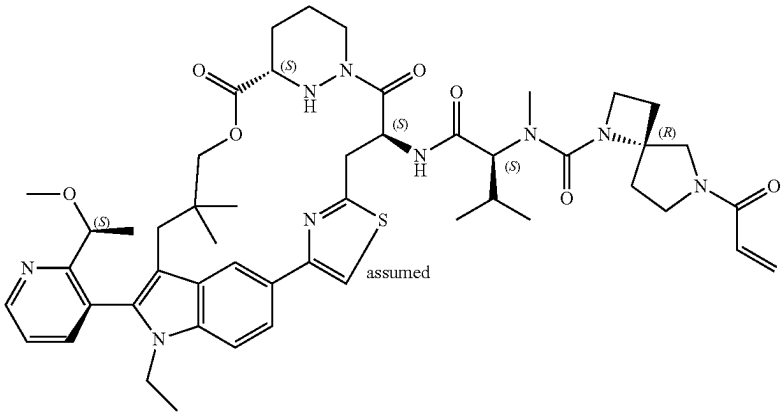
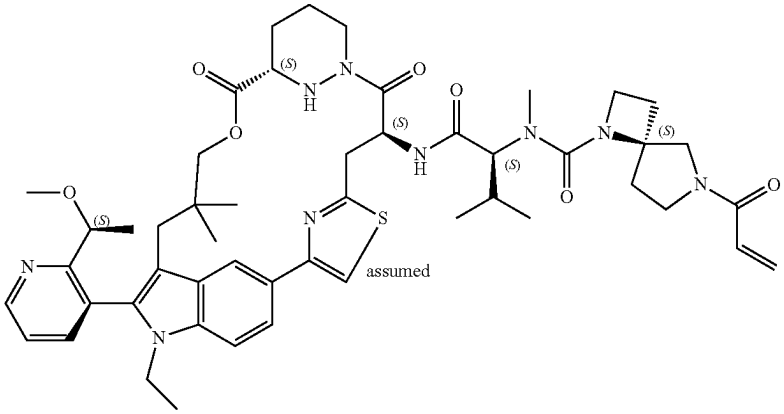
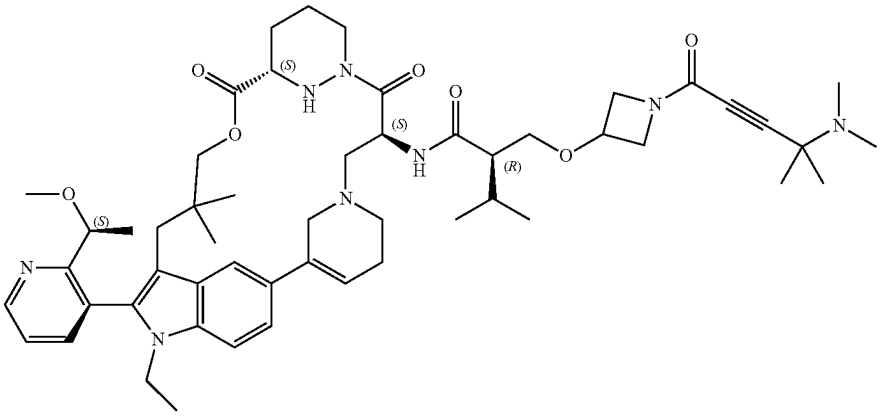
Certain Compounds of the Present Invention	
Ex#	Structure
A661	
A662	
A663	

TABLE 1-continued

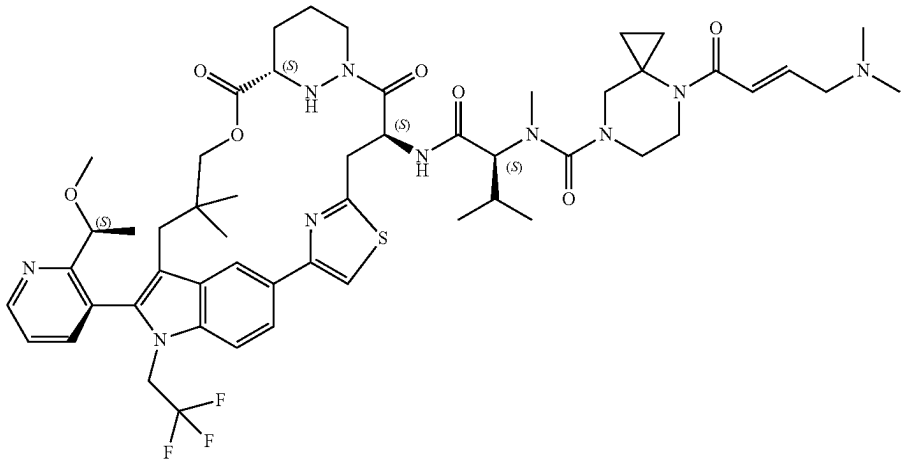
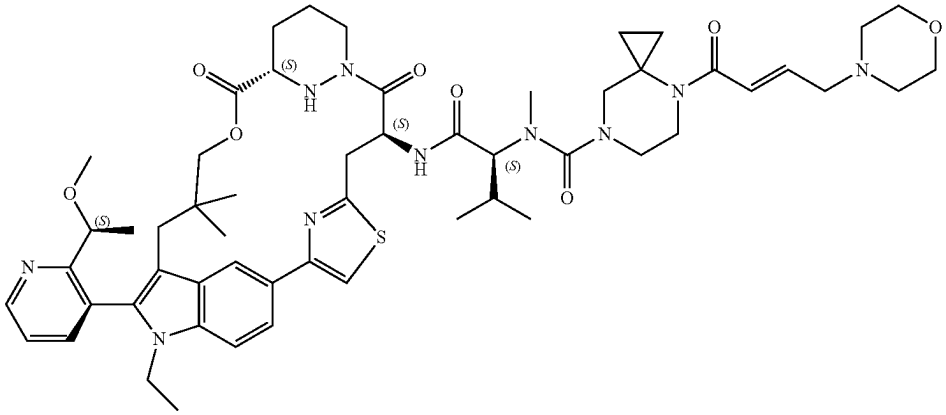
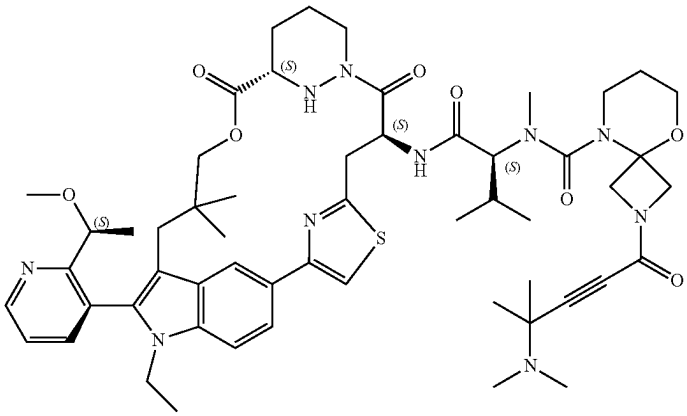
Certain Compounds of the Present Invention	
Ex#	Structure
A664	
A665	
A666	

TABLE 1-continued

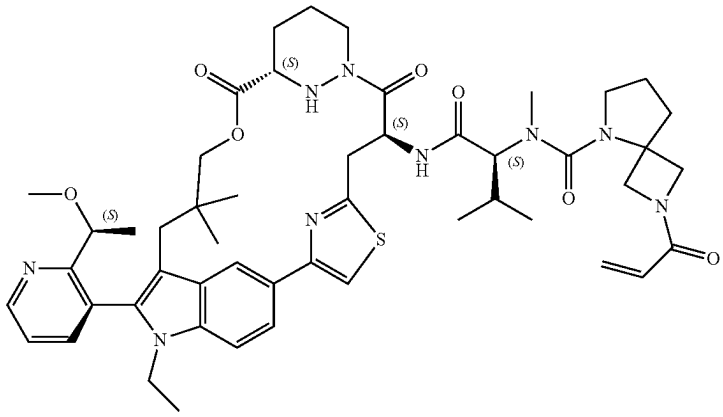
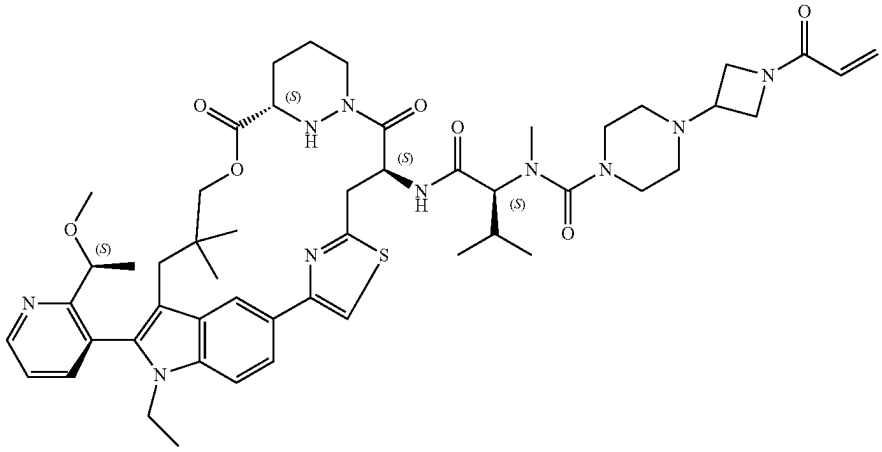
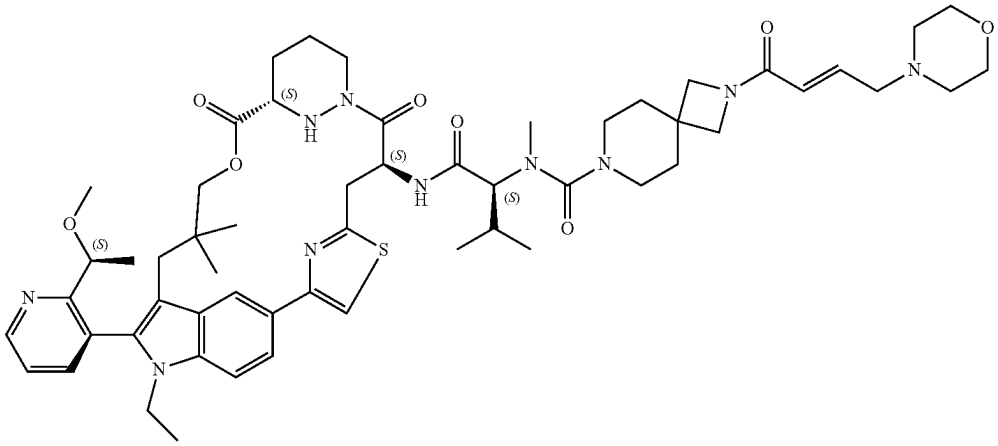
Certain Compounds of the Present Invention	
Ex#	Structure
A667	
A668	
A669	

TABLE 1-continued

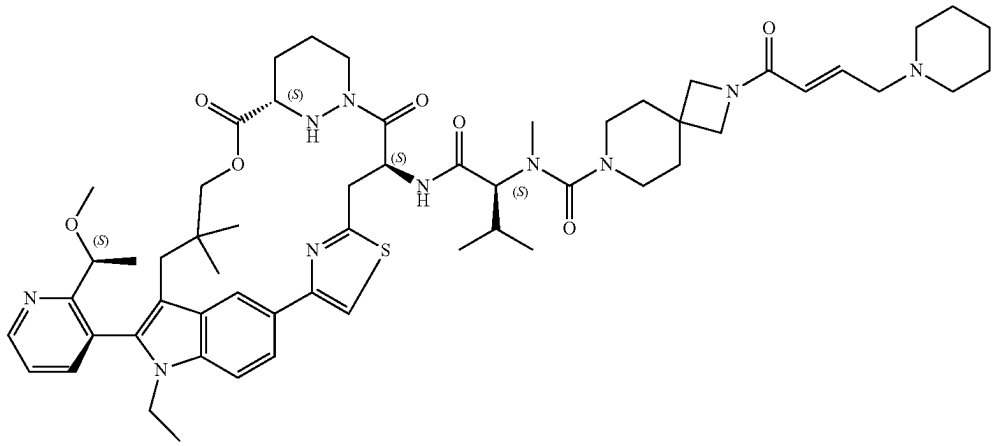
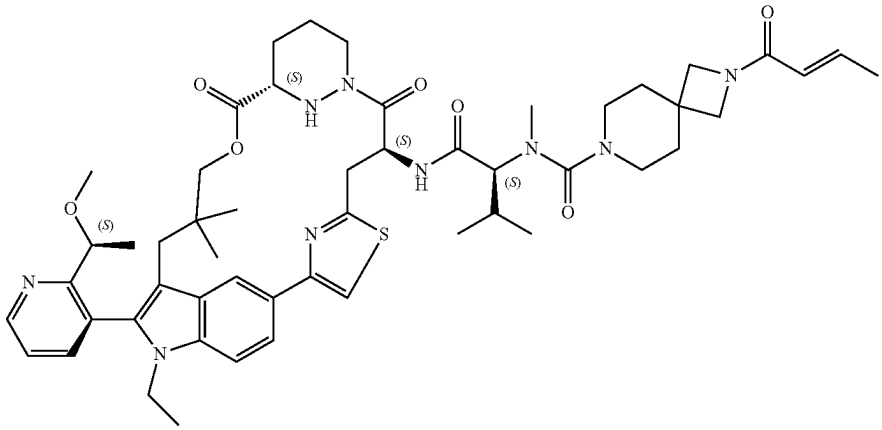
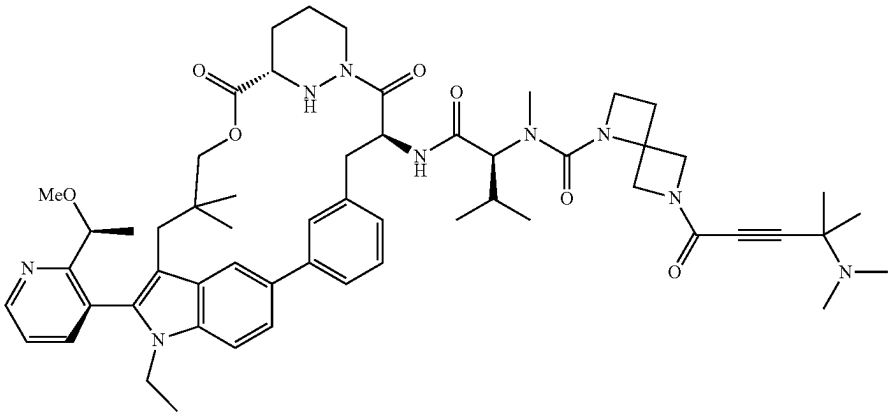
Certain Compounds of the Present Invention	
Ex#	Structure
A670	
A671	
A672	

TABLE 1-continued

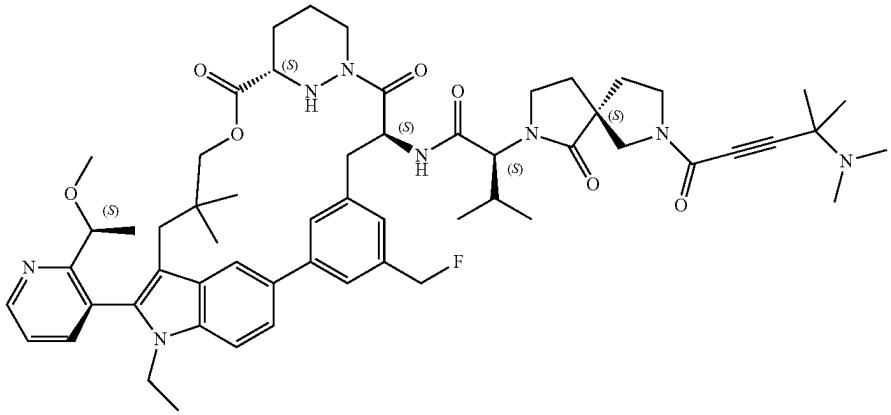
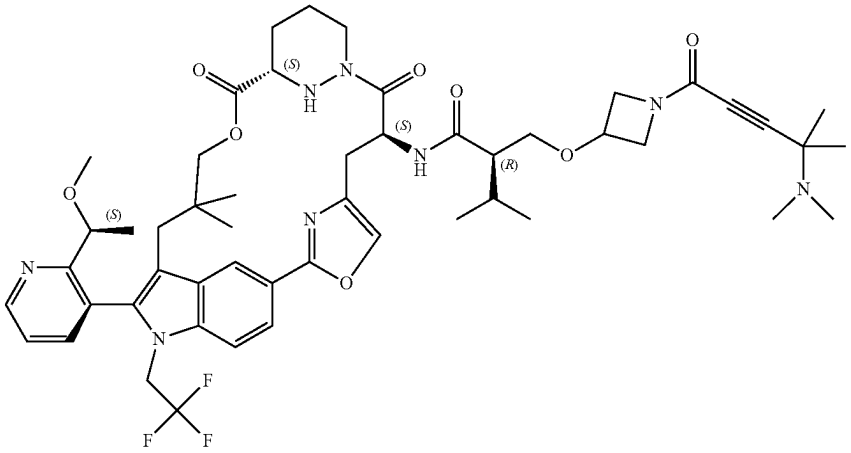
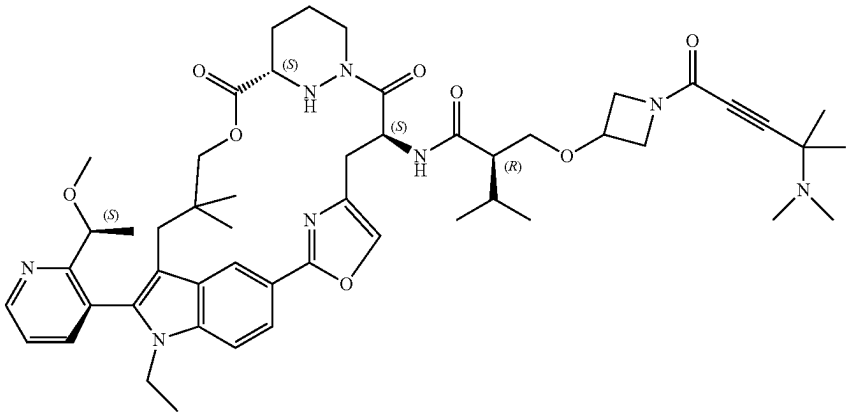
Certain Compounds of the Present Invention	
Ex#	Structure
A673	
A674	
A675	

TABLE 1-continued

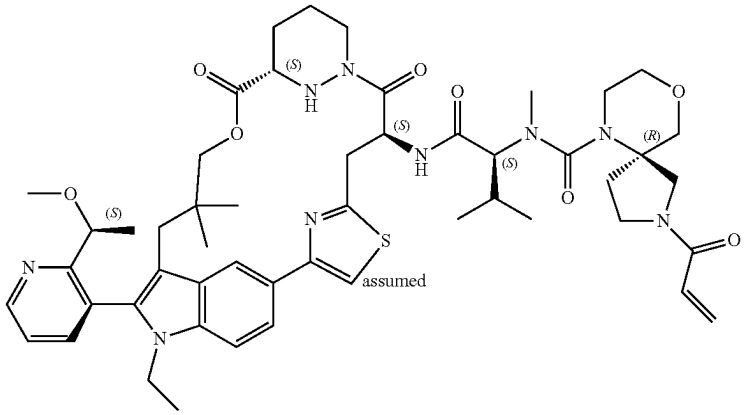
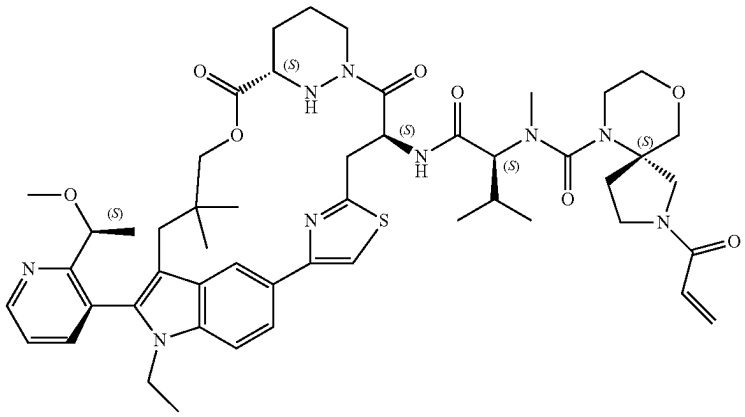
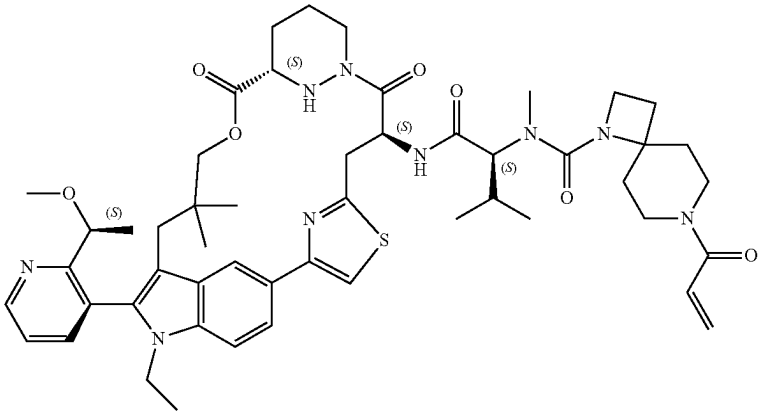
Certain Compounds of the Present Invention	
Ex#	Structure
A676	
A677	
A678	

TABLE 1-continued

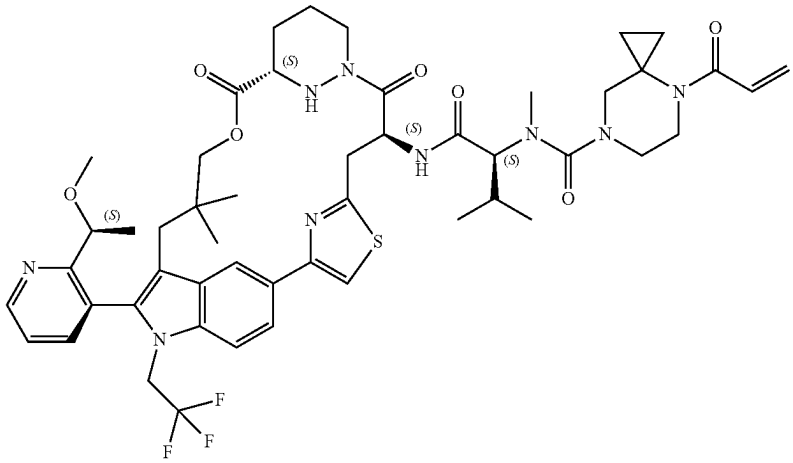
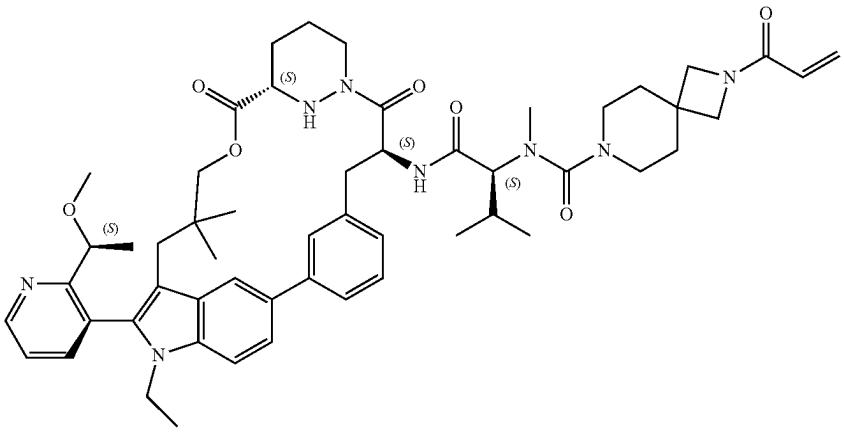
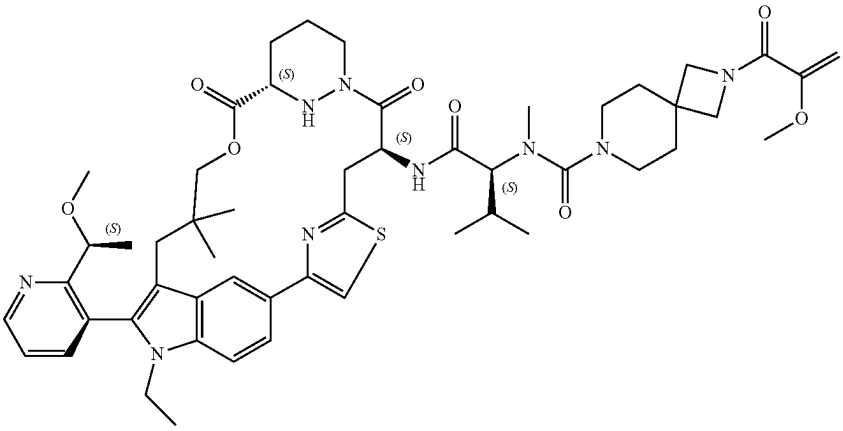
Certain Compounds of the Present Invention	
Ex#	Structure
A679	
A680	
A681	

TABLE 1-continued

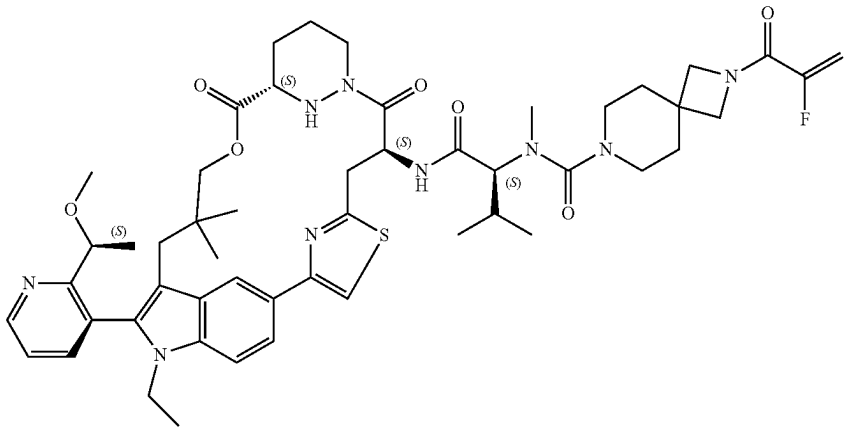
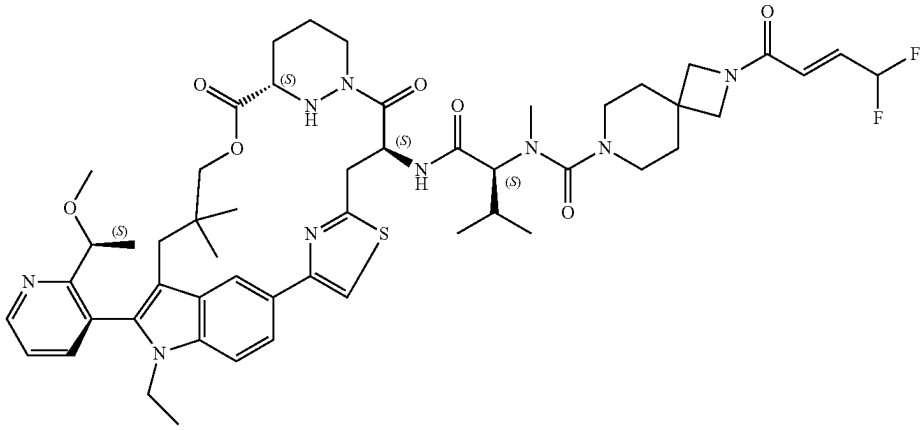
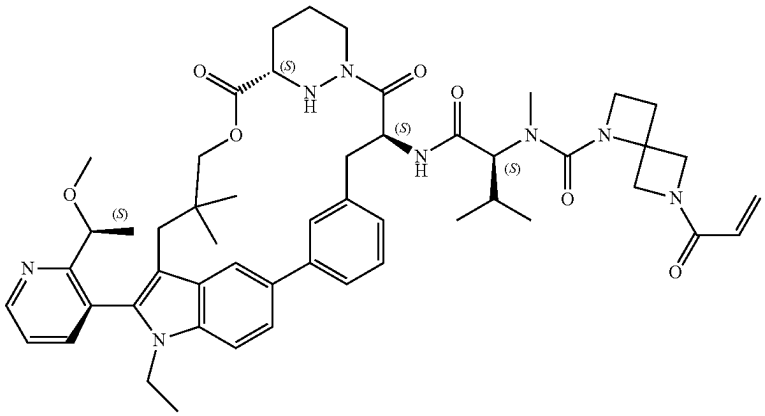
Certain Compounds of the Present Invention	
Ex#	Structure
A682	
A683	
A684	

TABLE 1-continued

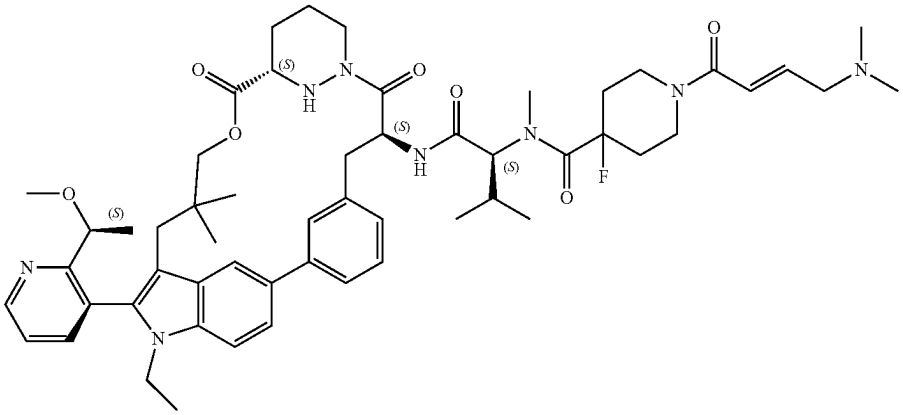
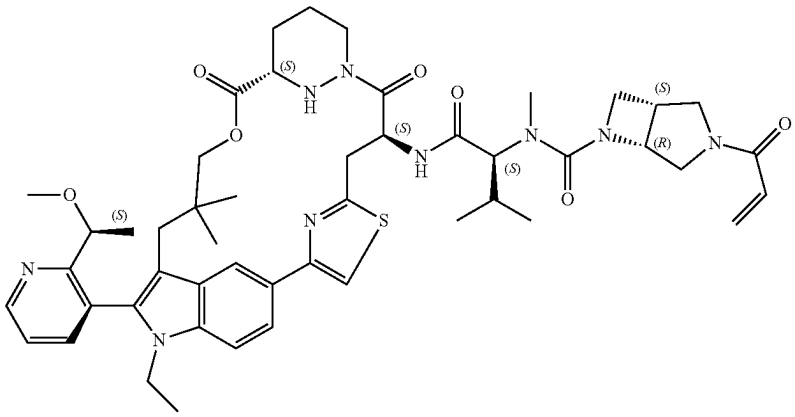
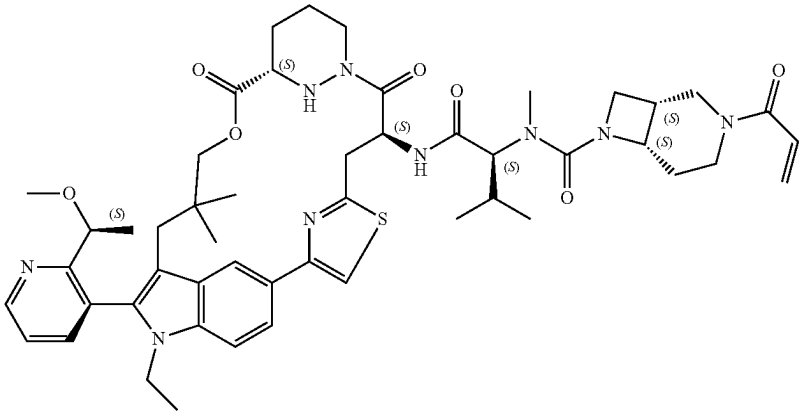
Certain Compounds of the Present Invention	
Ex#	Structure
A685	
A686	
A687	

TABLE 1-continued

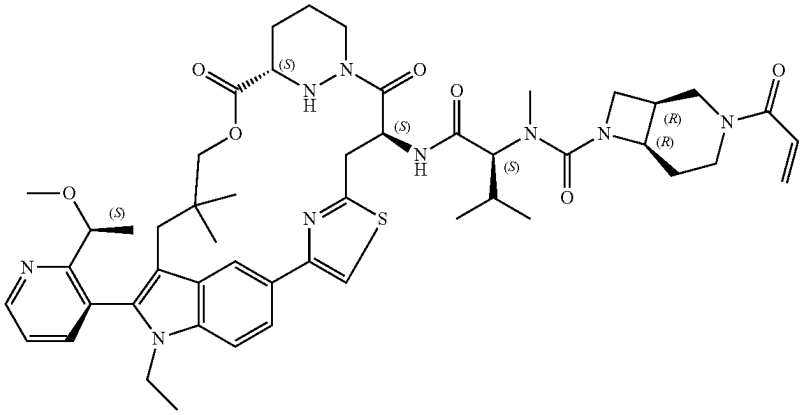
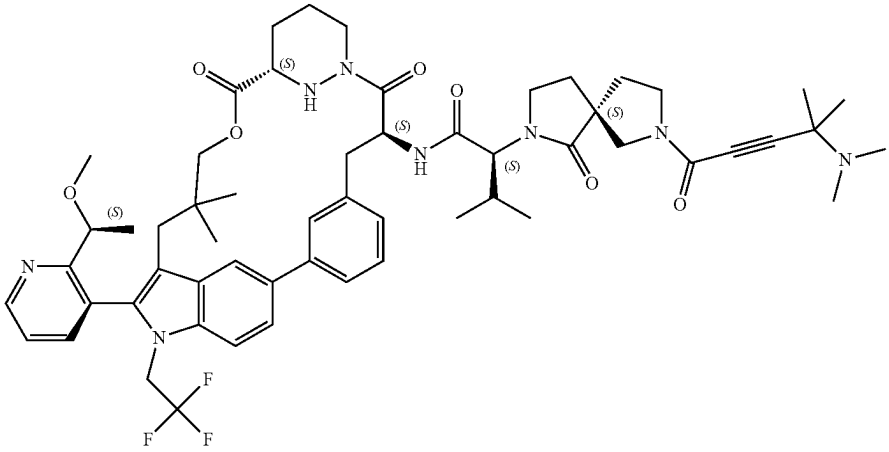
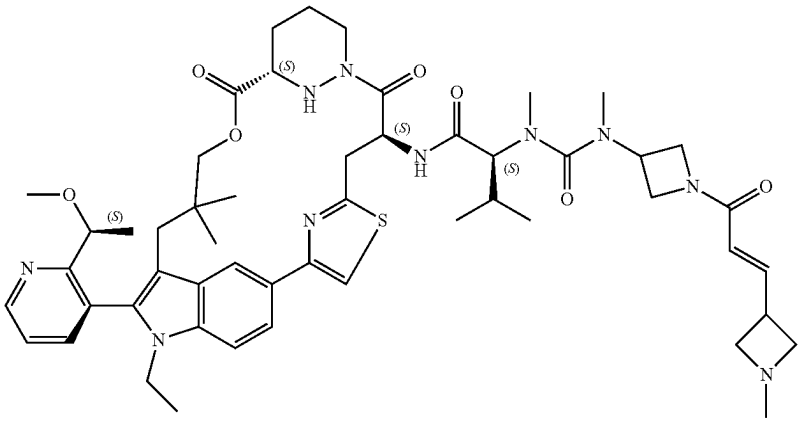
Certain Compounds of the Present Invention	
Ex#	Structure
A688	
A689	
A690	

TABLE 1-continued

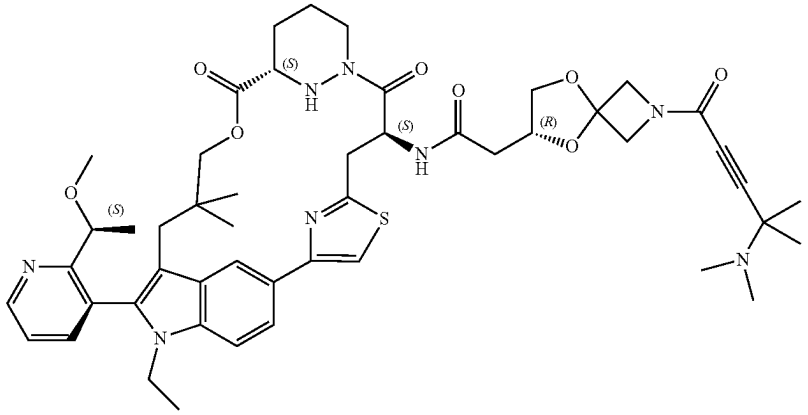
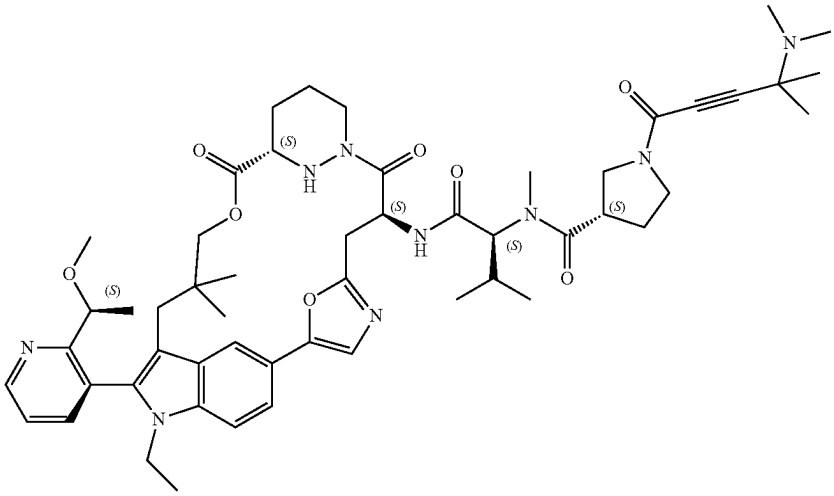
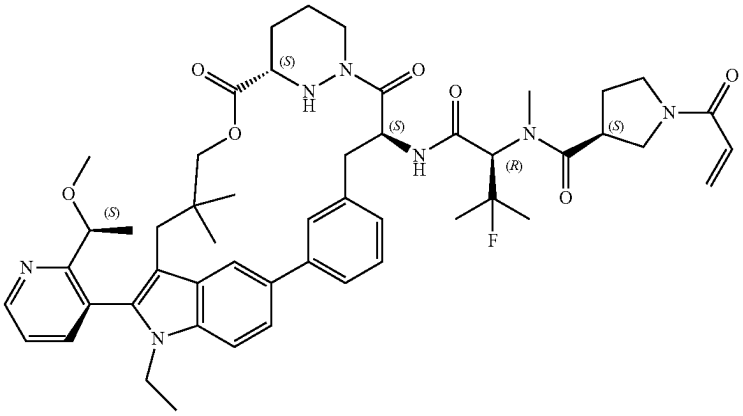
Certain Compounds of the Present Invention	
Ex#	Structure
A691	
A692	
A693	

TABLE 1-continued

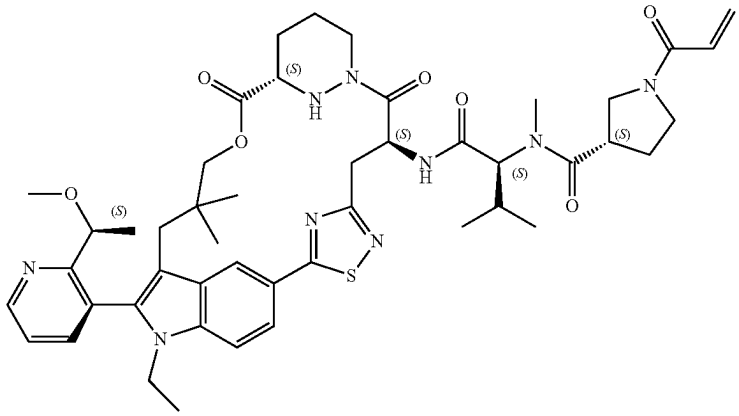
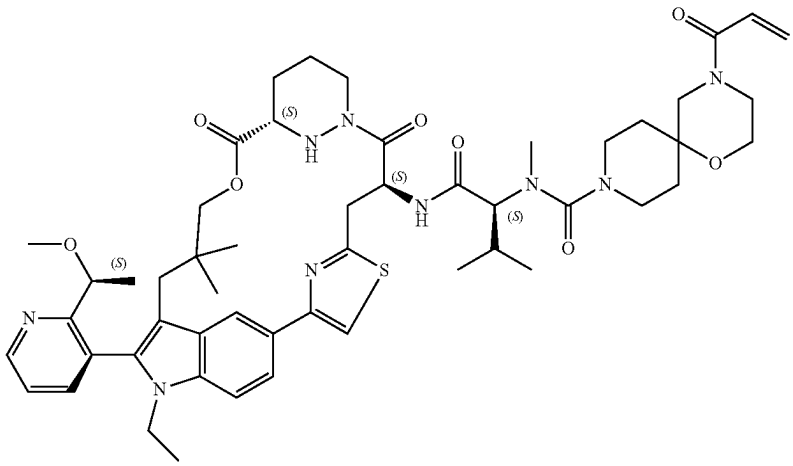
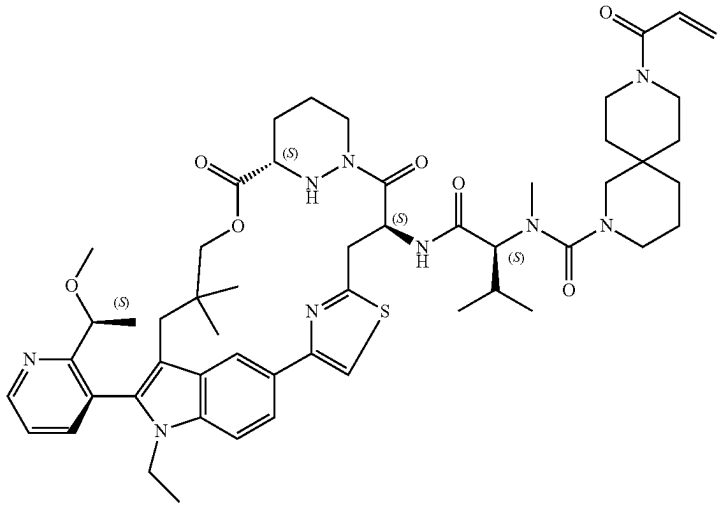
Certain Compounds of the Present Invention	
Ex#	Structure
A694	
A695	
A696	

TABLE 1-continued

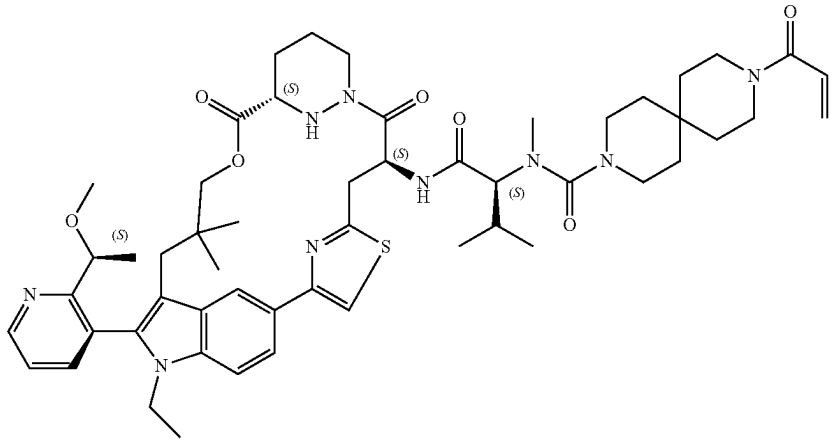
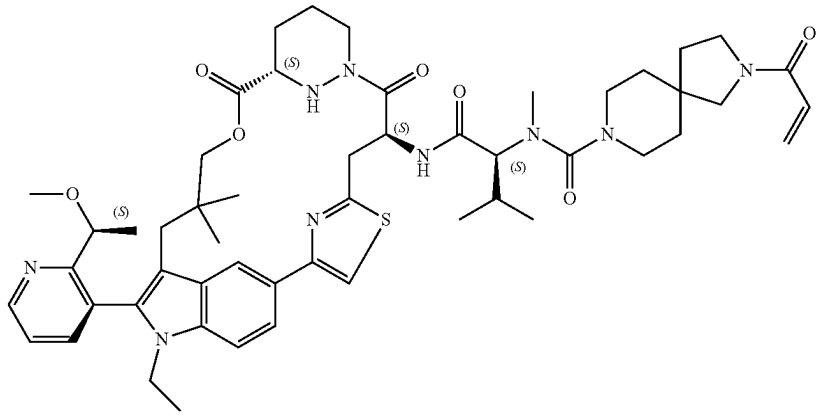
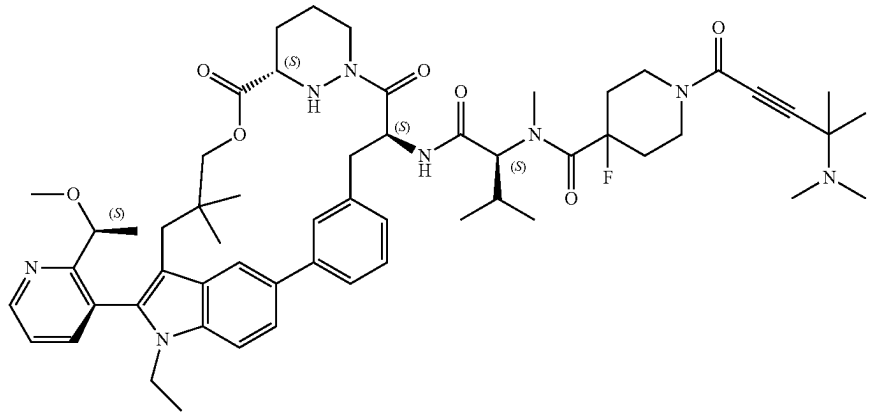
Certain Compounds of the Present Invention	
Ex#	Structure
A697	
A698	
A699	

TABLE 1-continued

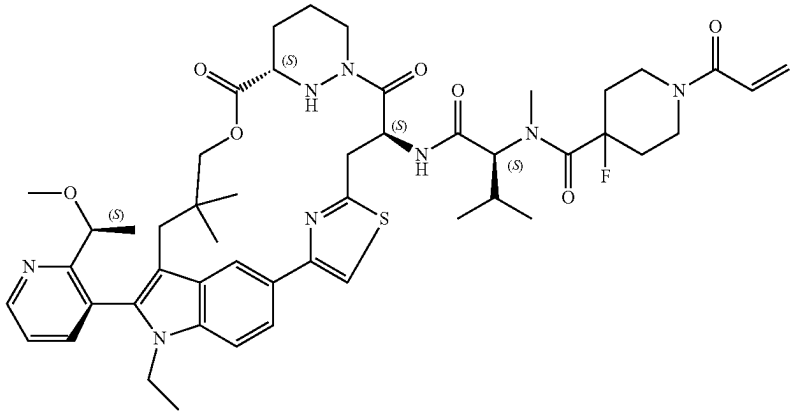
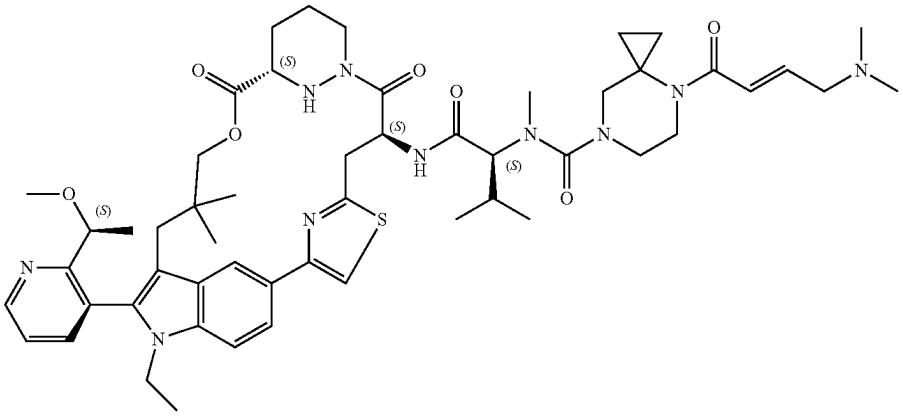
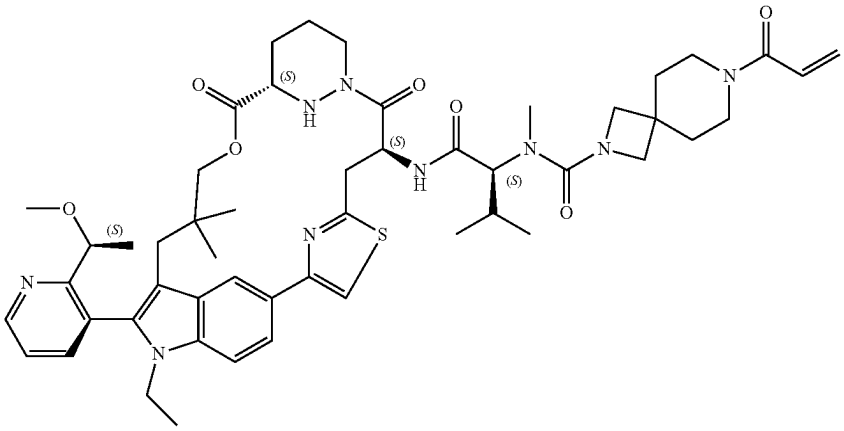
Certain Compounds of the Present Invention	
Ex#	Structure
A700	
A701	
A702	

TABLE 1-continued

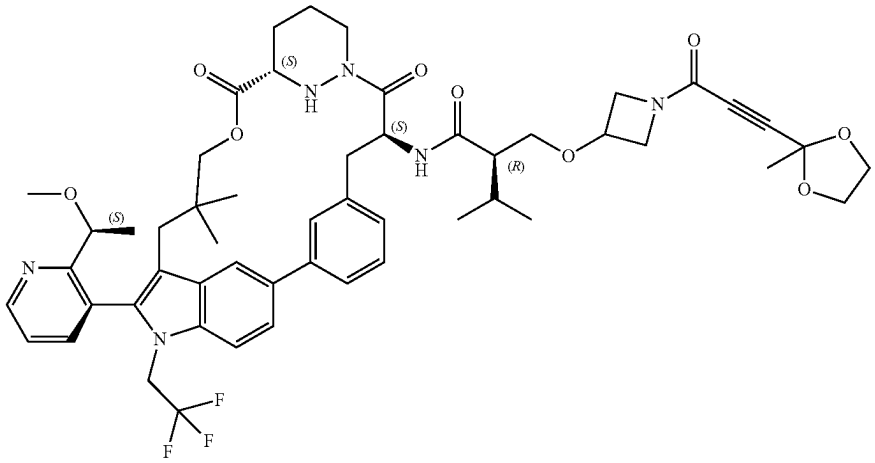
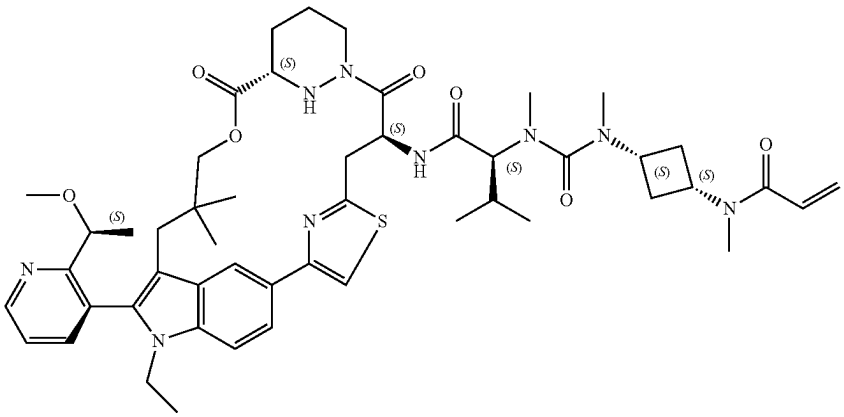
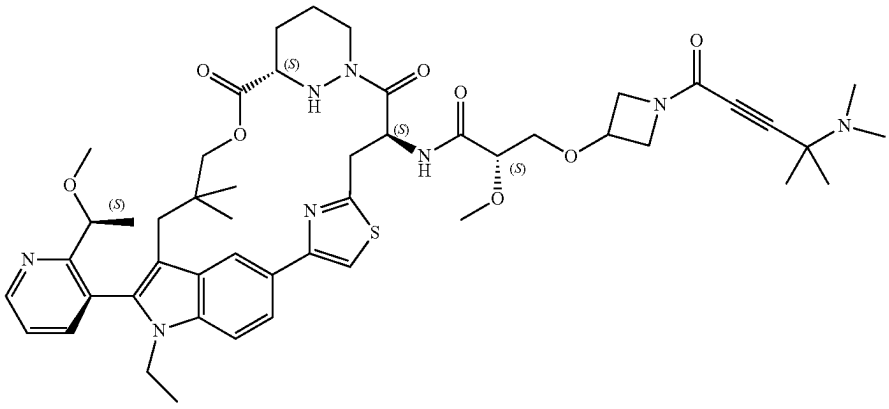
Certain Compounds of the Present Invention	
Ex#	Structure
A703	
A704	
A705	

TABLE 1-continued

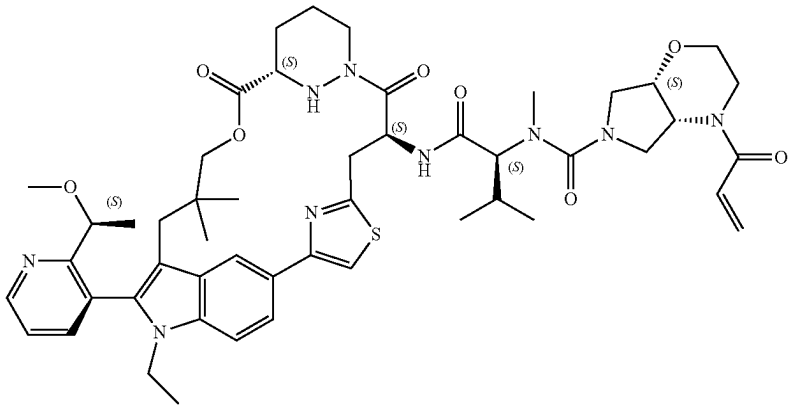
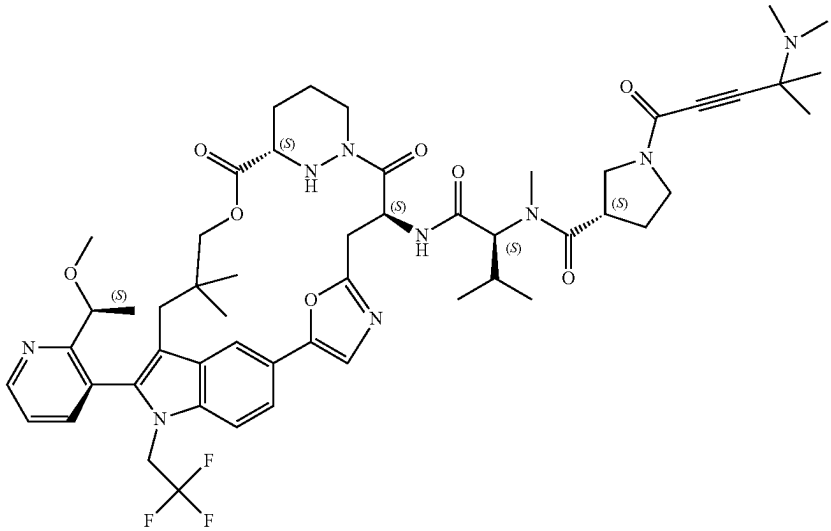
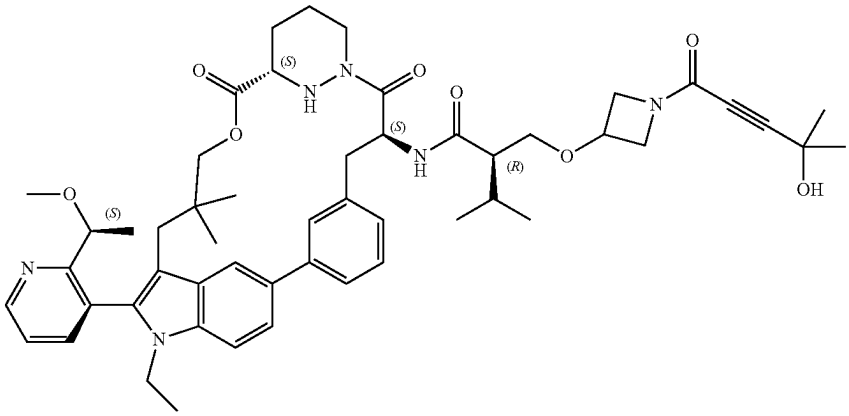
Certain Compounds of the Present Invention	
Ex#	Structure
A706	
A707	
A708	

TABLE 1-continued

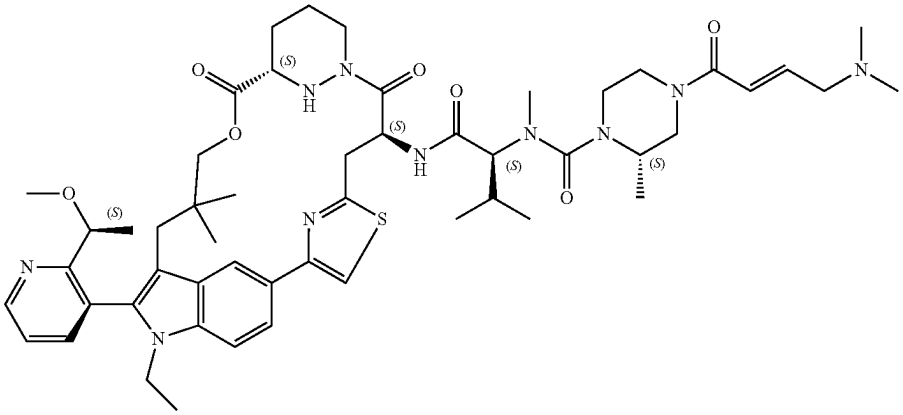
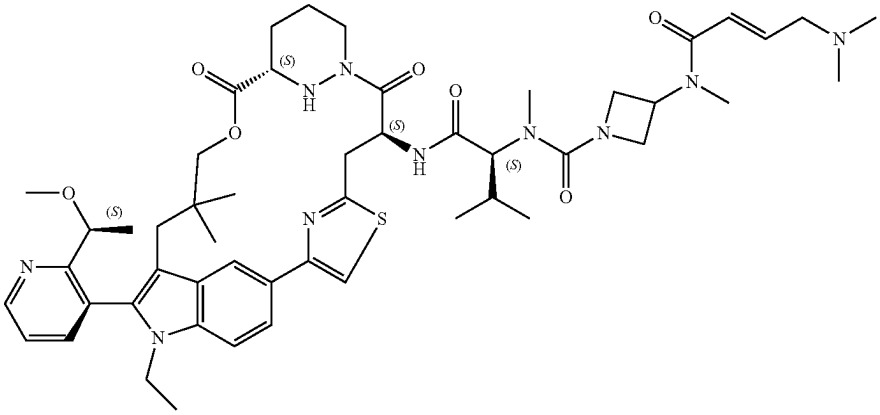
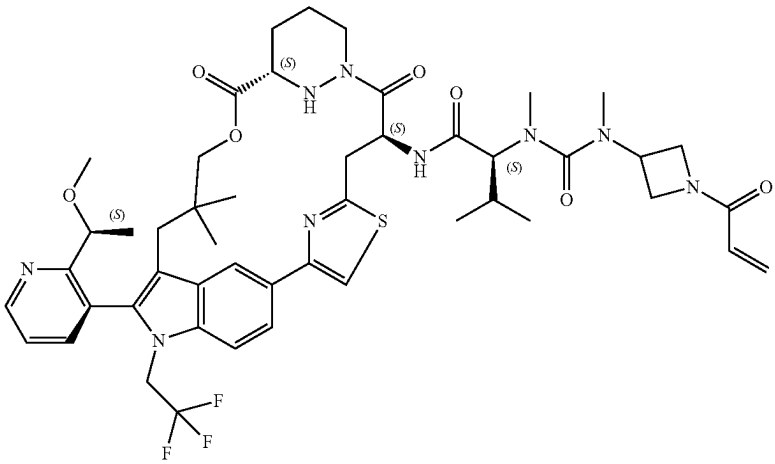
Certain Compounds of the Present Invention	
Ex#	Structure
A709	
A710	
A711	

TABLE 1-continued

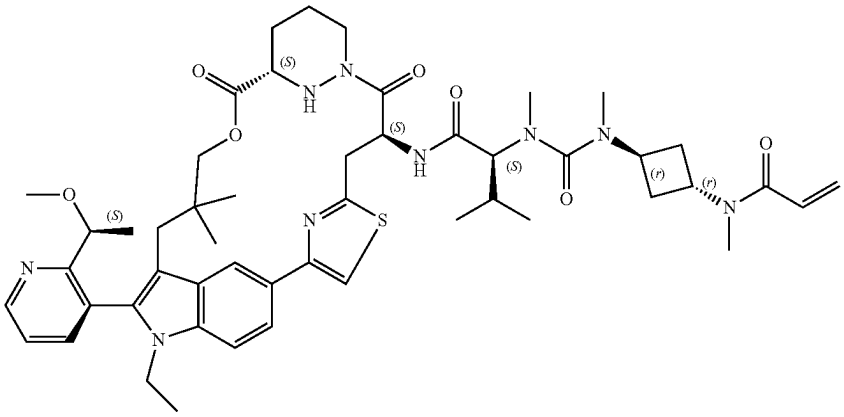
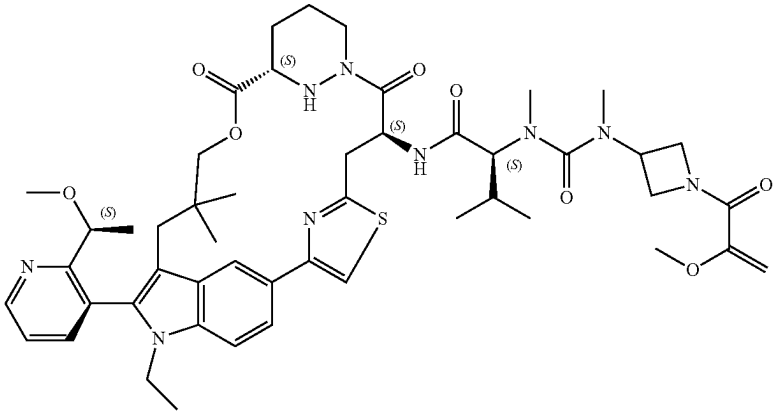
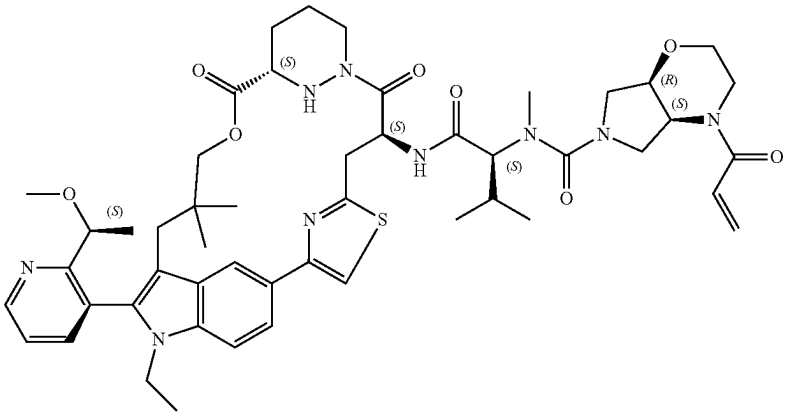
Certain Compounds of the Present Invention	
Ex#	Structure
A712	
A713	
A714	

TABLE 1-continued

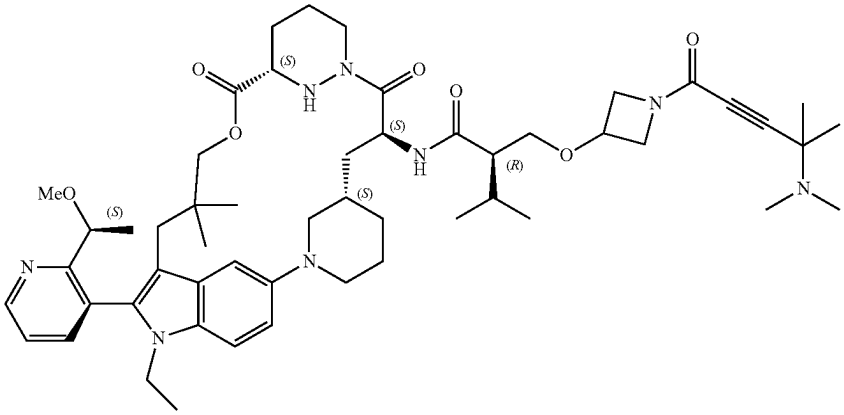
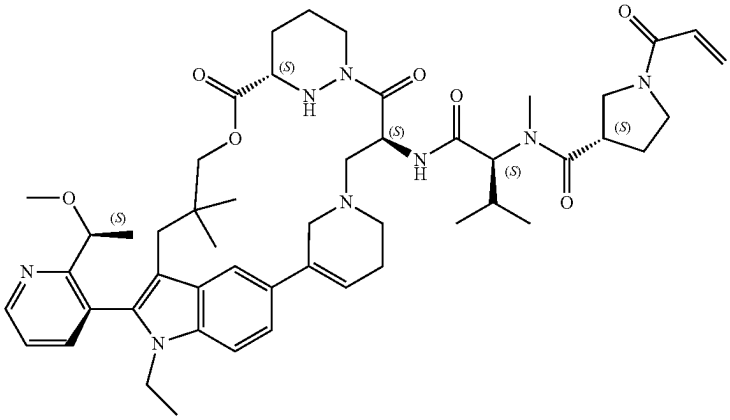
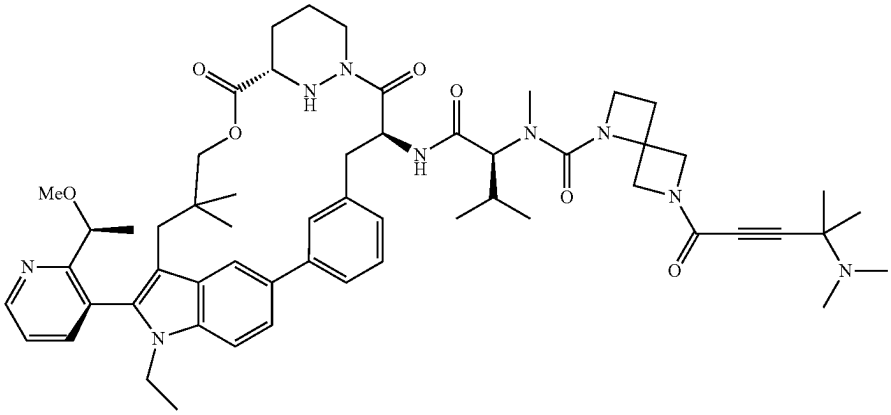
Certain Compounds of the Present Invention	
Ex#	Structure
A715	
A716	
A717	

TABLE 1-continued

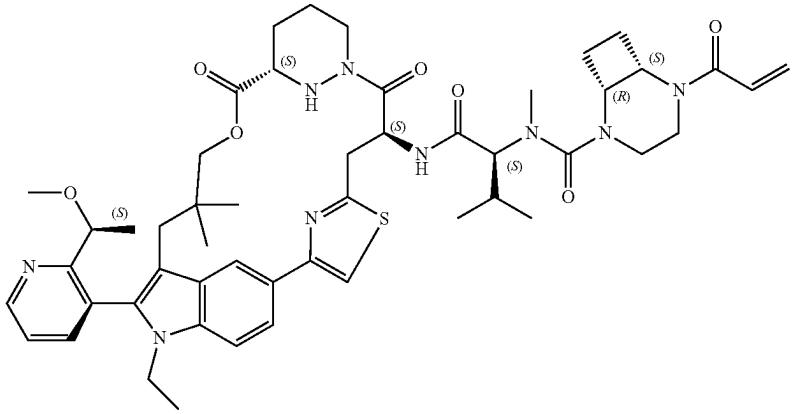
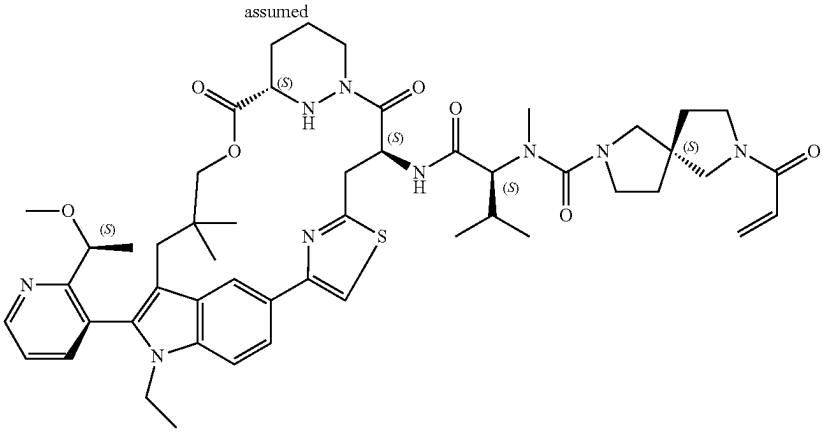
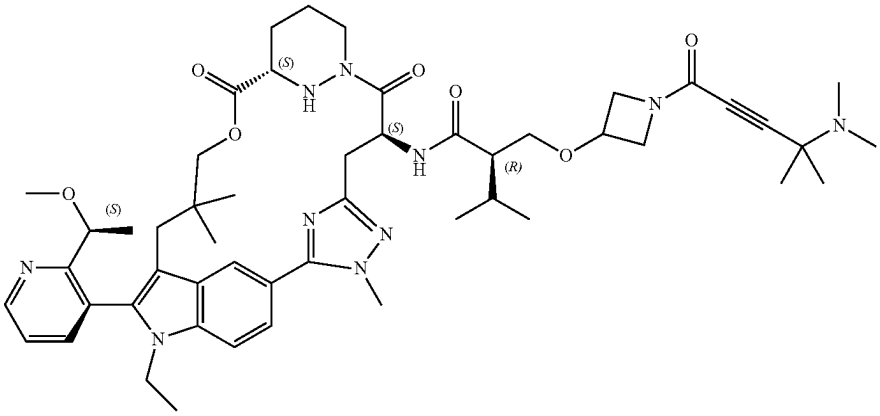
Certain Compounds of the Present Invention	
Ex#	Structure
A718	
A719	assumed 
A720	

TABLE 1-continued

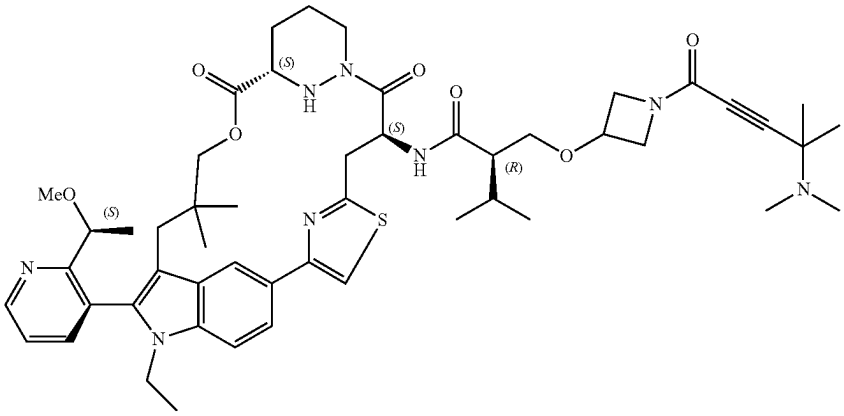
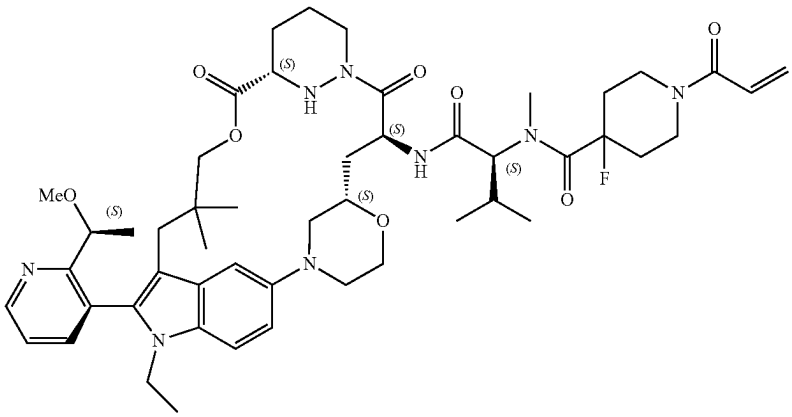
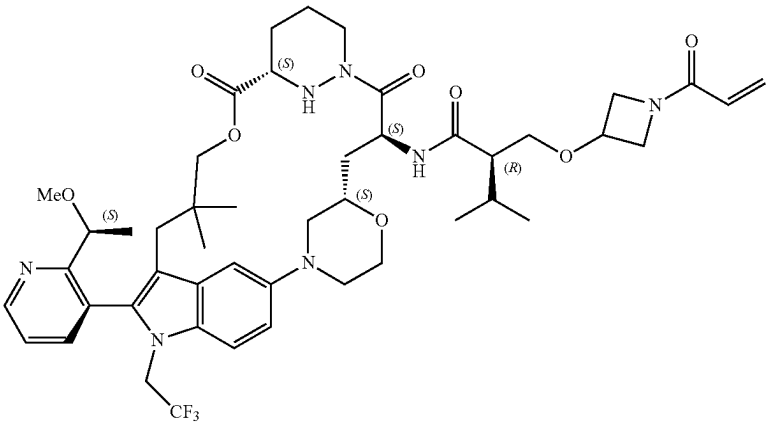
Certain Compounds of the Present Invention	
Ex#	Structure
A721	
A722	
A723	

TABLE 1-continued

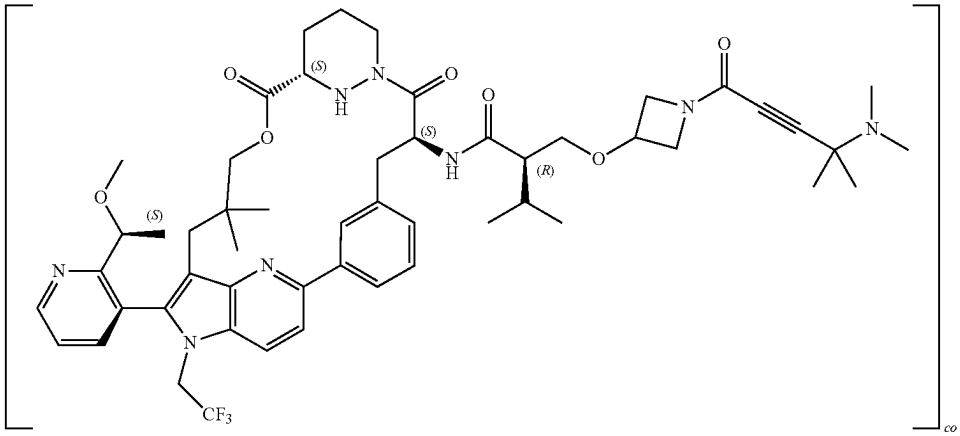
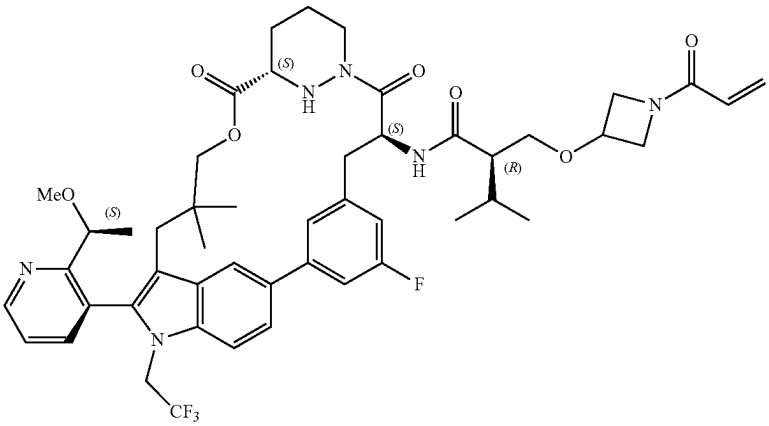
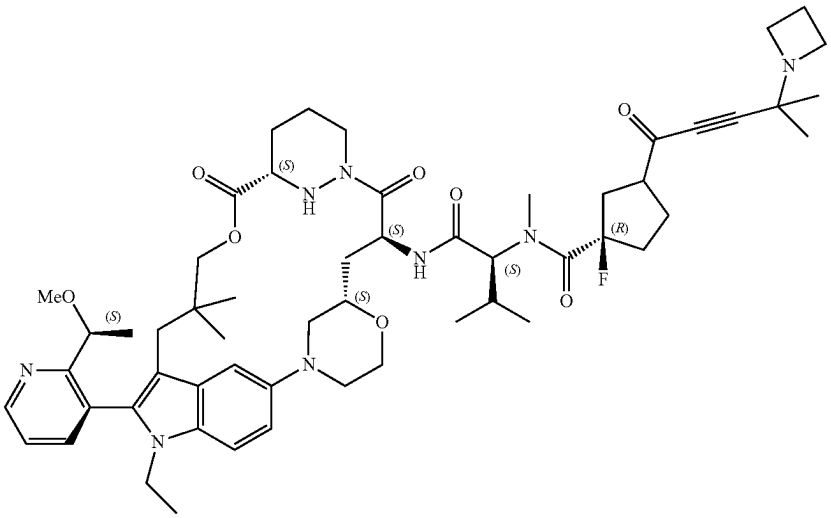
Certain Compounds of the Present Invention	
Ex#	Structure
A724	
A725	
A726	

TABLE 1-continued

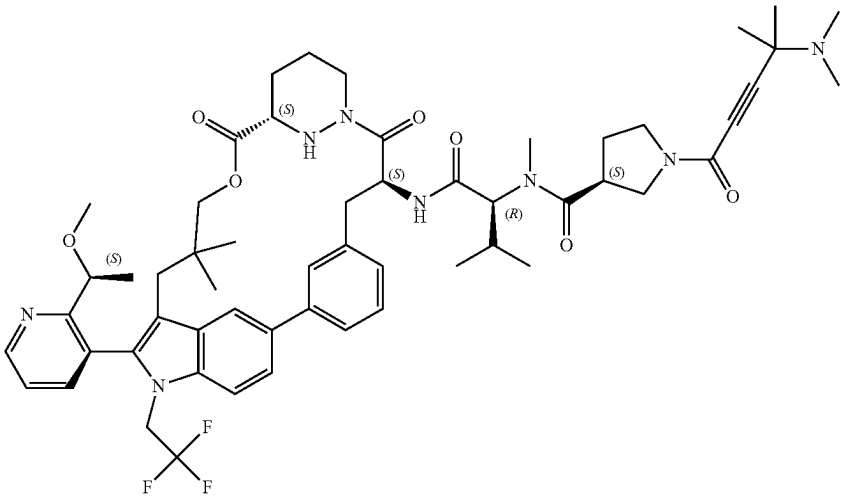
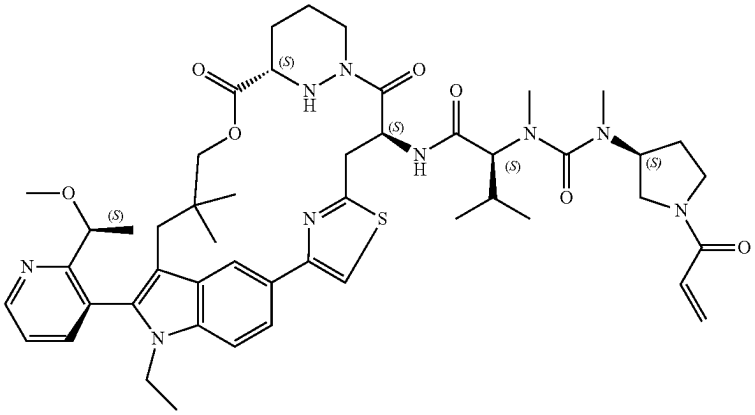
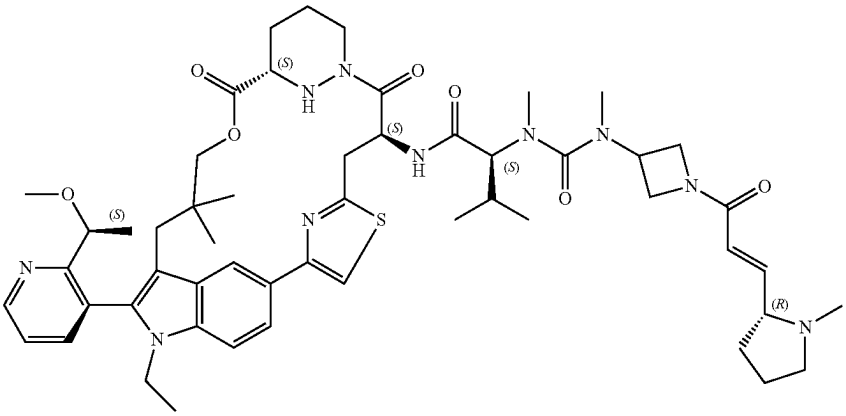
Certain Compounds of the Present Invention	
Ex#	Structure
A727	
A728	
A729	

TABLE 1-continued

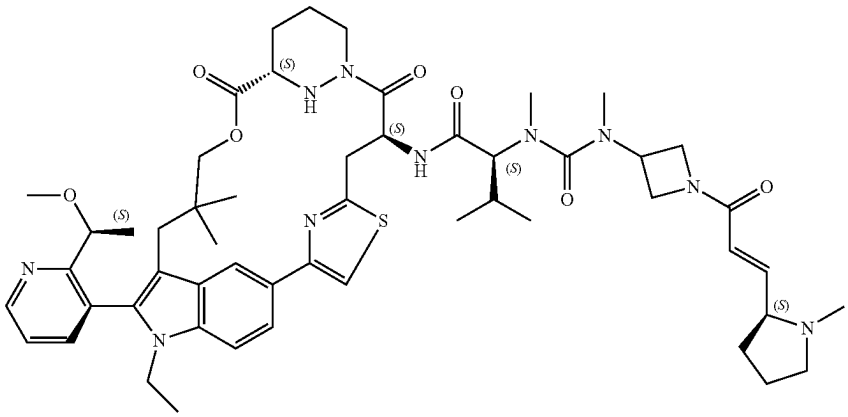
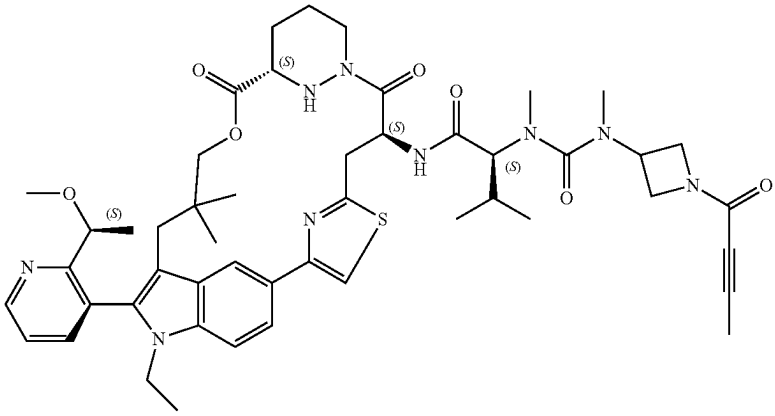
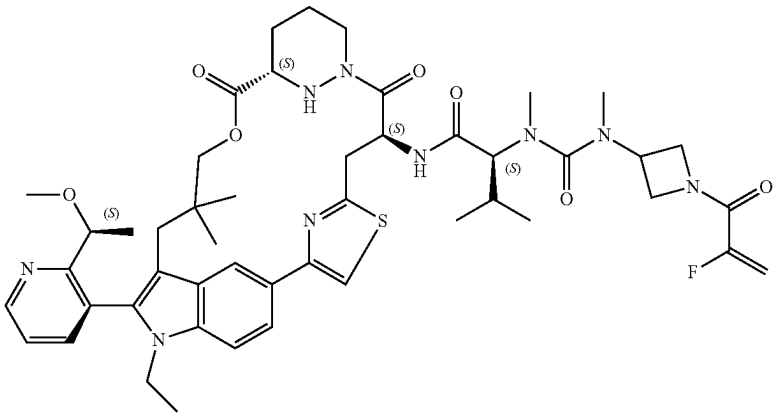
Certain Compounds of the Present Invention	
Ex#	Structure
A730	
A731	
A732	

TABLE 1-continued

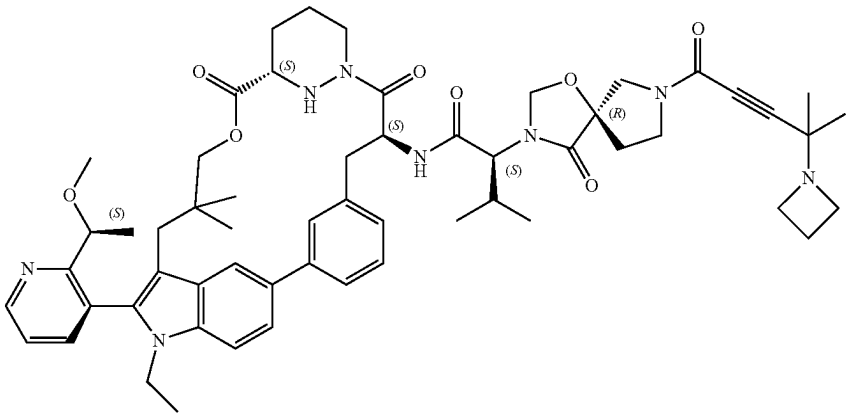
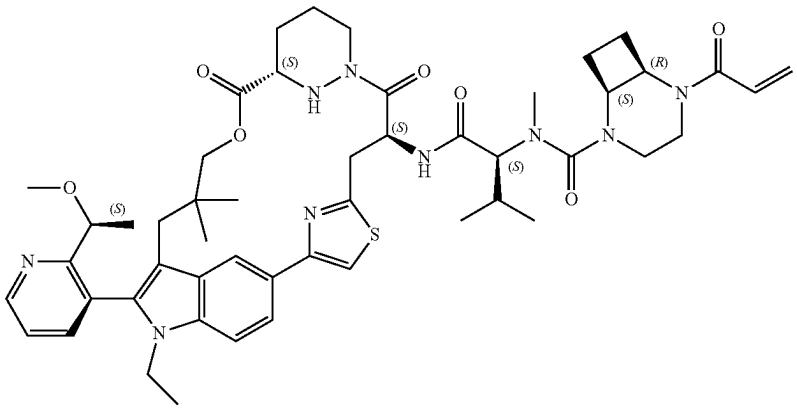
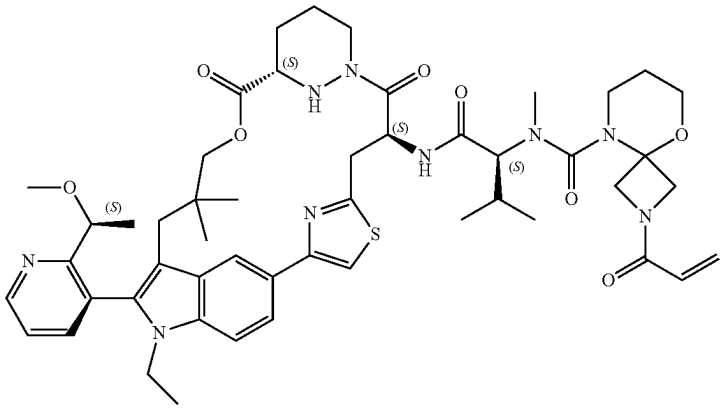
Certain Compounds of the Present Invention	
Ex#	Structure
A733	
A734	
A735	

TABLE 1-continued

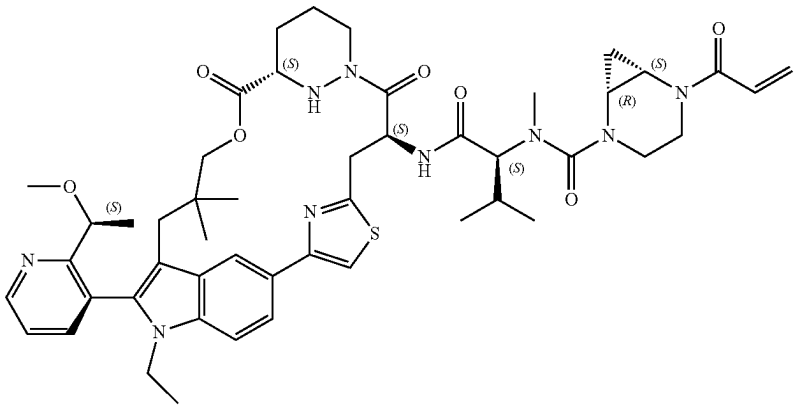
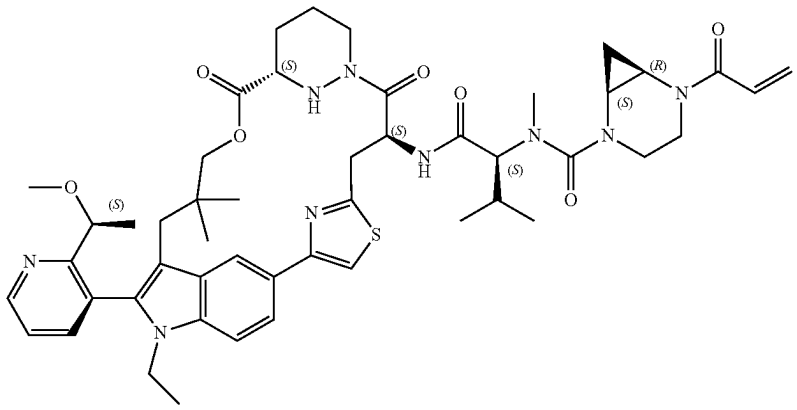
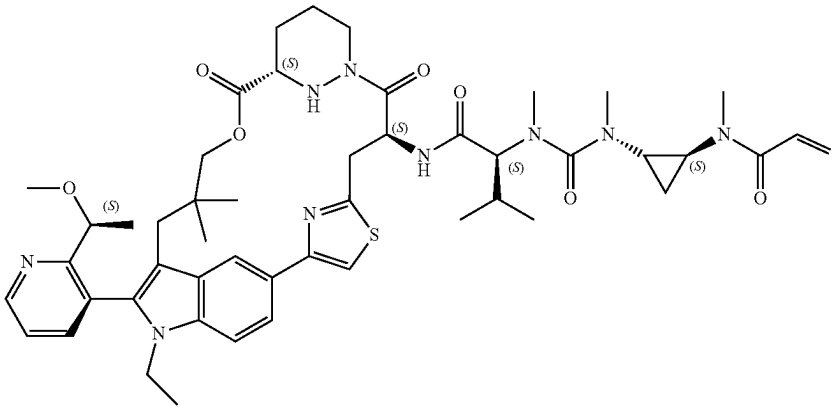
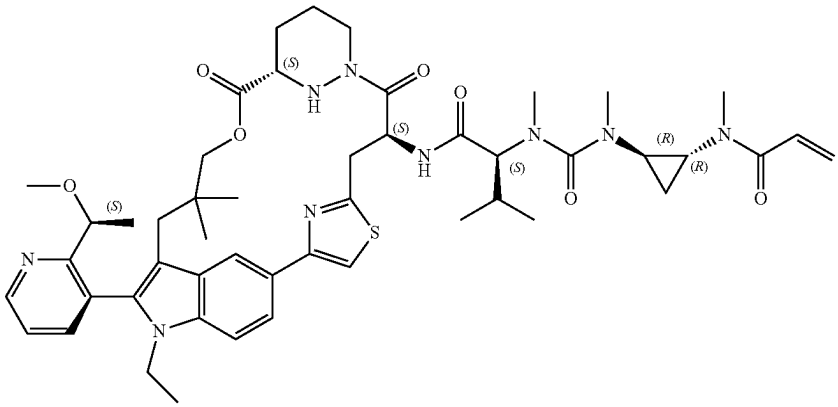
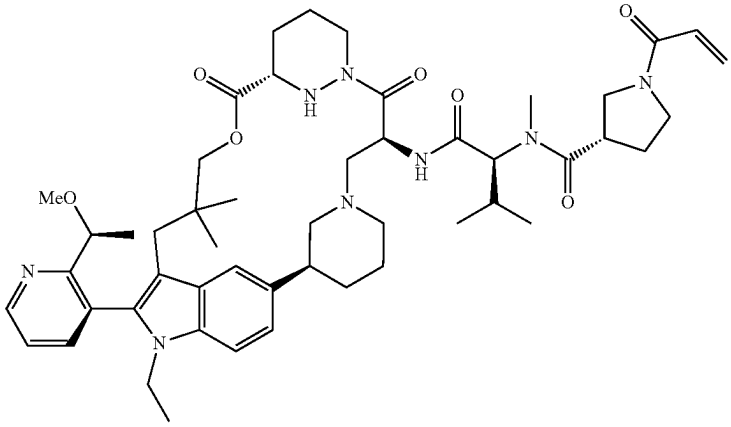
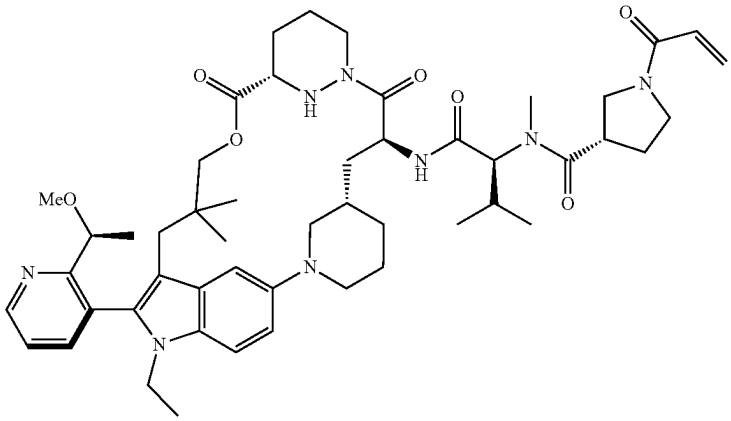
Certain Compounds of the Present Invention	
Ex#	Structure
A736	
A737	
A738	

TABLE 1-continued

Certain Compounds of the Present Invention

Ex#	Structure
A739	
A740	
A741	

Note

that some compounds are shown with bonds as flat or wedged. In some instances, the relative stereochemistry of stereoisomers has been determined; in some instances, the absolute stereochemistry has been determined. In some instances, a single Example number corresponds to a mixture of stereoisomers. All stereoisomers of the compounds of the foregoing table are contemplated by the present invention. In particular embodiments, an atropisomer of a compound of the foregoing table is contemplated.

Brackets are to be ignored.

*The activity of this stereoisomer may, in fact, be attributable to the presence of a small amount of the stereoisomer with the (S) configuration at the —NC(O)—$\underline{C}$H(CH_3)$_2$—N(CH_3)— position.

In some embodiments, a compound of Table 2 is provided, or a pharmaceutically acceptable salt thereof. In some embodiments, a compound of the present invention is selected from Table 2, or a pharmaceutically acceptable salt or atropisomer thereof.

TABLE 2

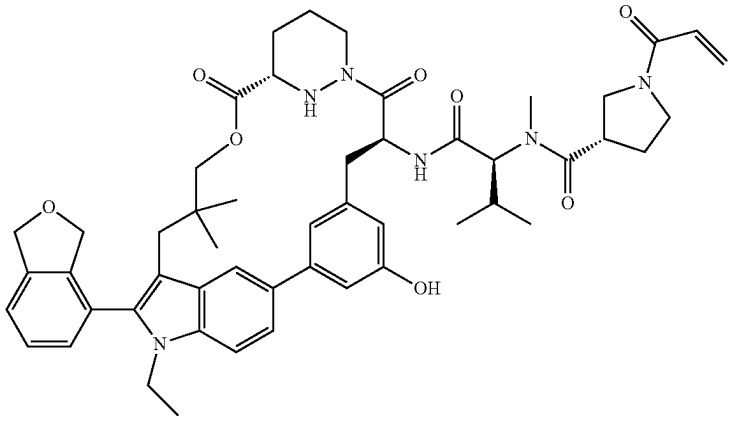
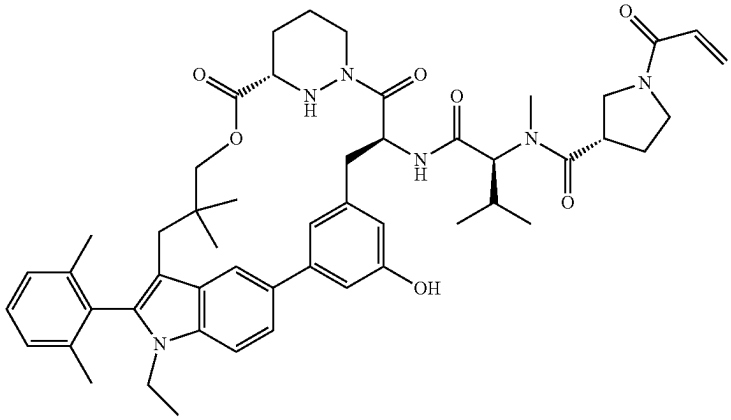
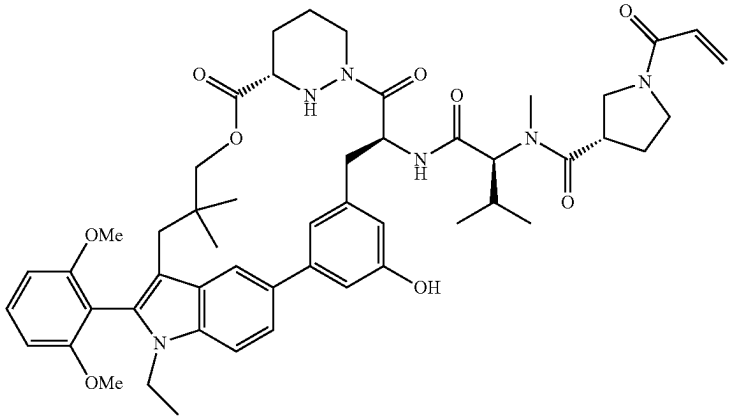
Certain Compounds of the Present Invention	
Ex #	Structure
B1	
B2	
B3	

TABLE 2-continued

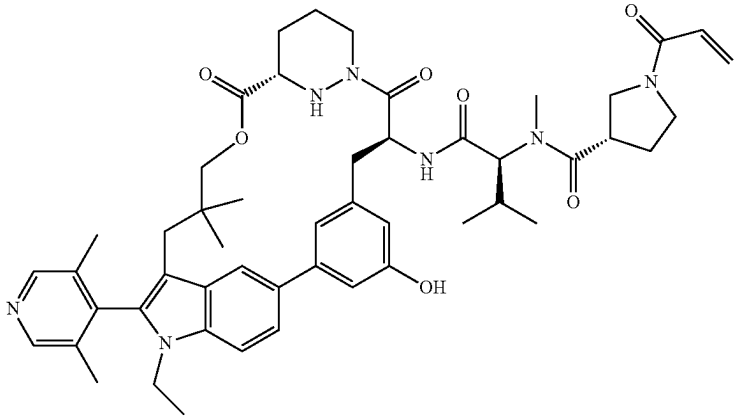
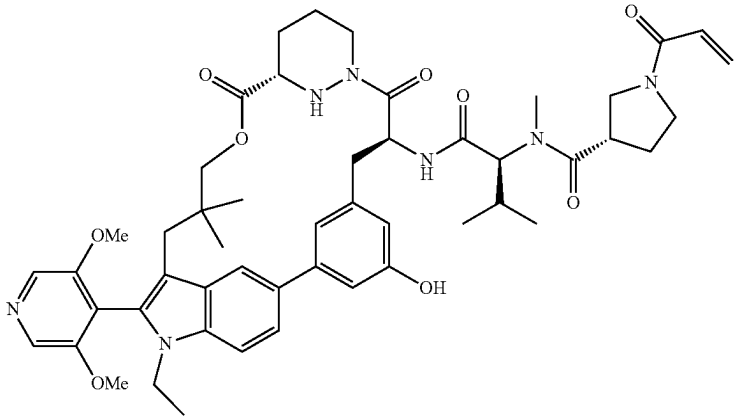
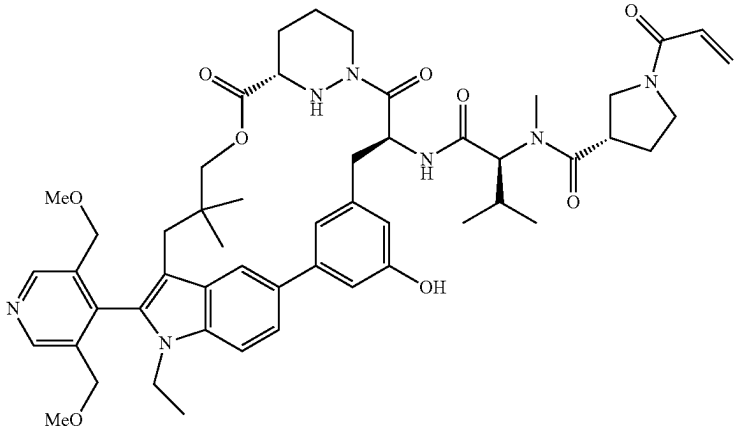
Certain Compounds of the Present Invention	
Ex #	Structure
B4	
B5	
B6	

TABLE 2-continued

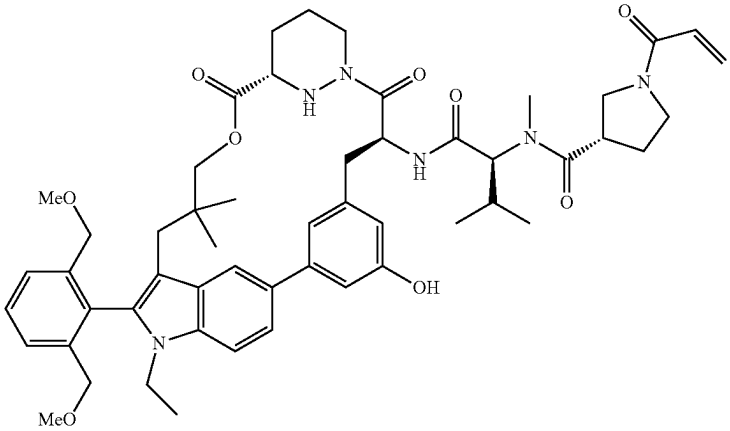
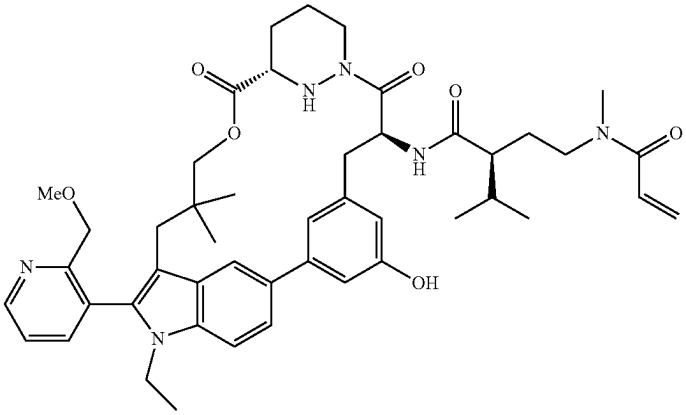
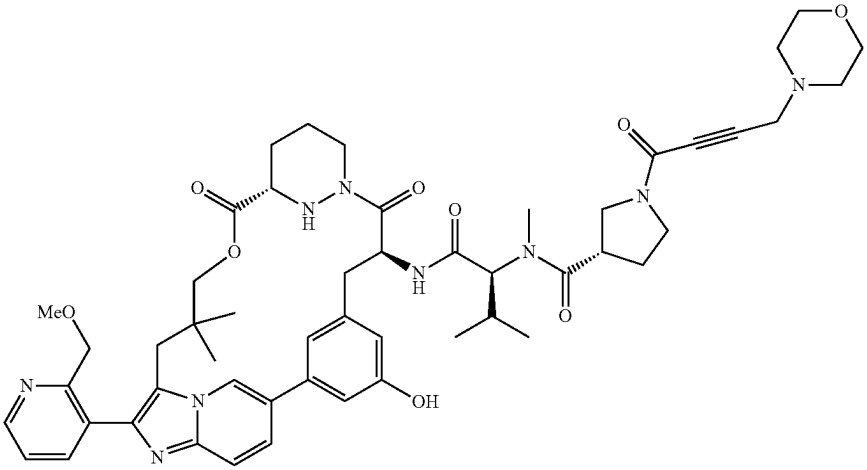
Certain Compounds of the Present Invention	
Ex #	Structure
B7	
B11	
B12	

TABLE 2-continued

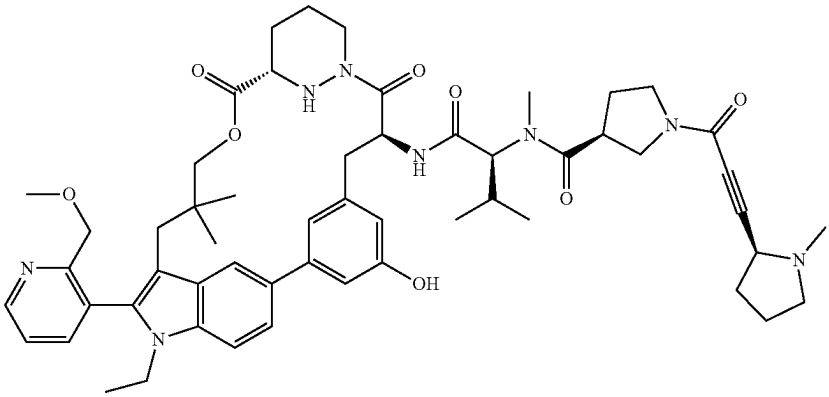
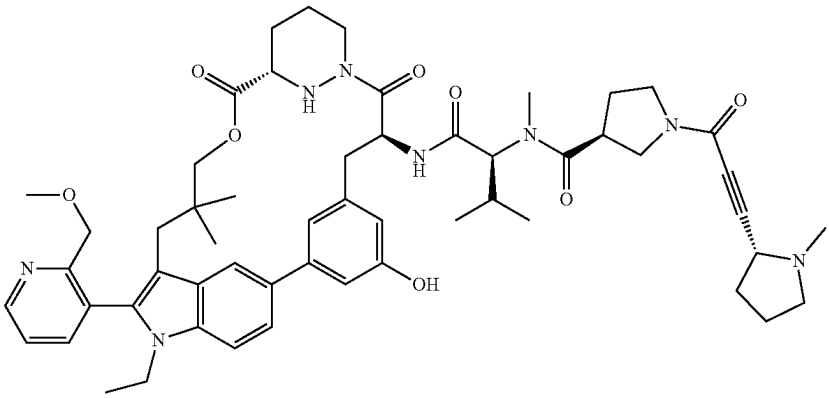
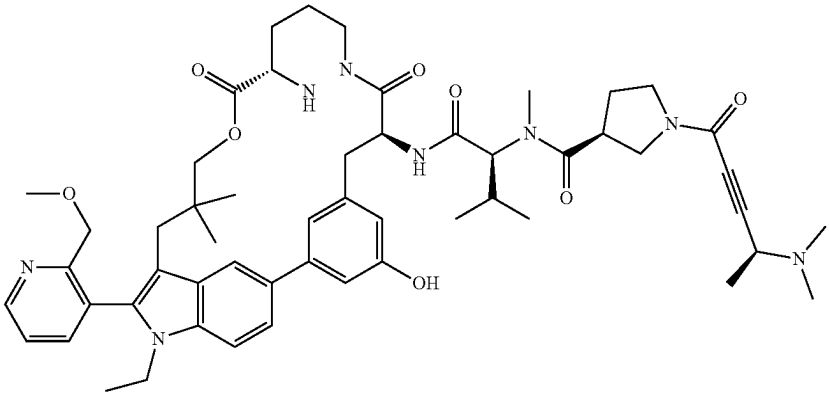
Certain Compounds of the Present Invention	
Ex #	Structure
B13	
B18	
B21	

TABLE 2-continued

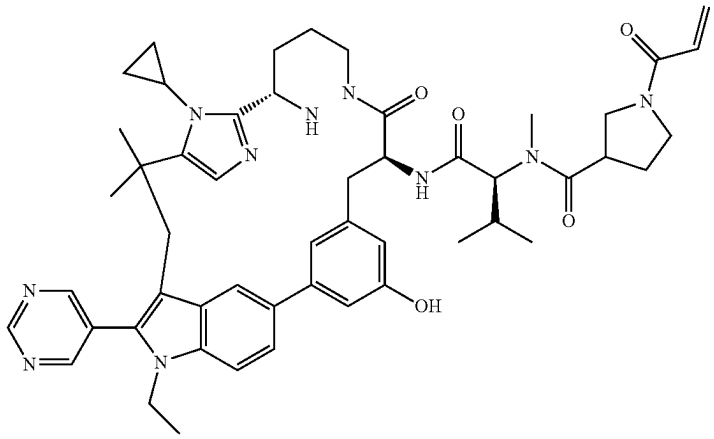
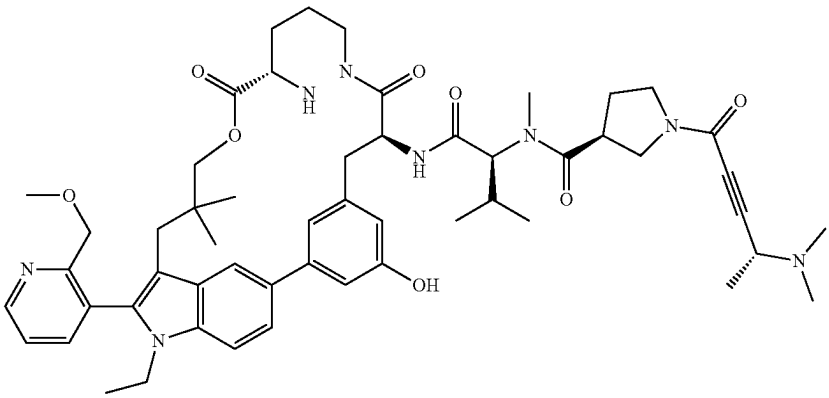
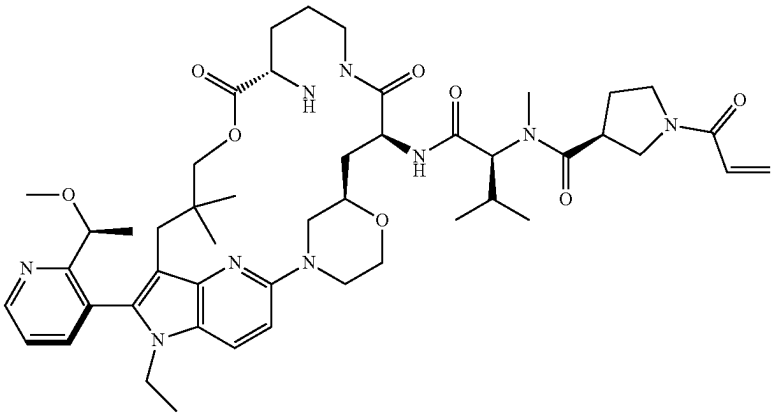
Certain Compounds of the Present Invention	
Ex #	Structure
B22	
B25	
B27	

TABLE 2-continued

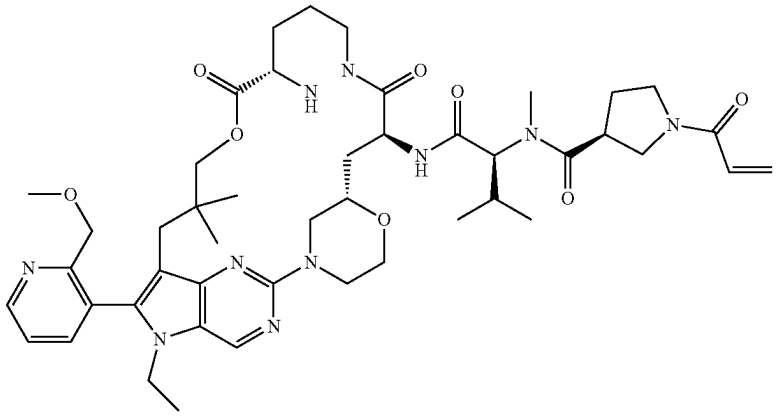
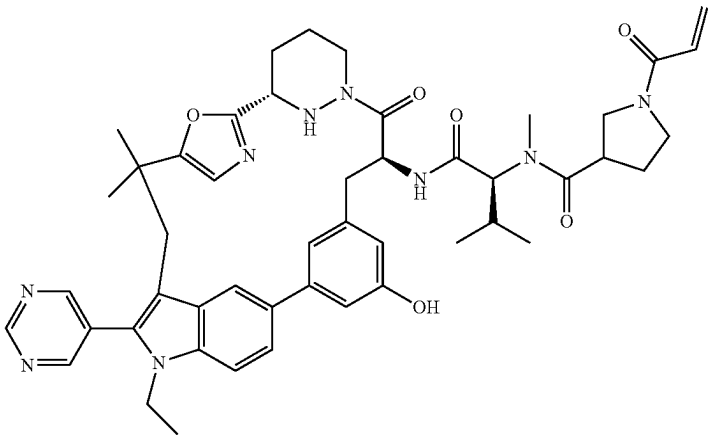
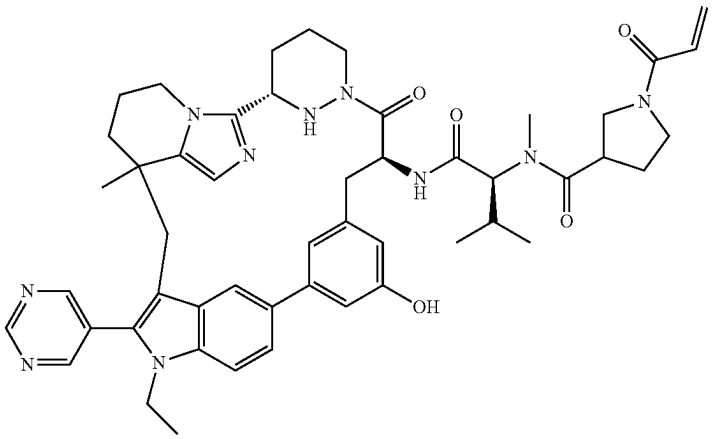
Certain Compounds of the Present Invention	
Ex #	Structure
B28	
B29	
B30	

TABLE 2-continued

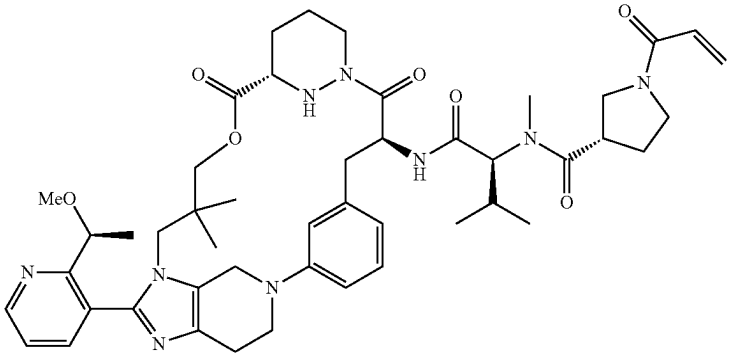
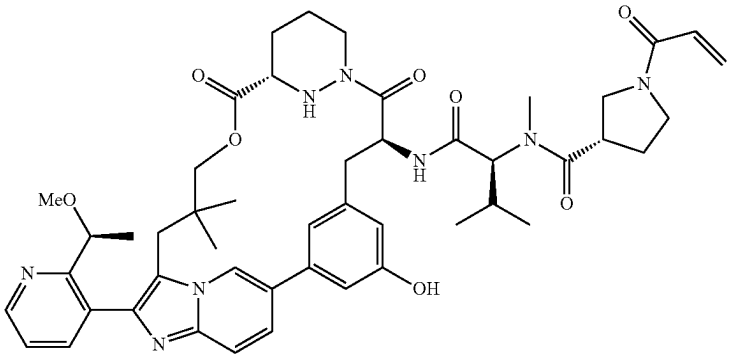
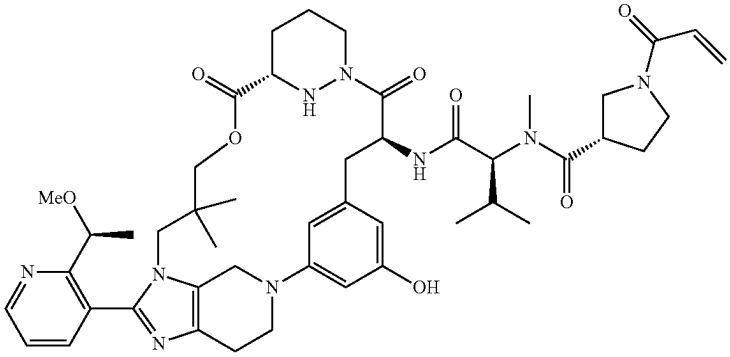
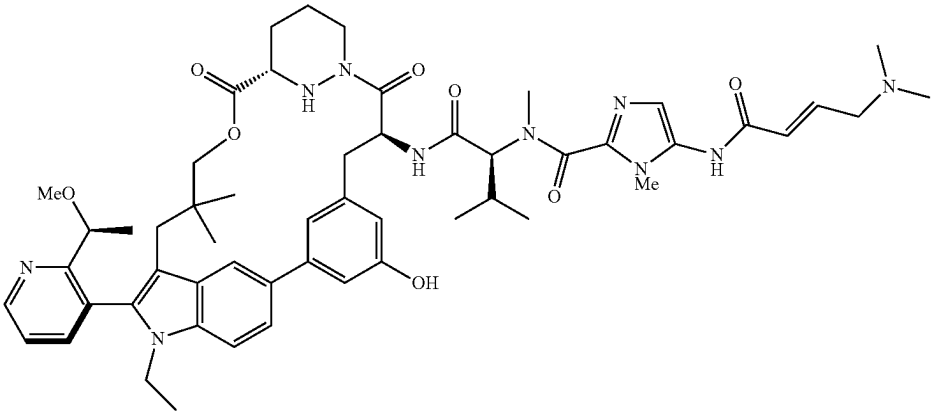
Certain Compounds of the Present Invention	
Ex #	Structure
B32	
B34	
B38	
B47	

TABLE 2-continued

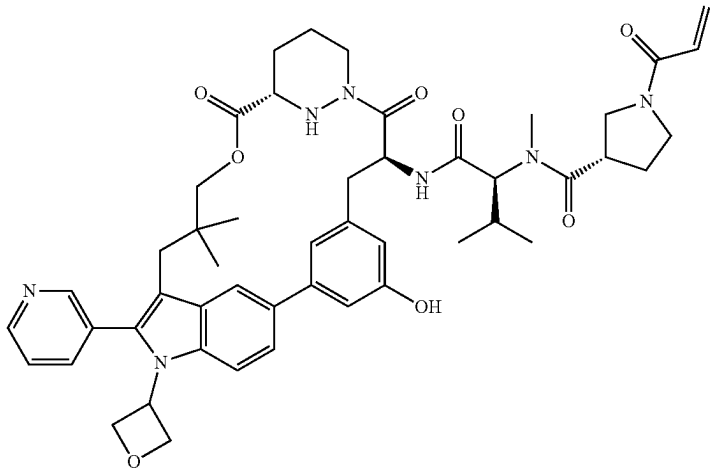
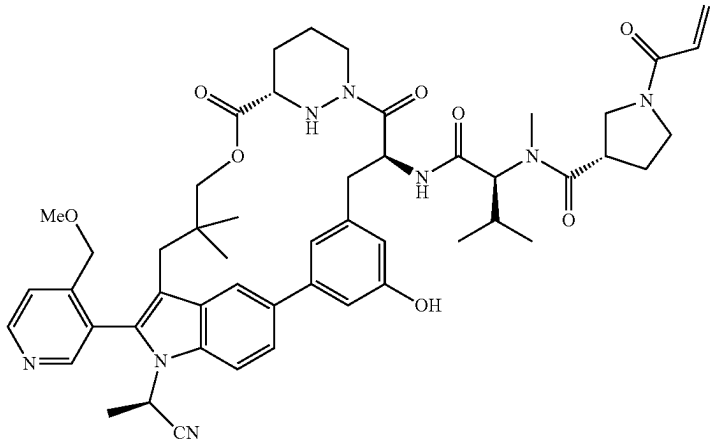
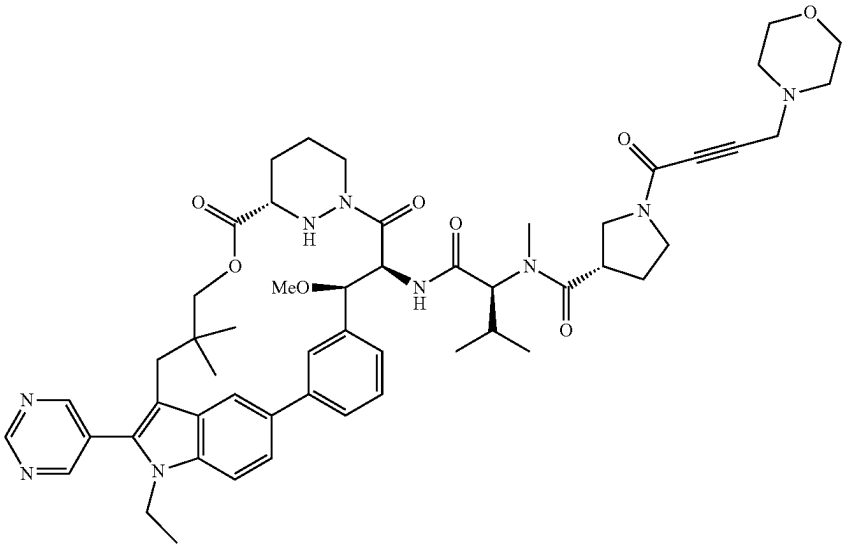
Certain Compounds of the Present Invention	
Ex #	Structure
B64	
B65	
B66	

TABLE 2-continued

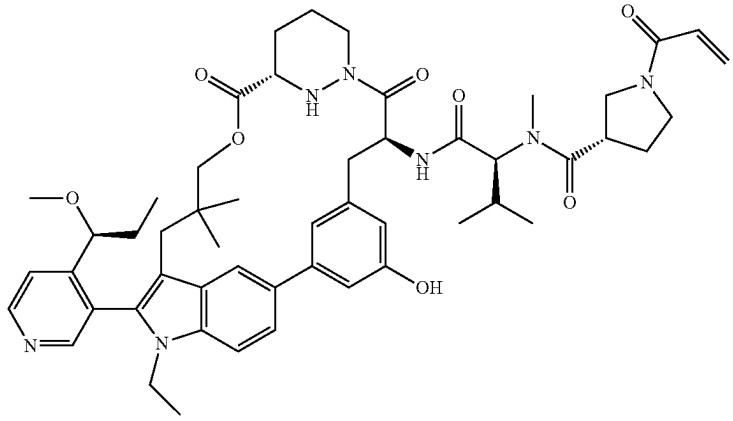
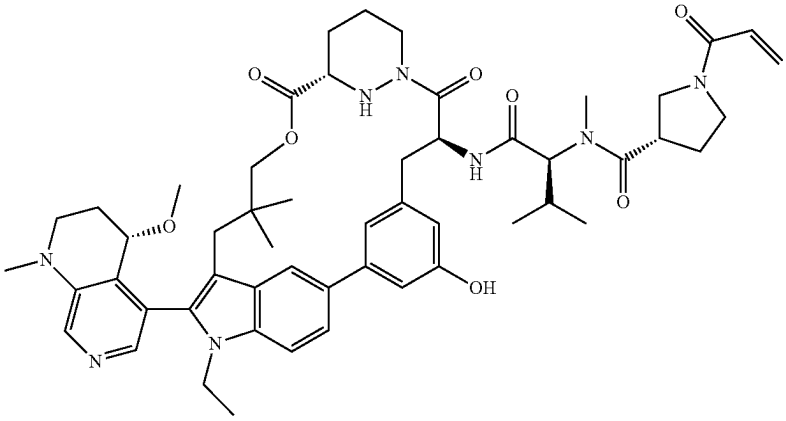
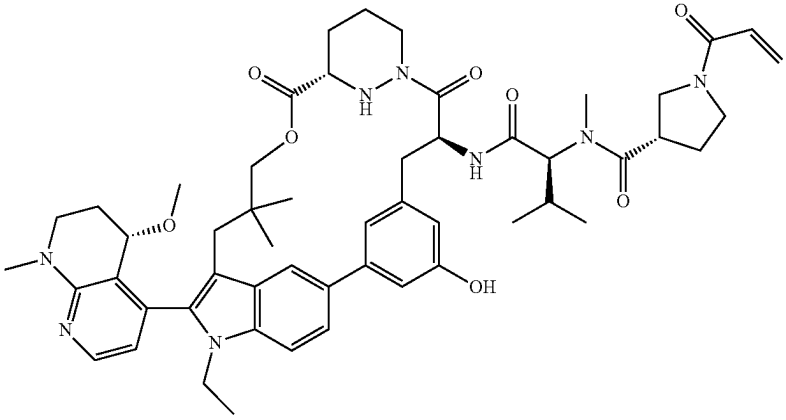
Certain Compounds of the Present Invention	
Ex #	Structure
B70	
B73	
B74	

TABLE 2-continued

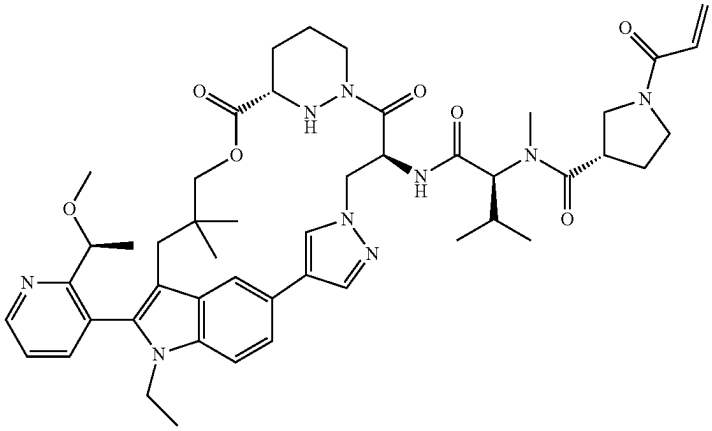
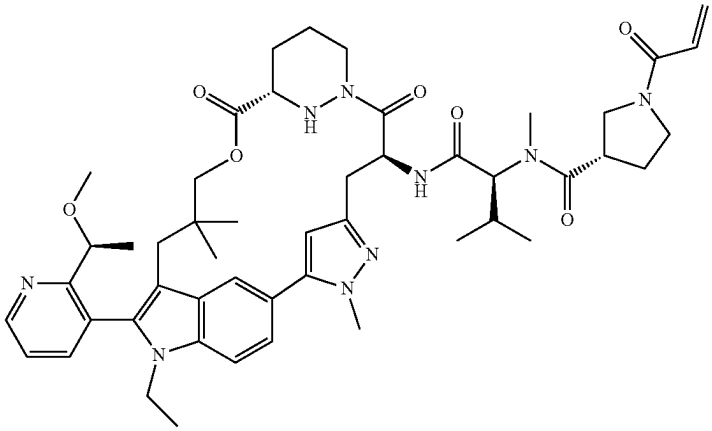
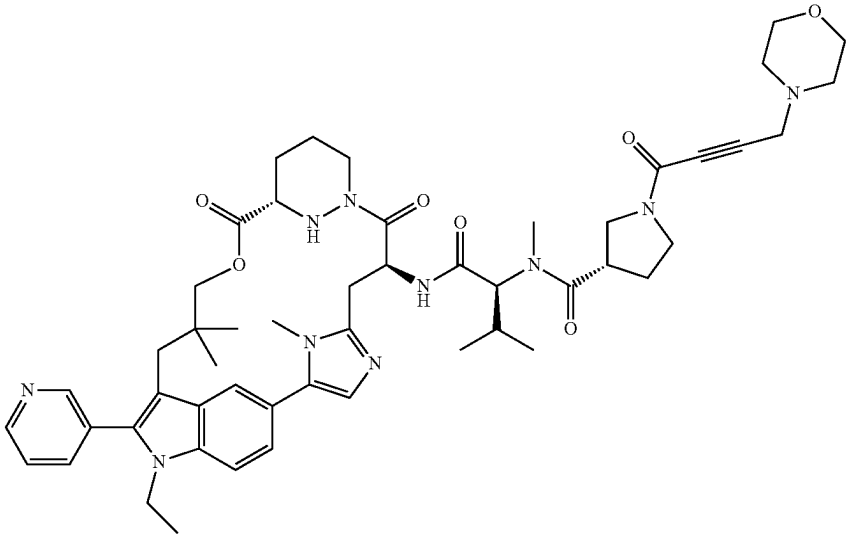
Certain Compounds of the Present Invention	
Ex #	Structure
B75	
B76	
B77	

TABLE 2-continued

Certain Compounds of the Present Invention	
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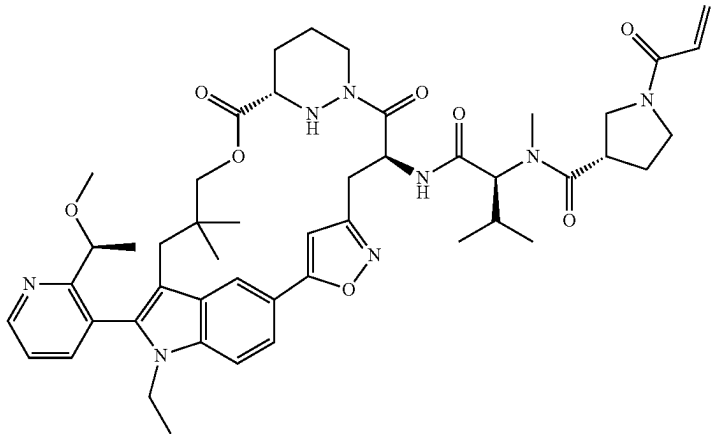
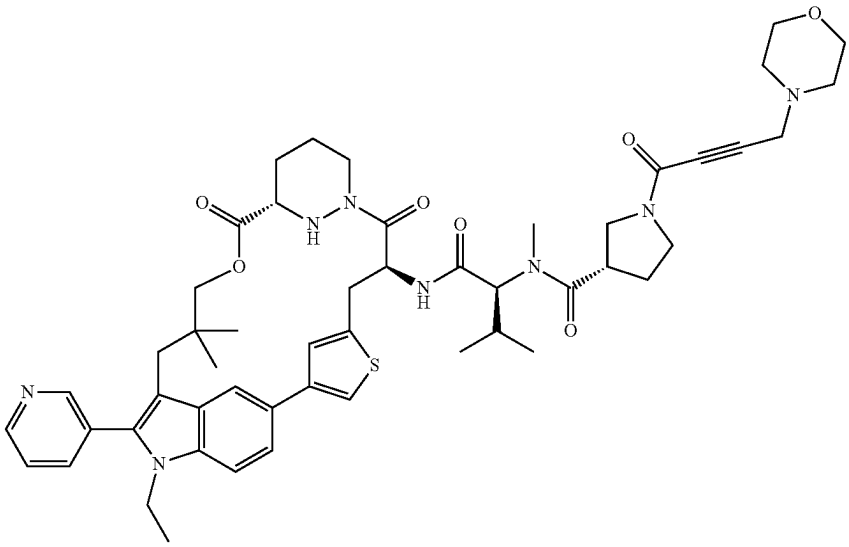
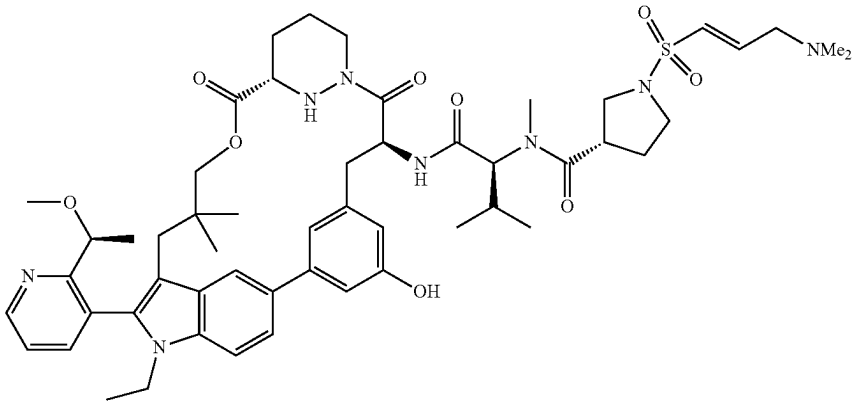
B81	
B83	
B85	

TABLE 2-continued

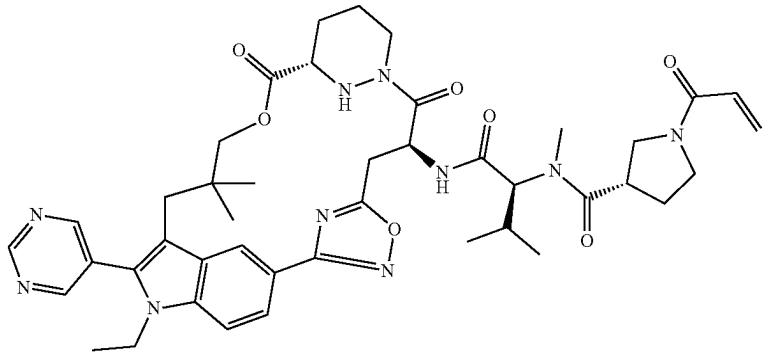
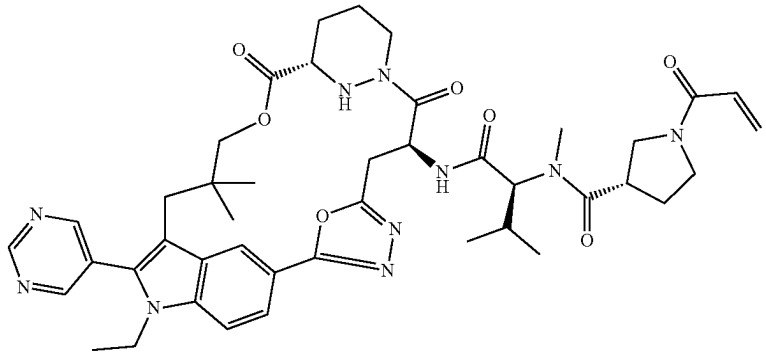
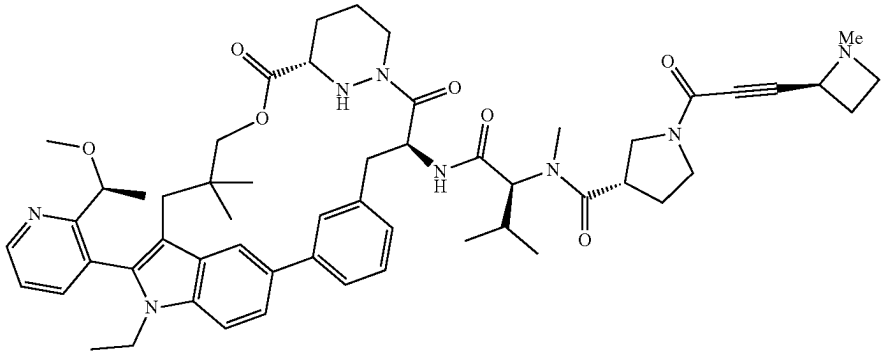
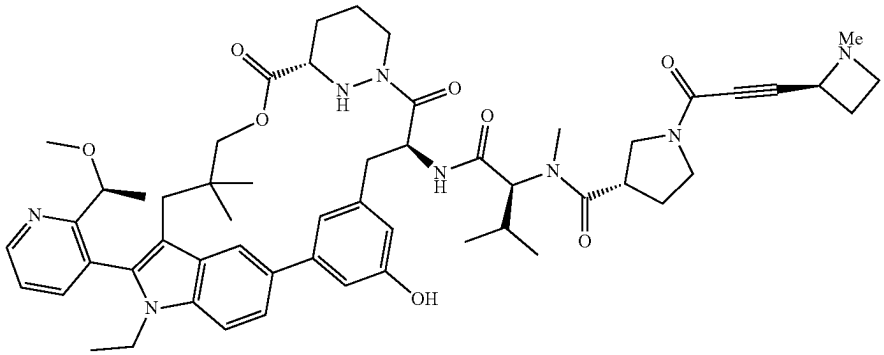
Certain Compounds of the Present Invention	
Ex #	Structure
B86	
B87	
B88	
B89	

TABLE 2-continued

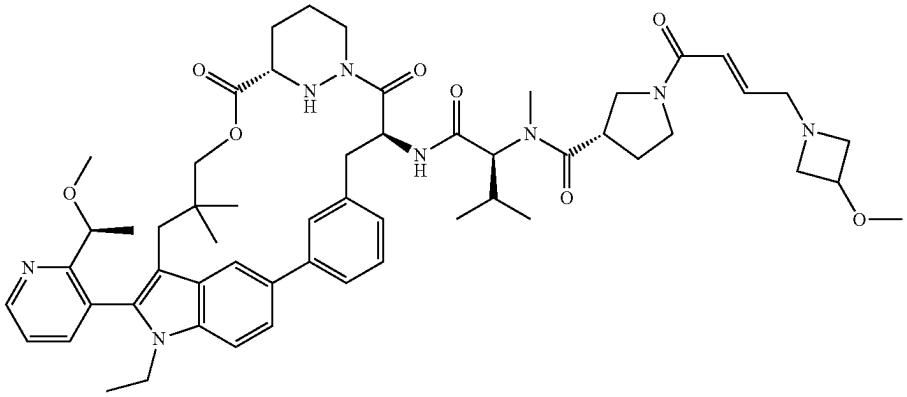
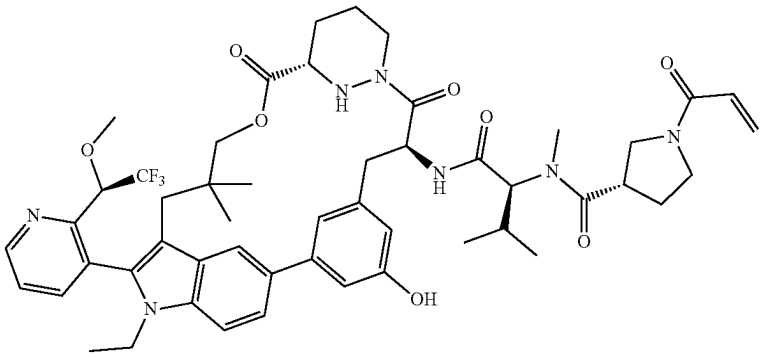
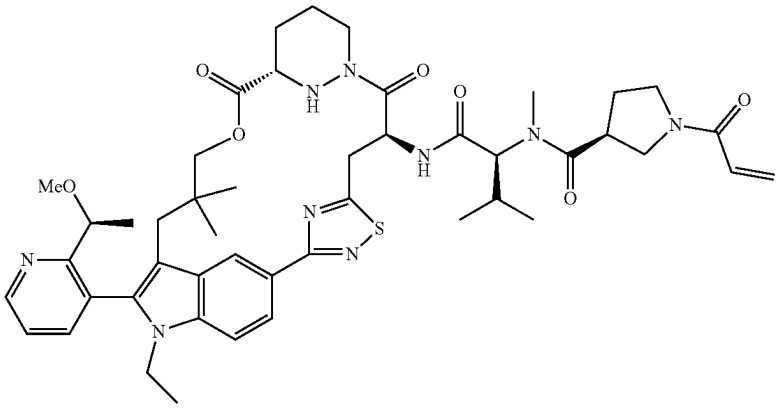
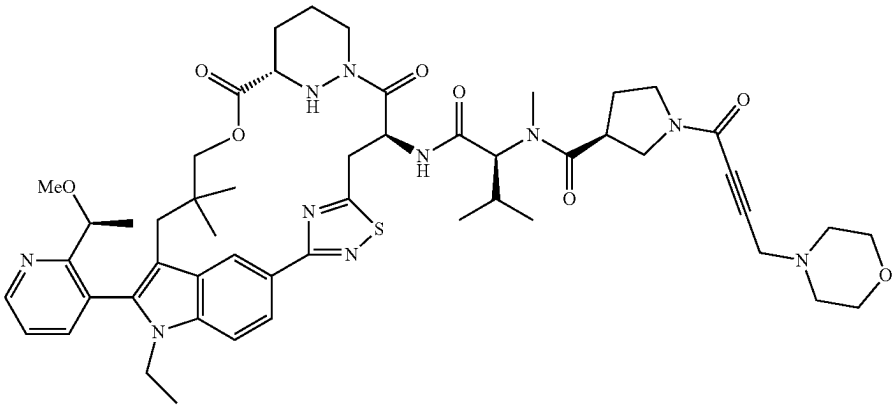
Certain Compounds of the Present Invention	
Ex #	Structure
B90	
B91	
B96	
B97	

TABLE 2-continued

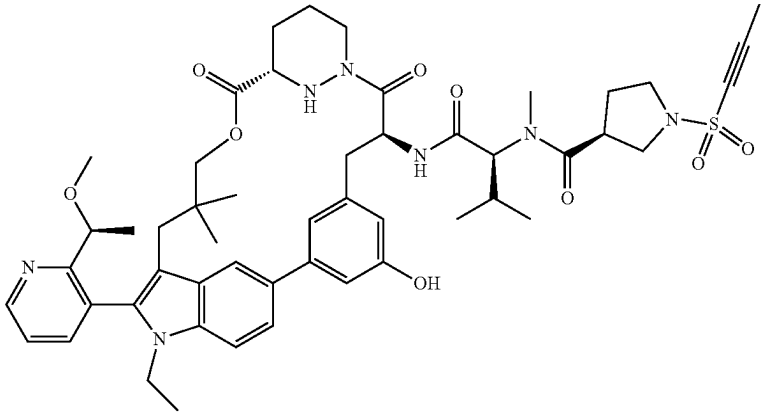
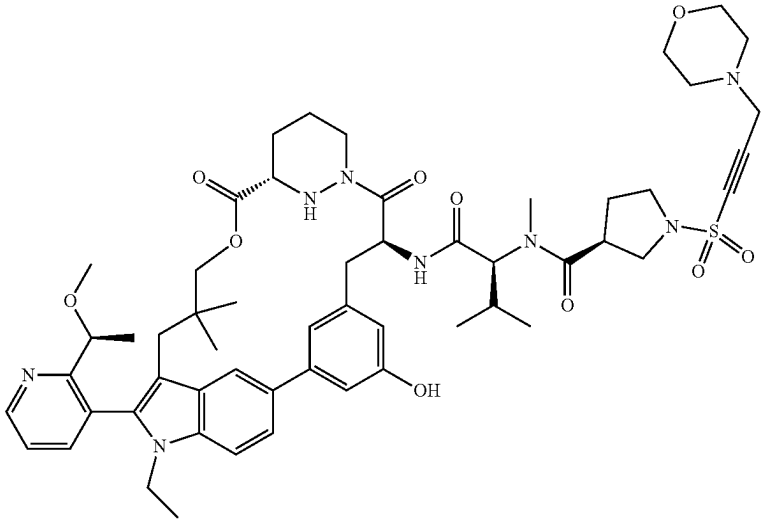
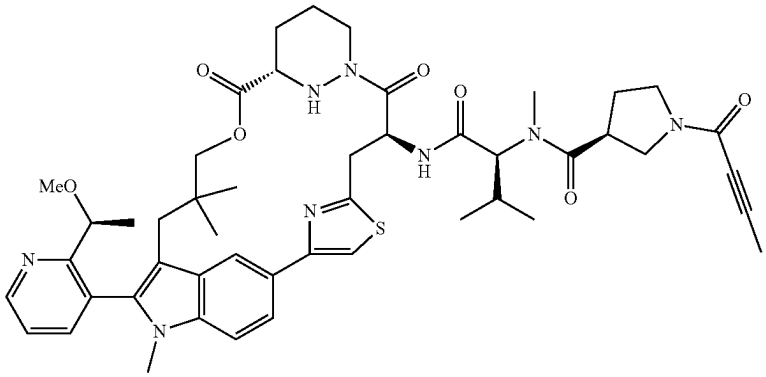
Certain Compounds of the Present Invention	
Ex #	Structure
B102	
B103	
B104	

TABLE 2-continued

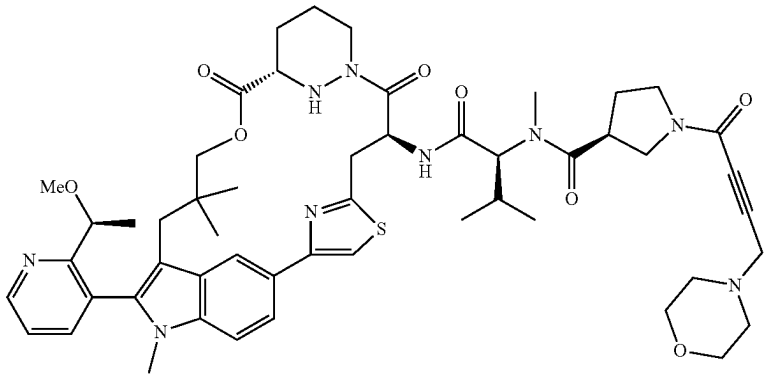
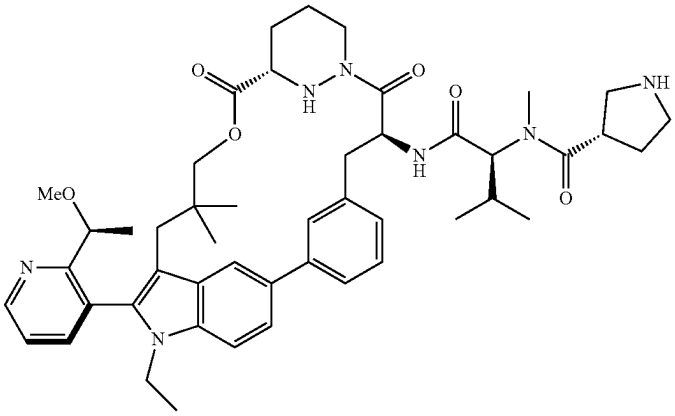
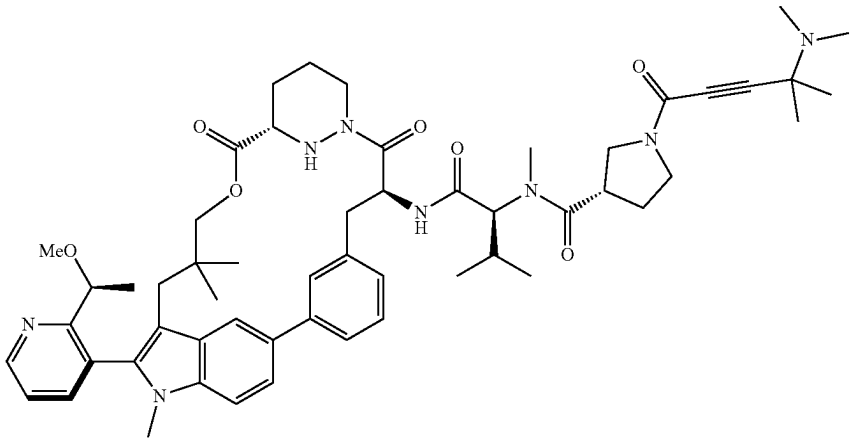
Certain Compounds of the Present Invention	
Ex #	Structure
B106	
B107	
B109	

TABLE 2-continued

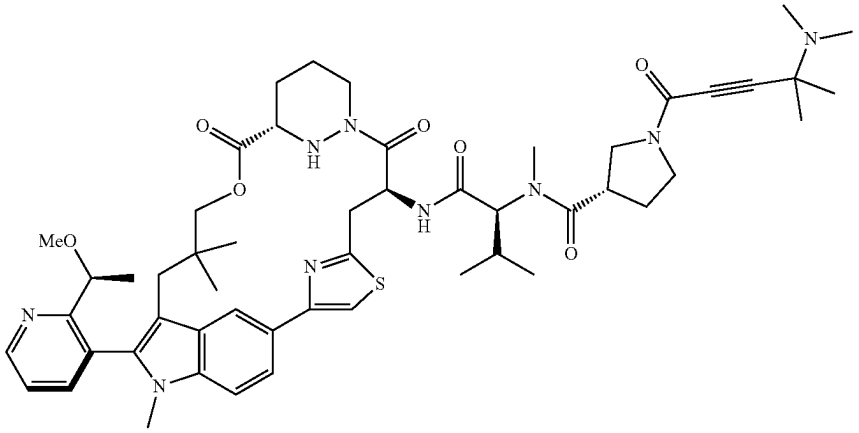
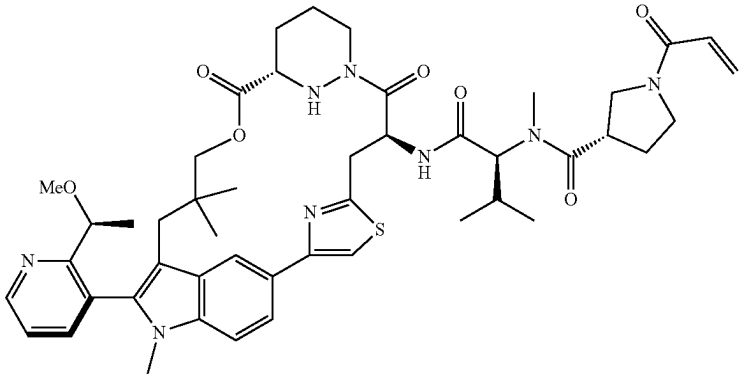
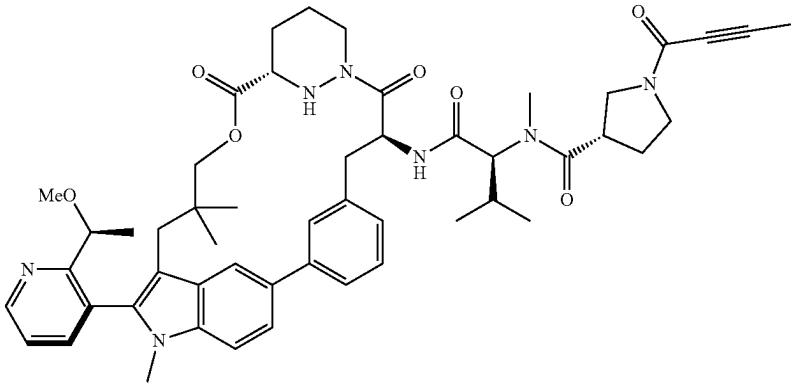
Certain Compounds of the Present Invention	
Ex #	Structure
B111	
B112	
B113	

TABLE 2-continued

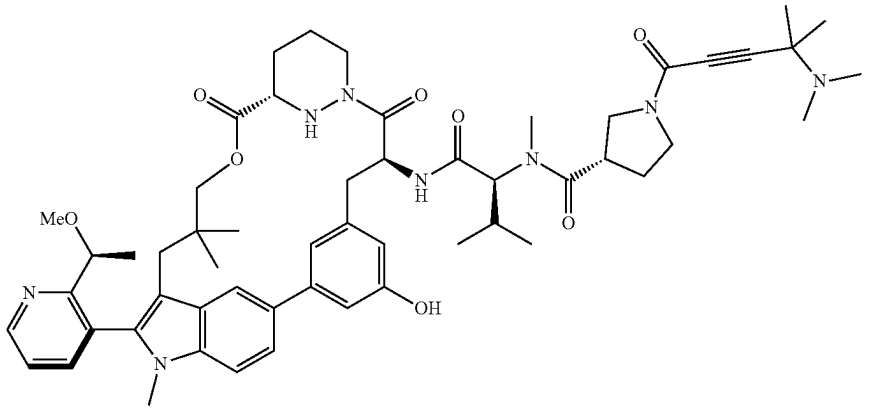
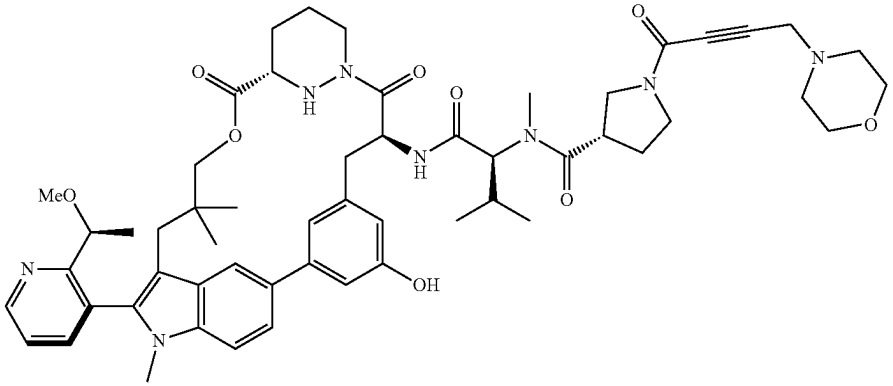
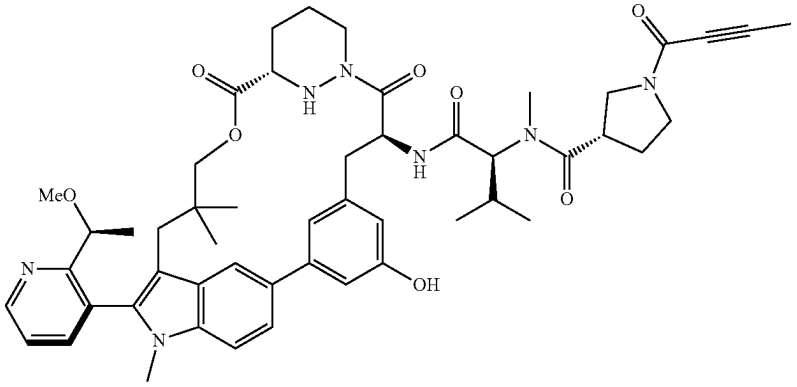
Certain Compounds of the Present Invention	
Ex #	Structure
B115	
B116	
B117	

TABLE 2-continued

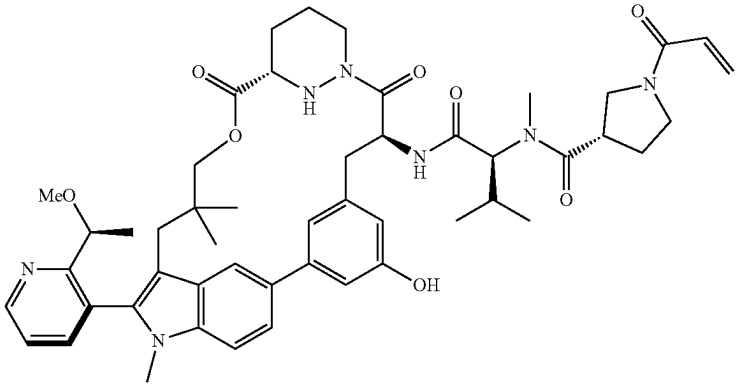
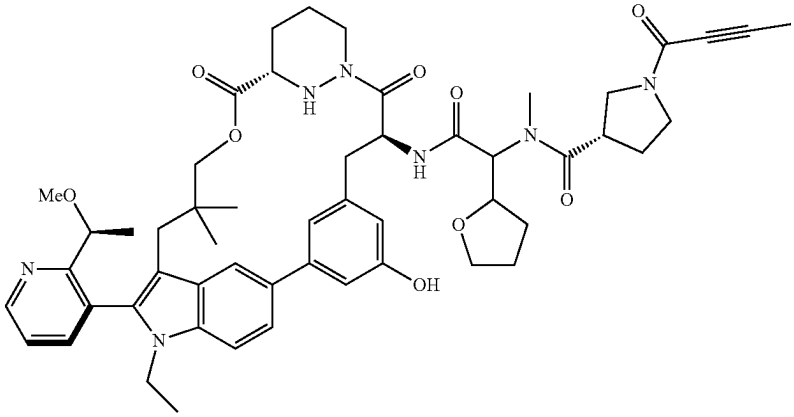
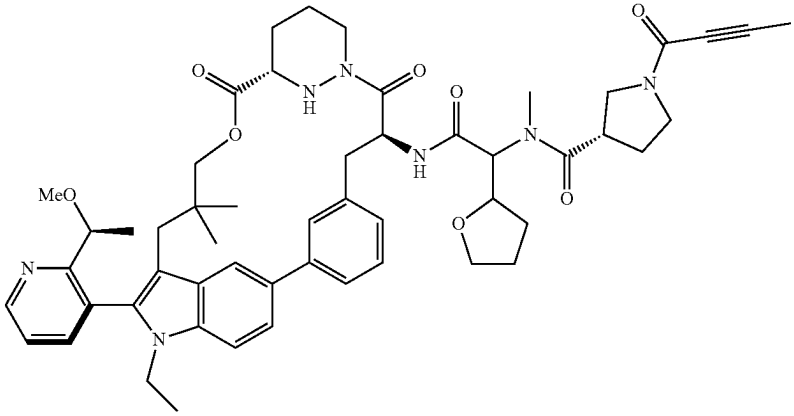
Certain Compounds of the Present Invention	
Ex #	Structure
B118	
B119	
B120	

TABLE 2-continued

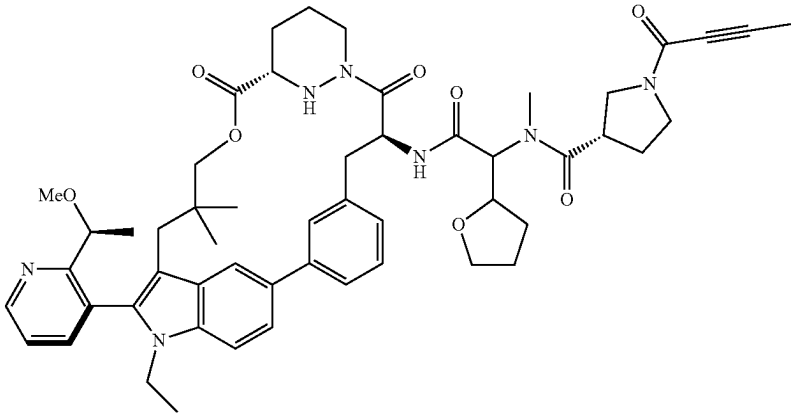
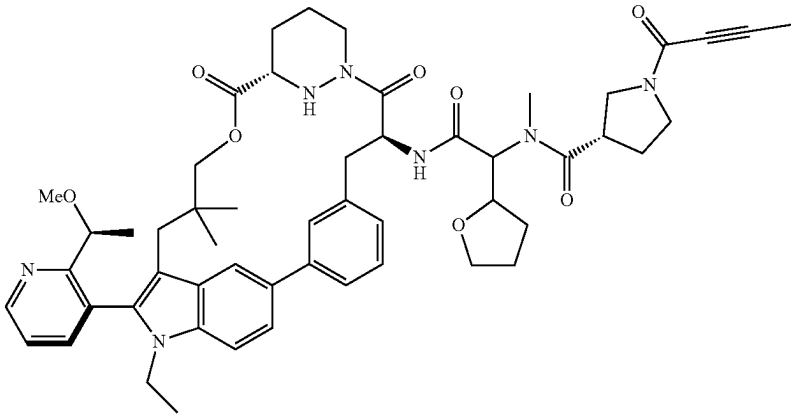
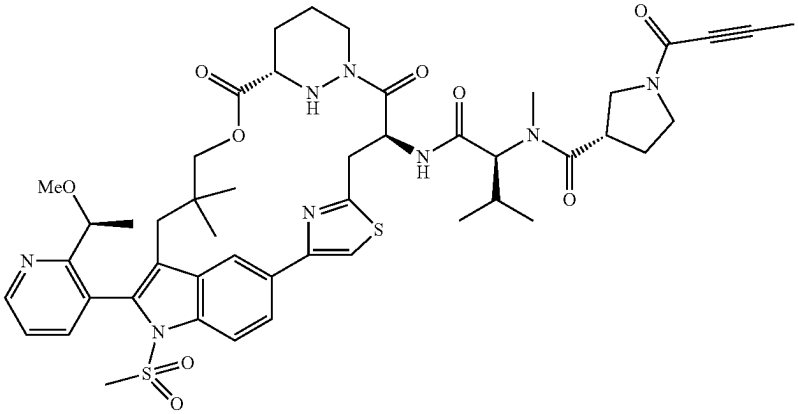
Certain Compounds of the Present Invention	
Ex #	Structure
B121	
B122	
B123	

TABLE 2-continued

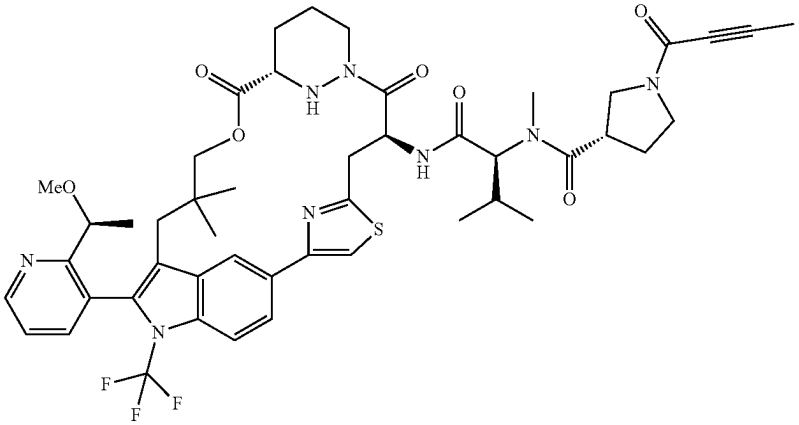
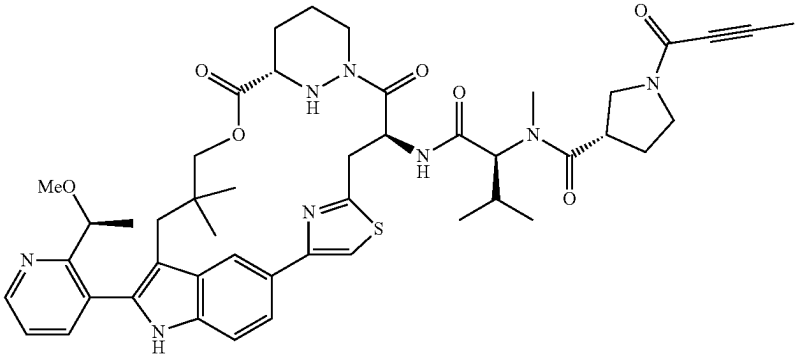
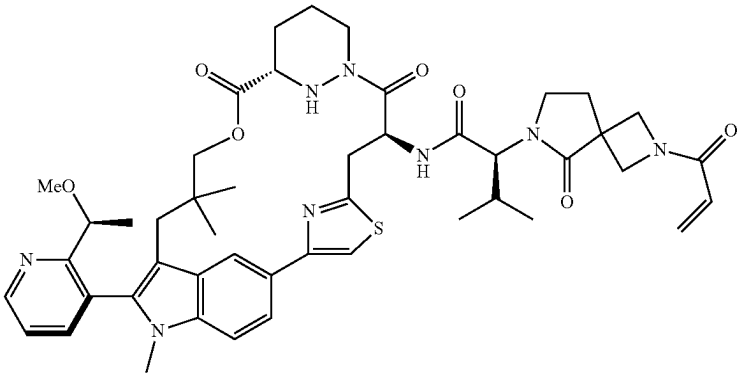
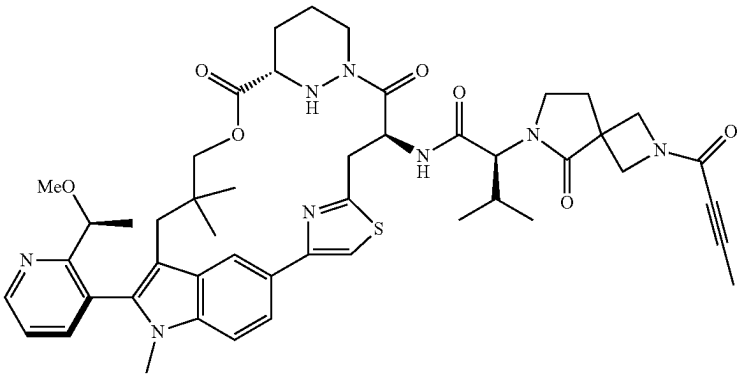
Certain Compounds of the Present Invention	
Ex #	Structure
B124	
B126	
B127	
B128	

TABLE 2-continued

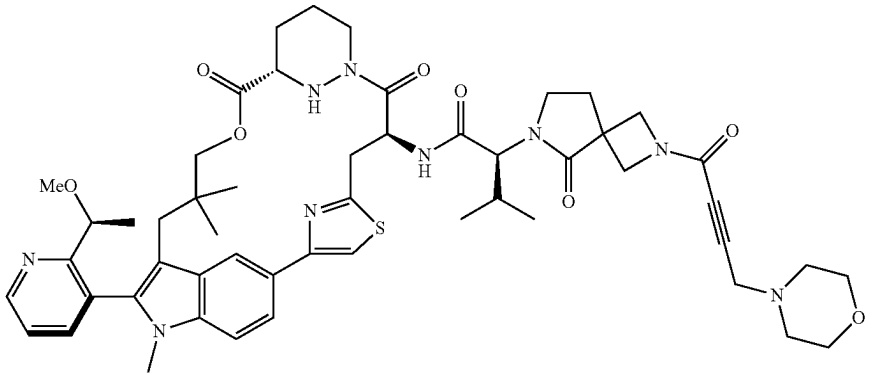
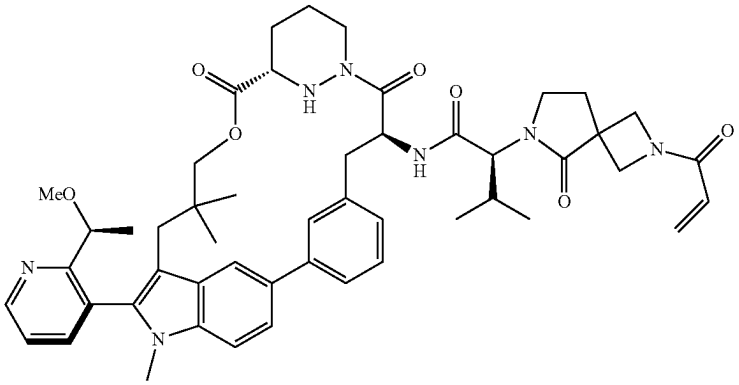
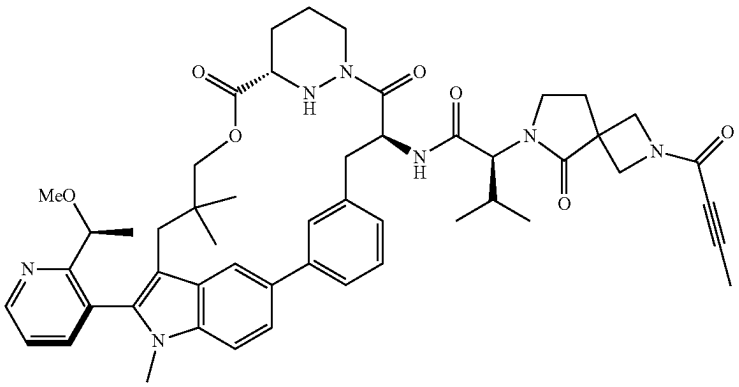
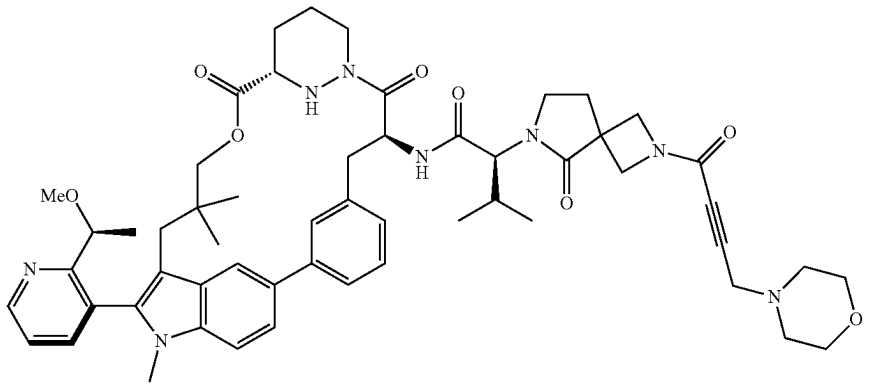
Certain Compounds of the Present Invention	
Ex #	Structure
B129	
B130	
B131	
B132	

TABLE 2-continued

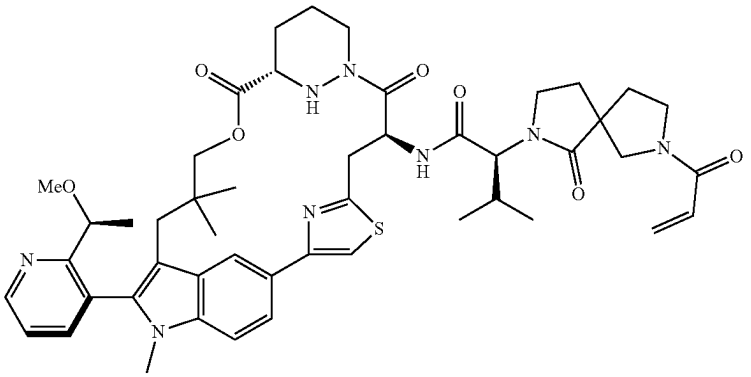
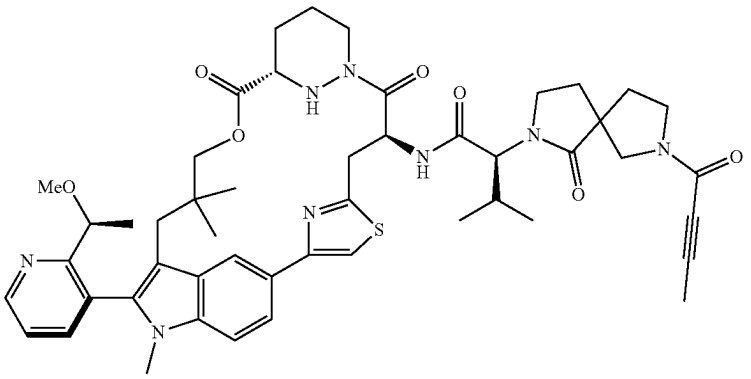
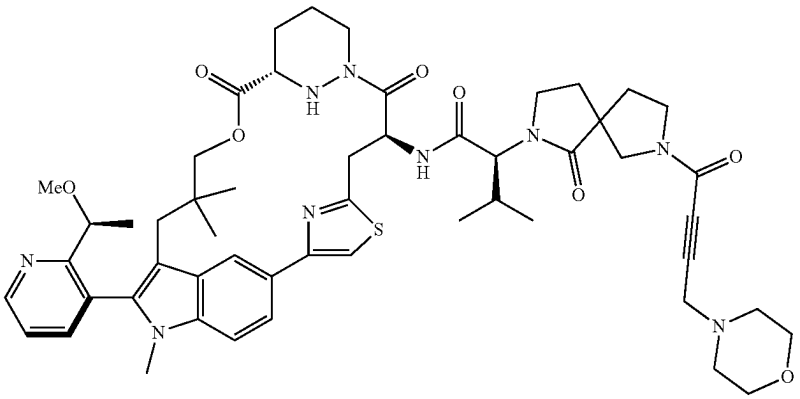
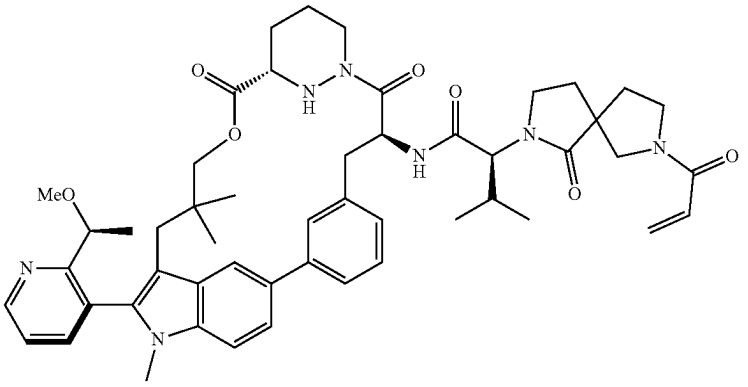
Certain Compounds of the Present Invention	
Ex #	Structure
B139	
B140	
B141	
B142	

TABLE 2-continued

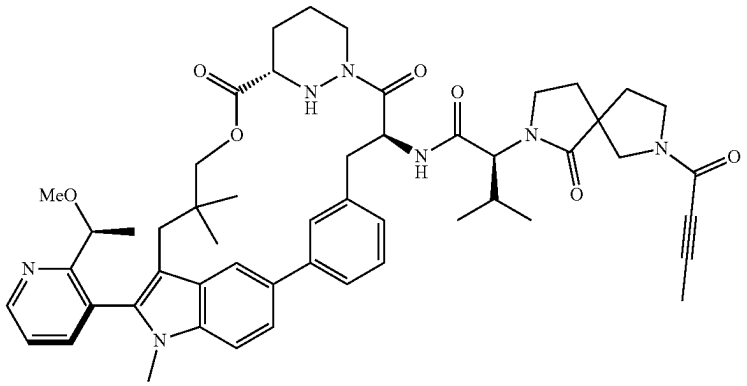
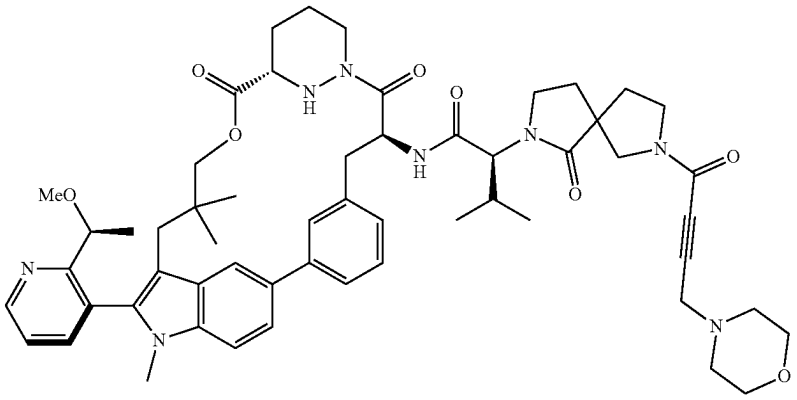
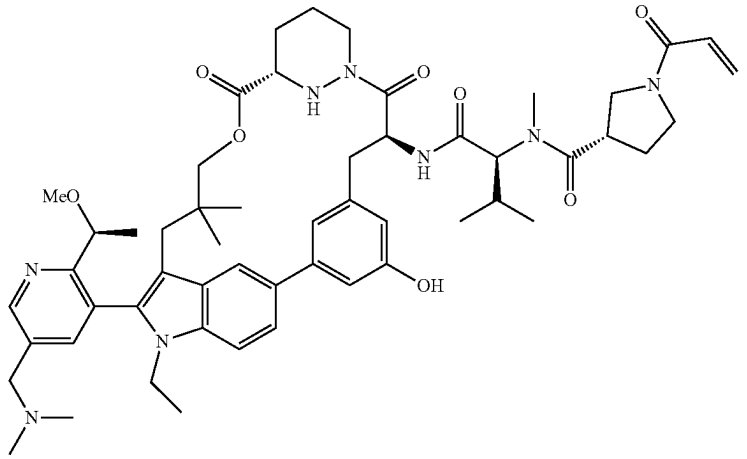
Certain Compounds of the Present Invention	
Ex #	Structure
B143	
B144	
B145	

TABLE 2-continued

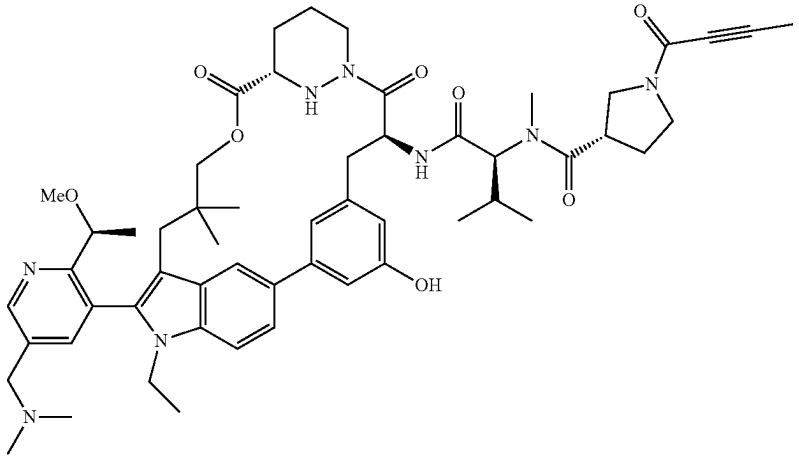
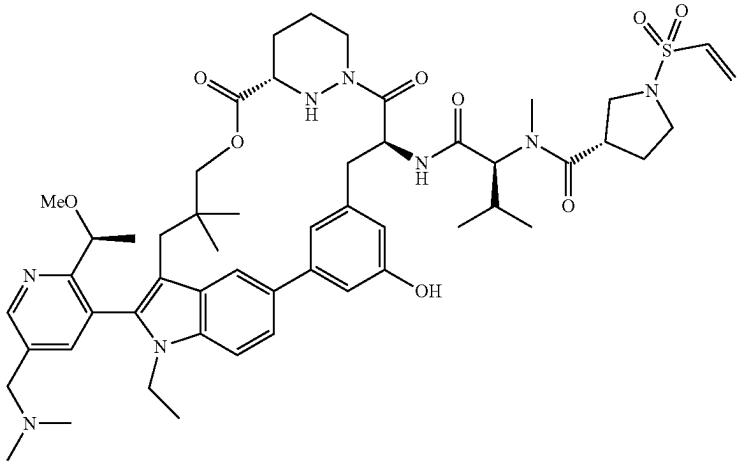
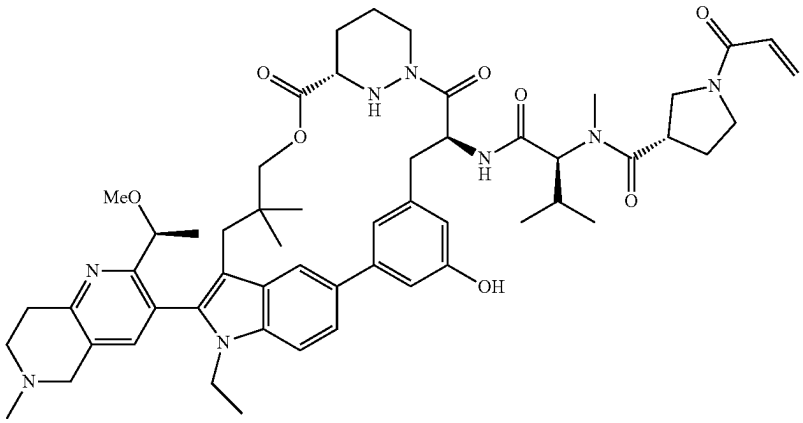
Certain Compounds of the Present Invention	
Ex #	Structure
B146	
B147	
B148	

TABLE 2-continued

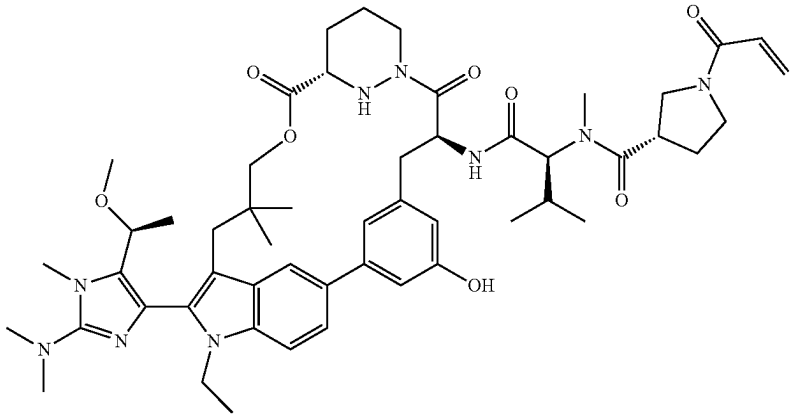
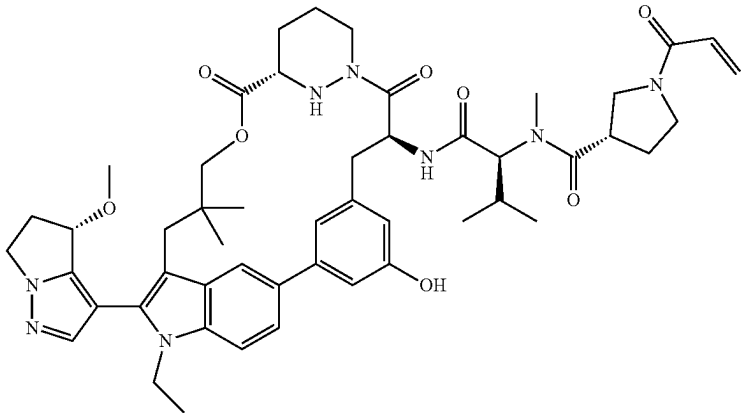
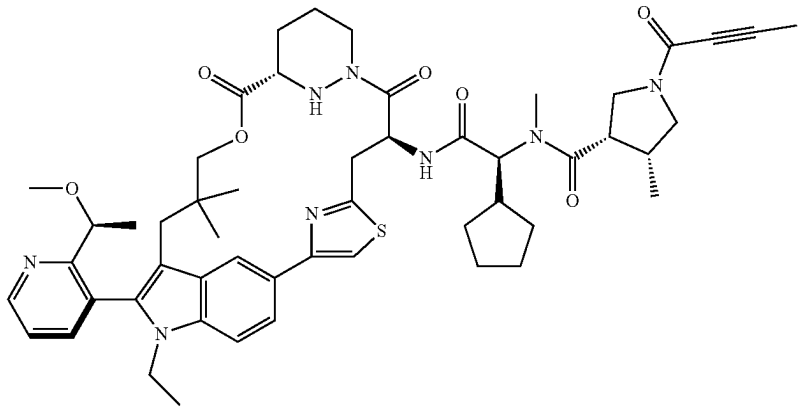
Certain Compounds of the Present Invention	
Ex #	Structure
B149	
B150	
B161	

TABLE 2-continued

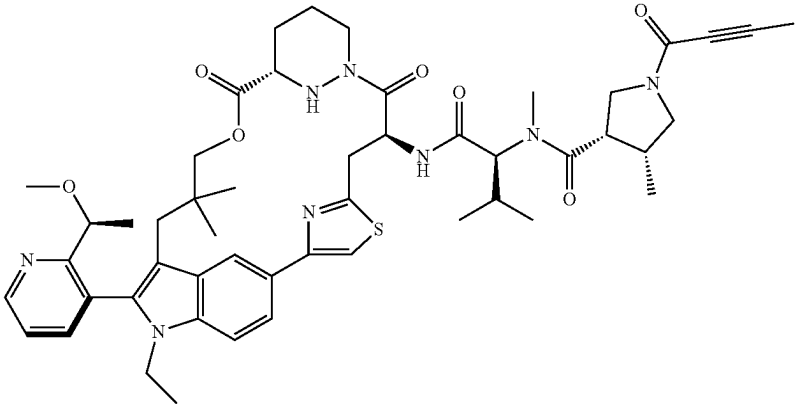
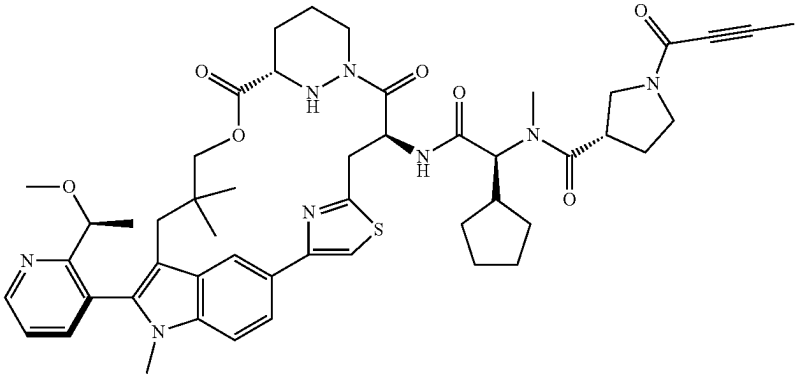
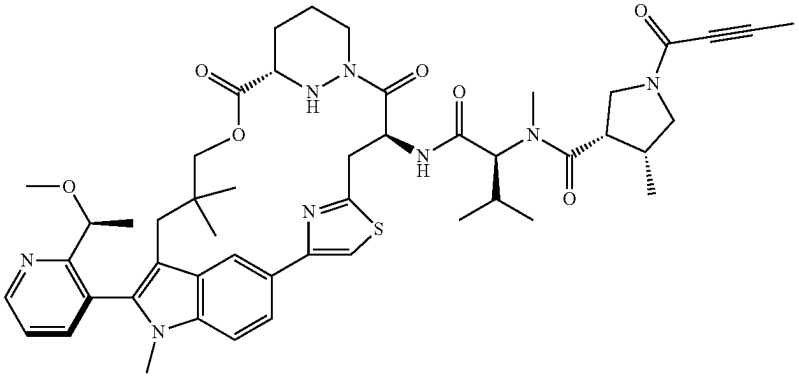
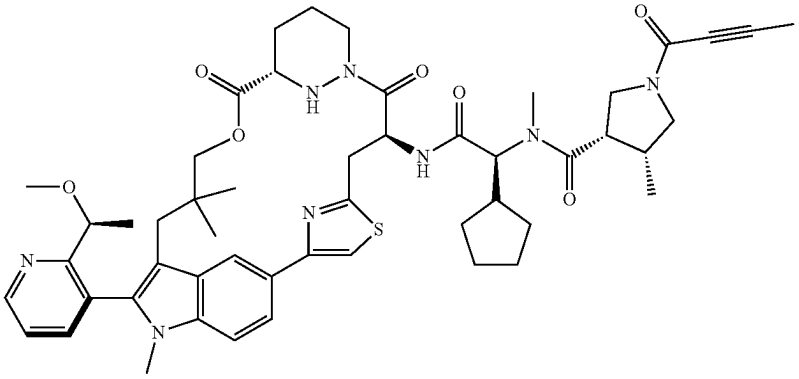
Certain Compounds of the Present Invention	
Ex #	Structure
B162	
B163	
B164	
B165	

TABLE 2-continued

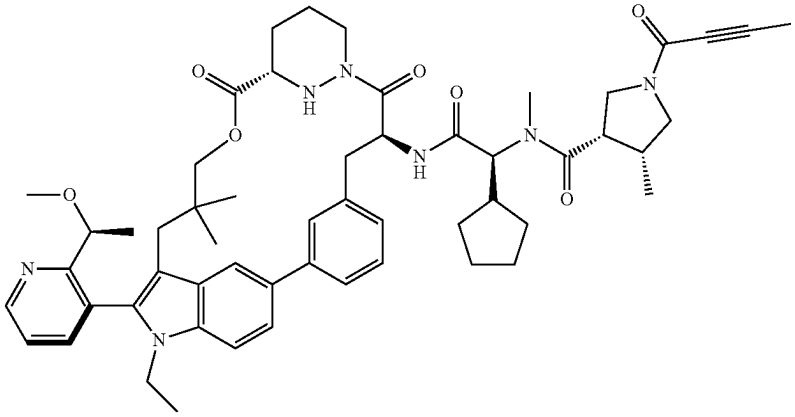
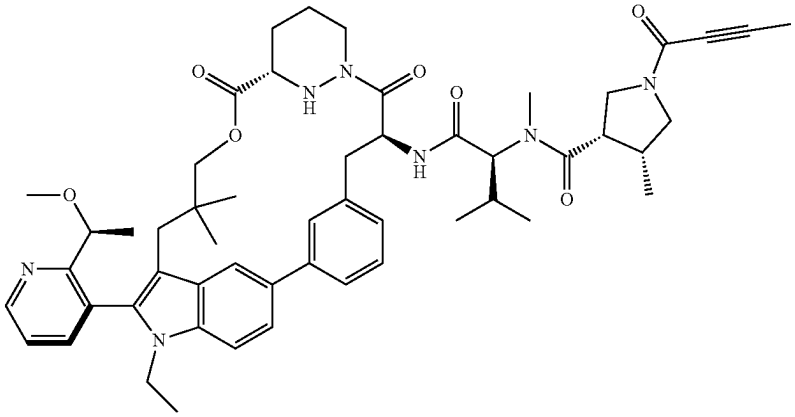
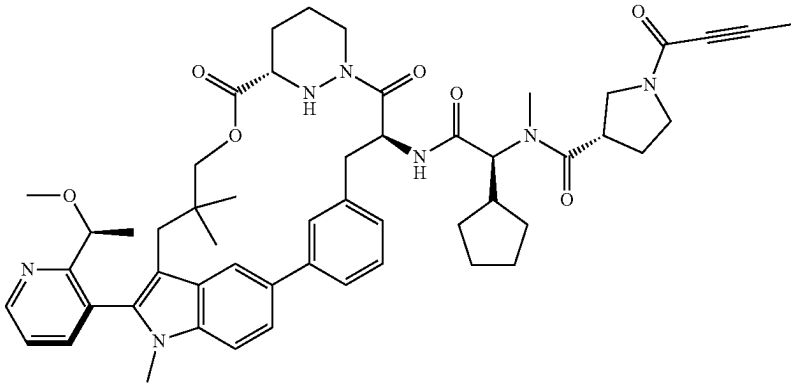
Certain Compounds of the Present Invention	
Ex #	Structure
B167	
B168	
B169	

TABLE 2-continued

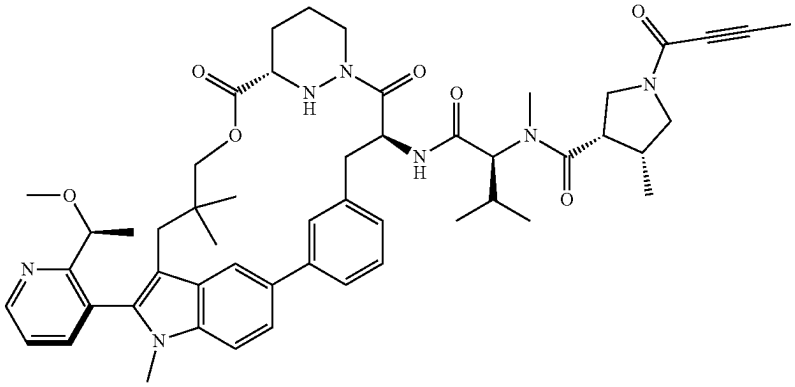
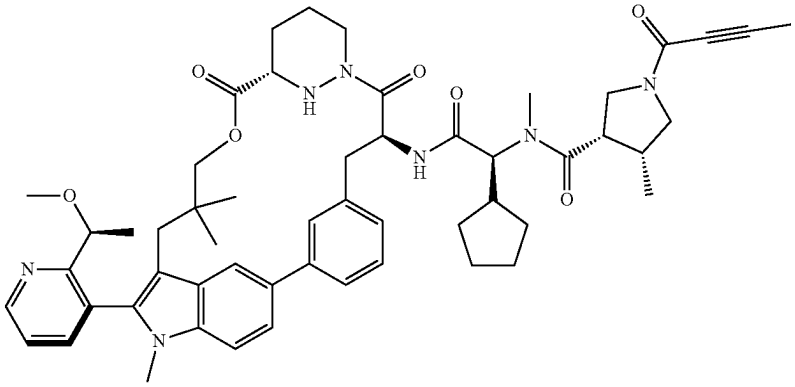
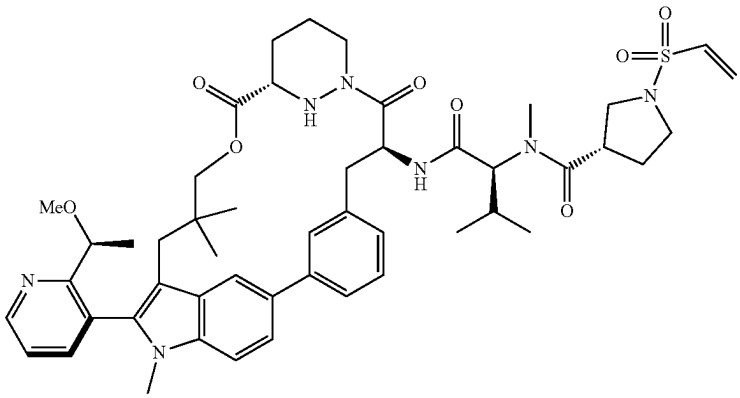
Certain Compounds of the Present Invention	
Ex #	Structure
B170	
B171	
B172	

TABLE 2-continued

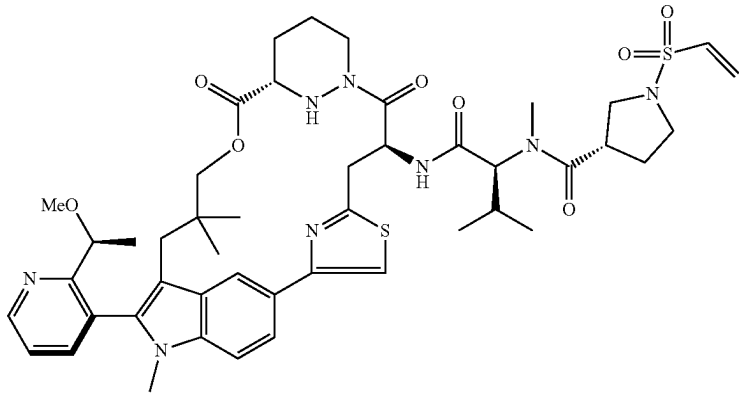
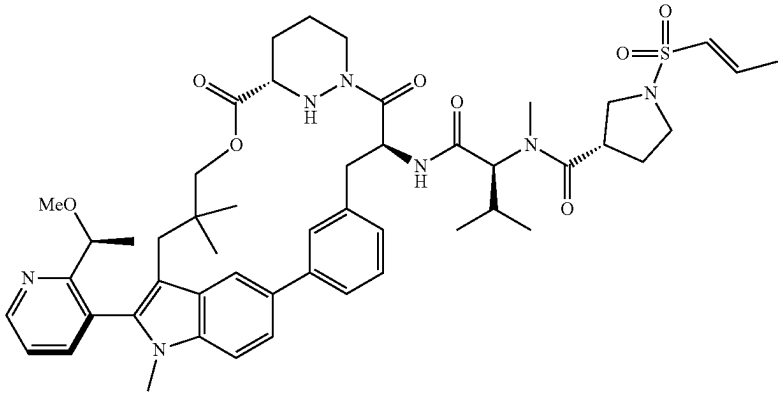
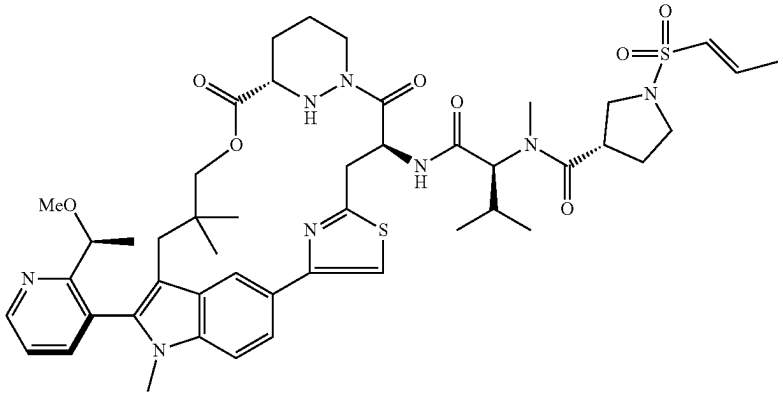
Certain Compounds of the Present Invention	
Ex #	Structure
B173	
B174	
B175	

TABLE 2-continued

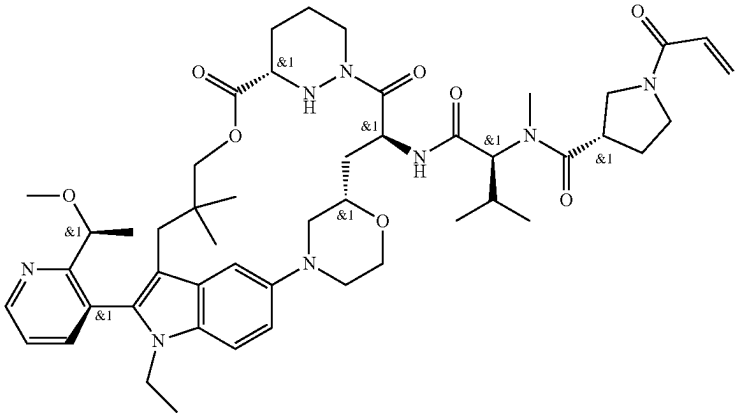
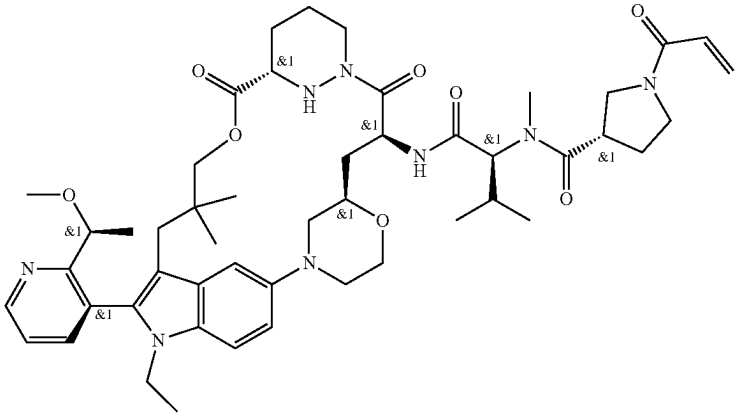
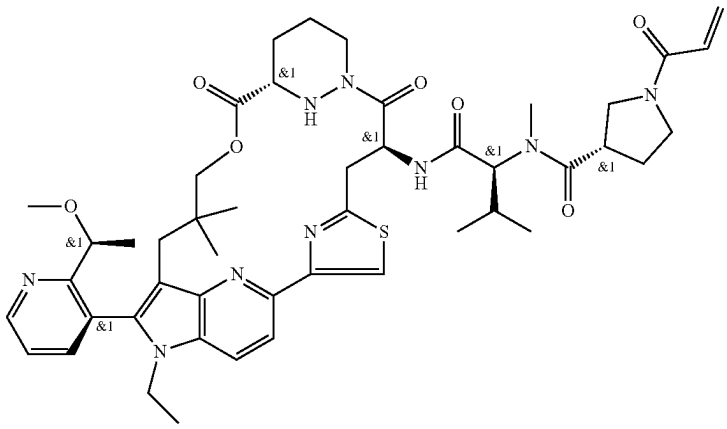
Certain Compounds of the Present Invention	
Ex #	Structure
B176	
B177	
B178	

TABLE 2-continued

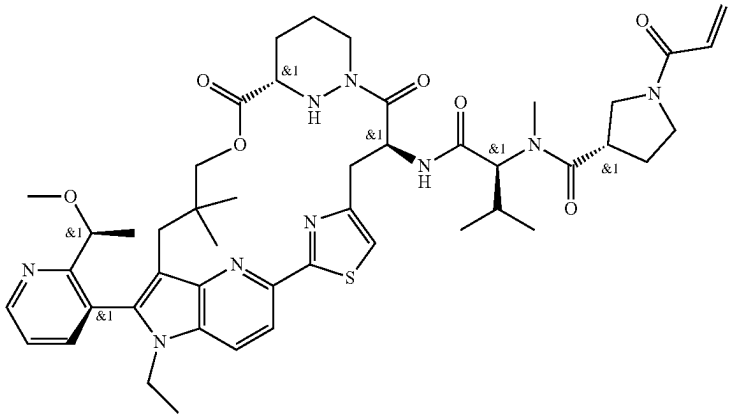
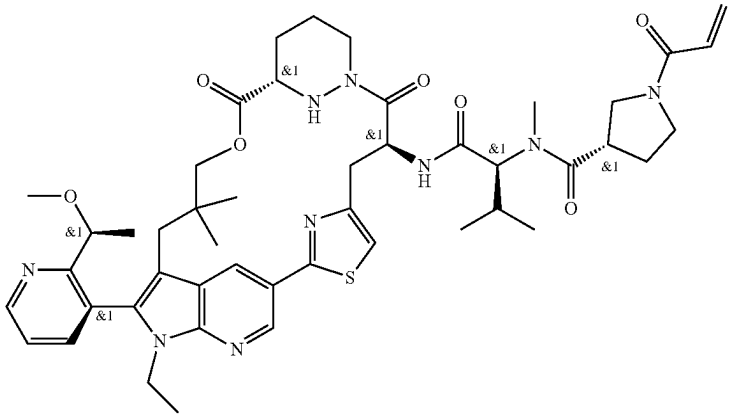
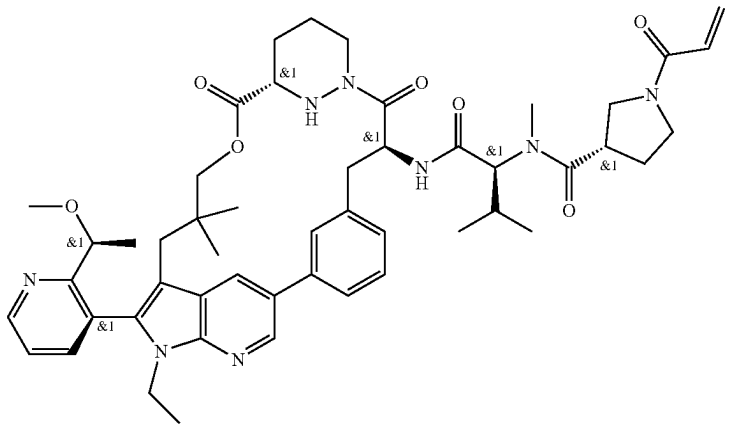
Certain Compounds of the Present Invention	
Ex #	Structure
B179	
B180	
B181	

TABLE 2-continued

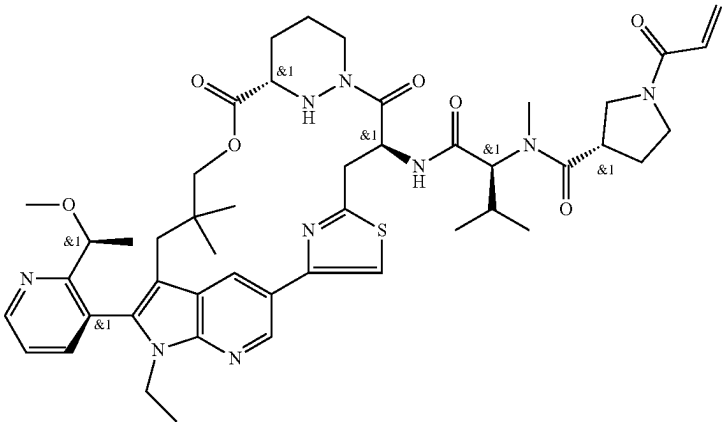
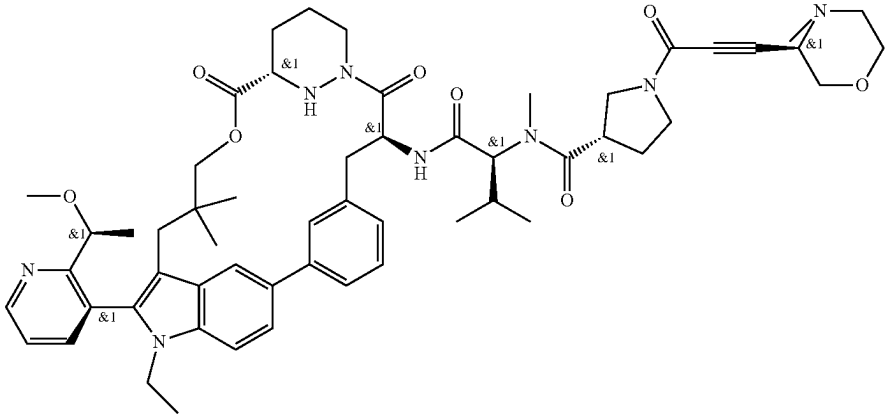
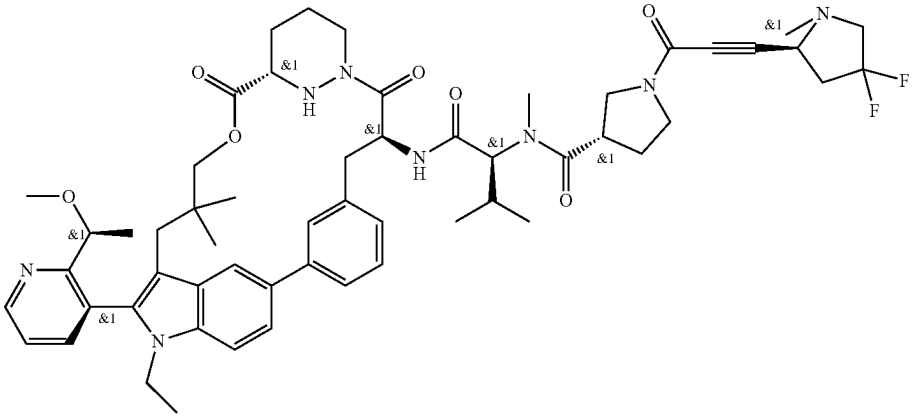
Certain Compounds of the Present Invention	
Ex #	Structure
B182	
B183	
B184	

TABLE 2-continued

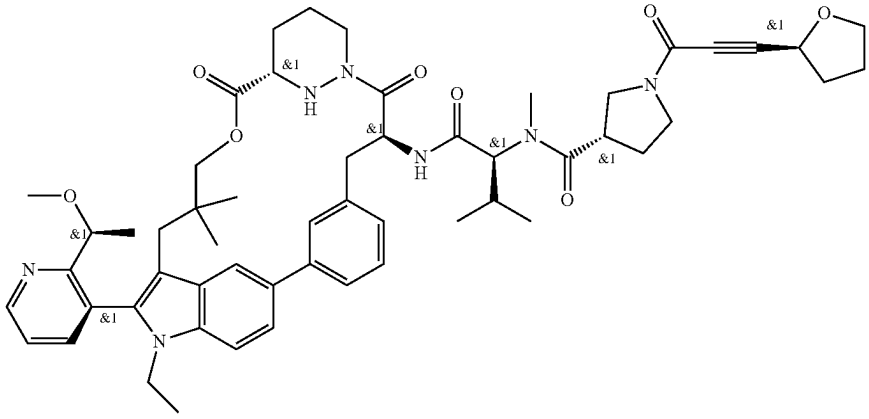
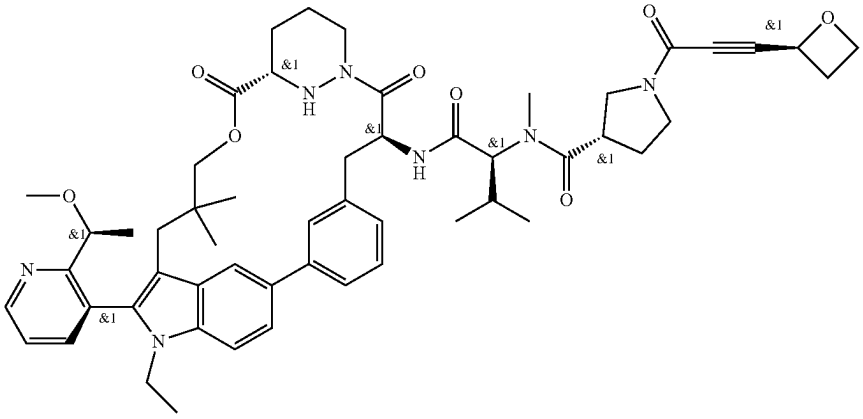
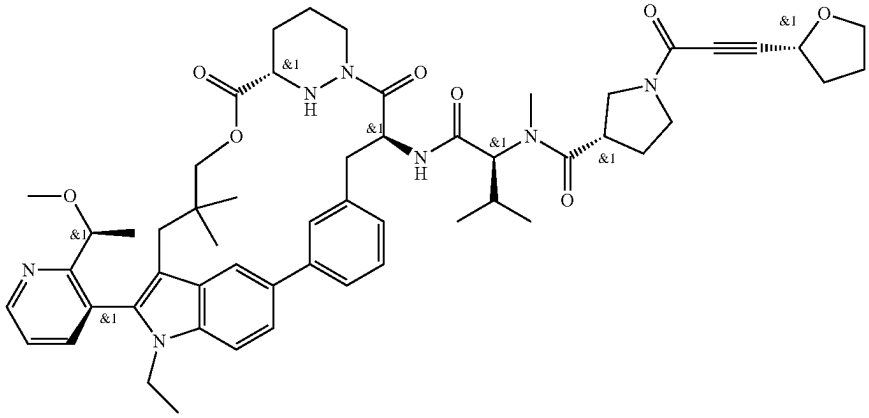
Certain Compounds of the Present Invention	
Ex #	Structure
B185	
B186	
B187	

TABLE 2-continued

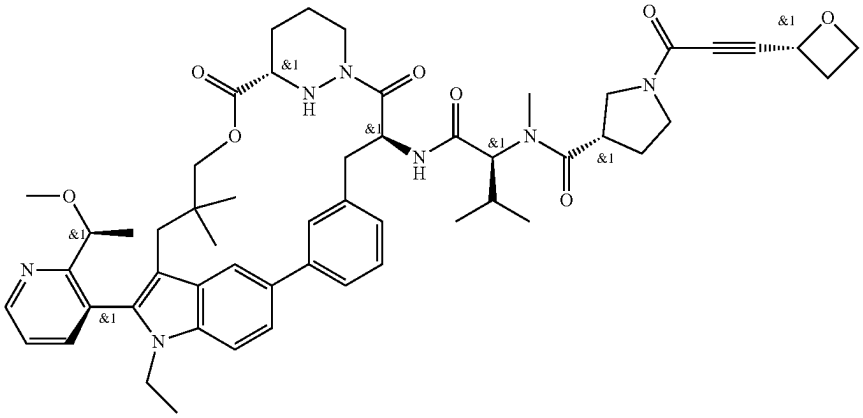
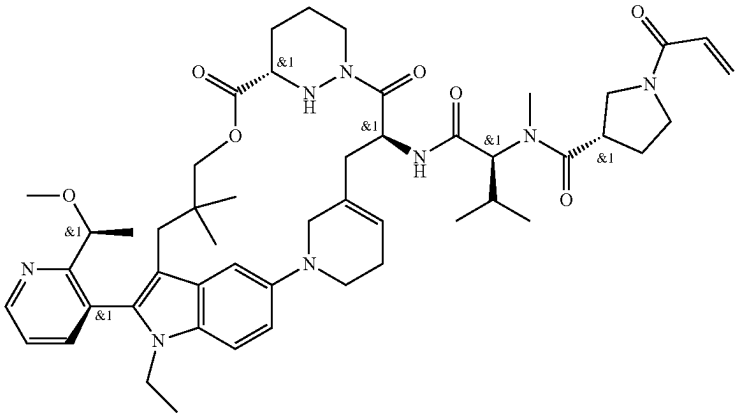
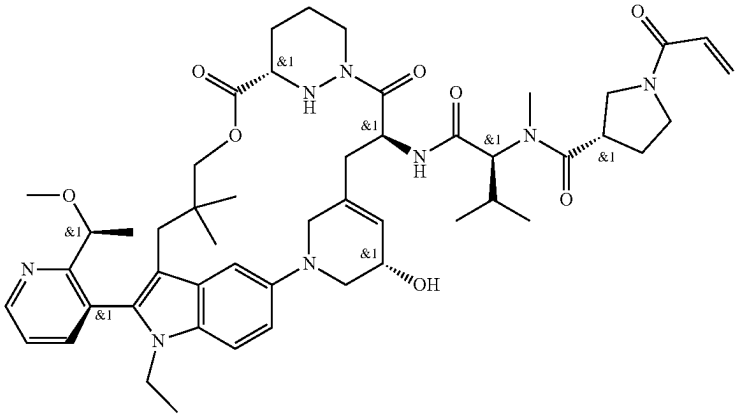
Certain Compounds of the Present Invention	
Ex #	Structure
B188	
B189	
B190	

TABLE 2-continued

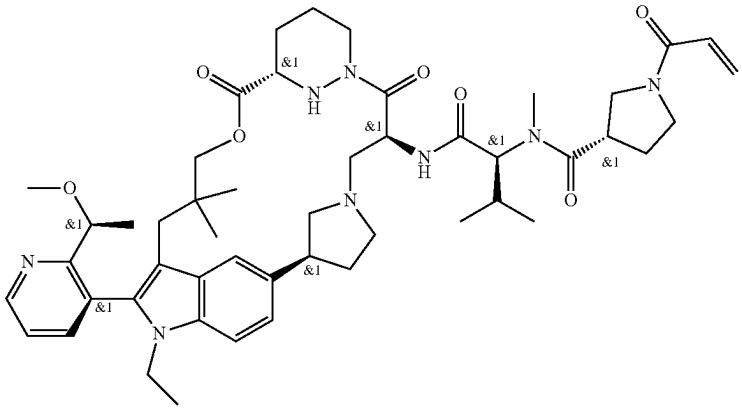
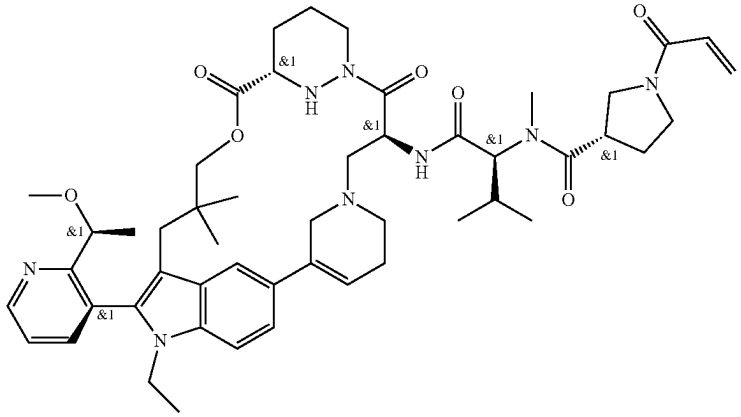
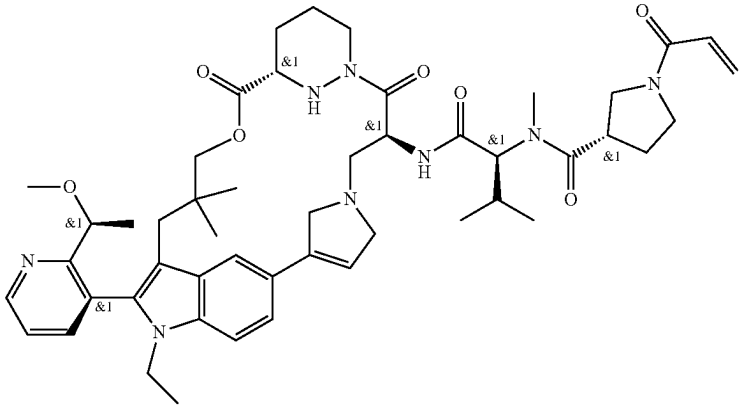
Certain Compounds of the Present Invention	
Ex #	Structure
B191	
B192	
B194	

TABLE 2-continued

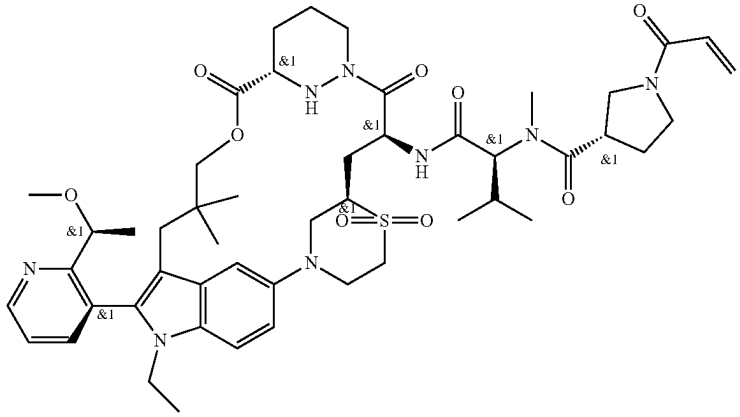
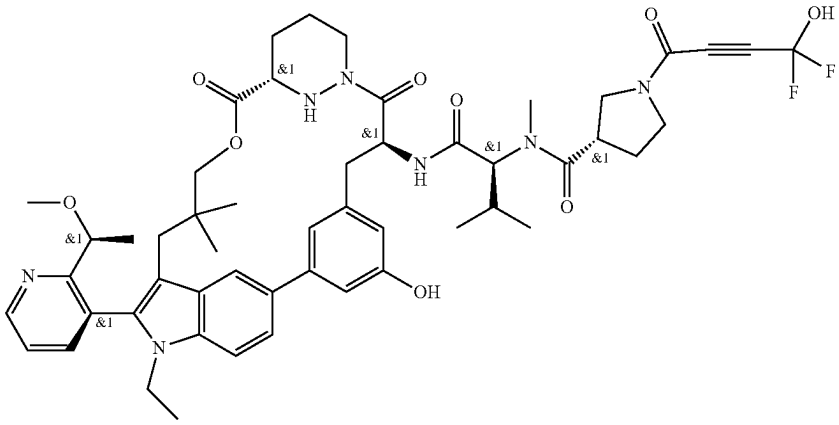
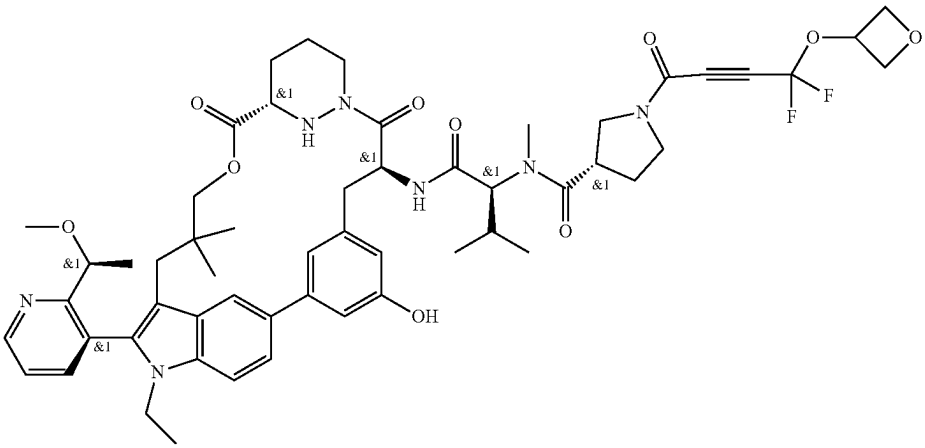
Certain Compounds of the Present Invention	
Ex #	Structure
B195	
B196	
B197	

TABLE 2-continued

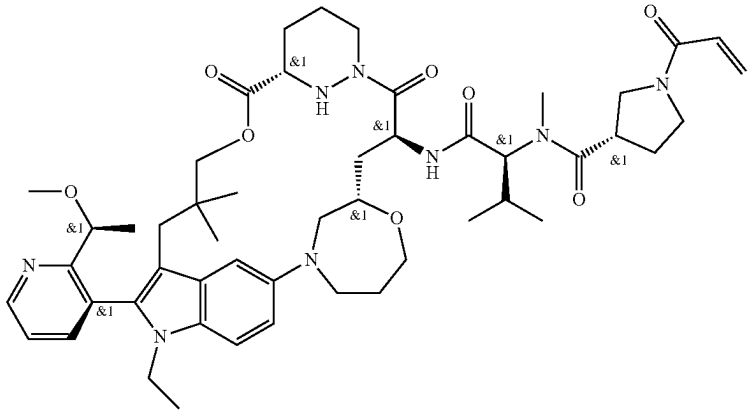
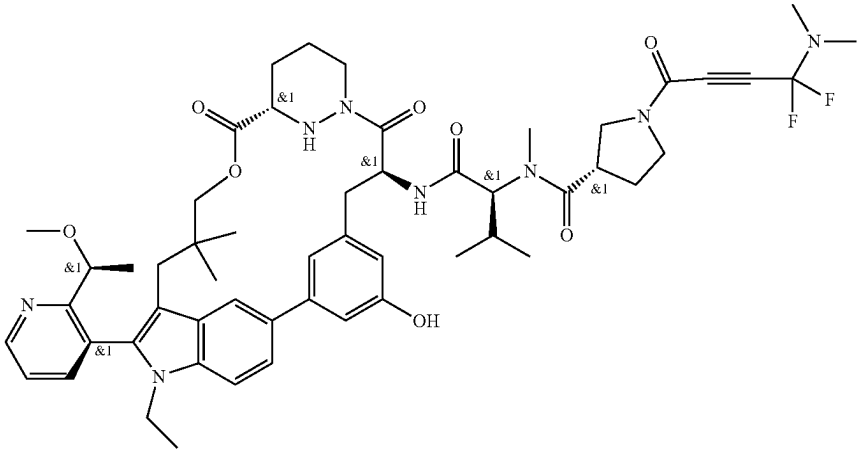
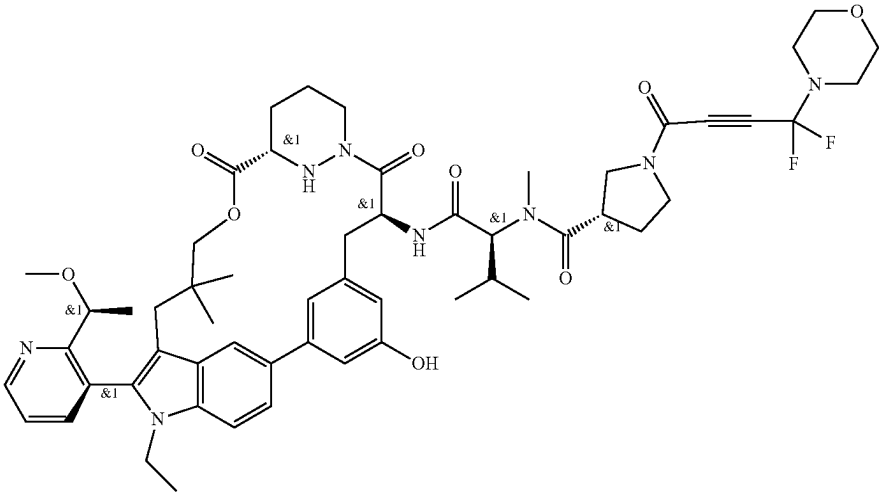
Certain Compounds of the Present Invention	
Ex #	Structure
B198	
B199	
B200	

TABLE 2-continued

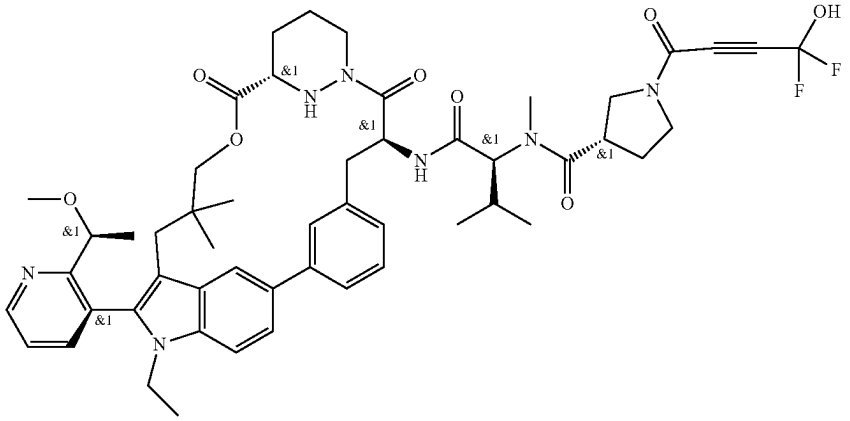
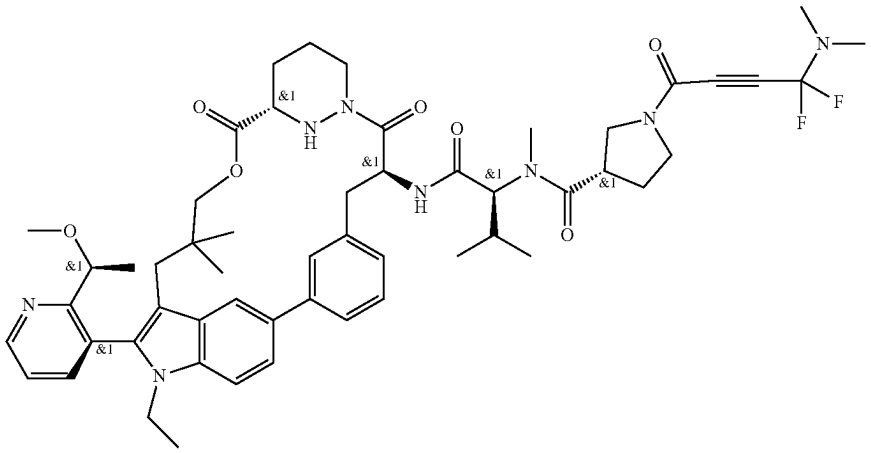
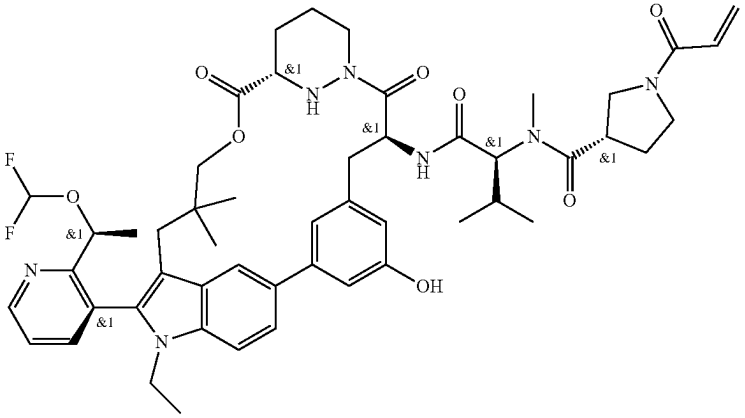
Certain Compounds of the Present Invention	
Ex #	Structure
B201	
B202	
B203	

TABLE 2-continued

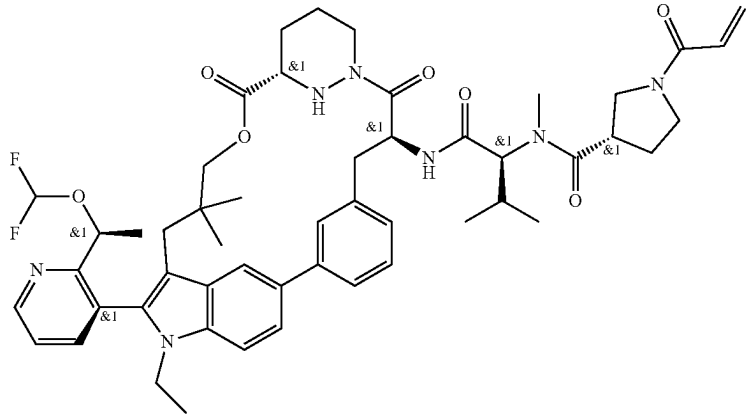
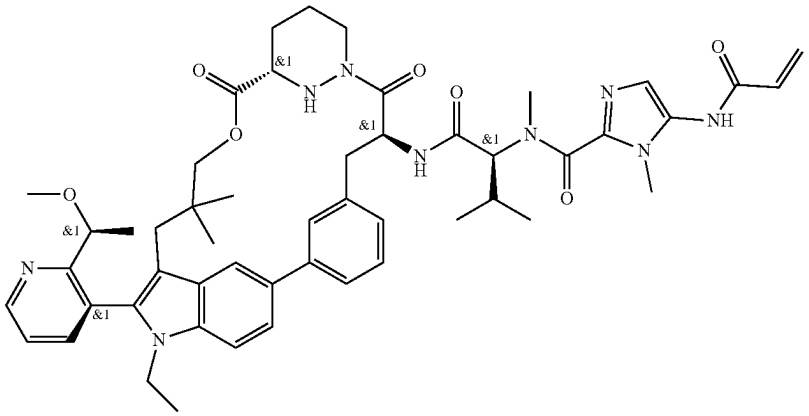
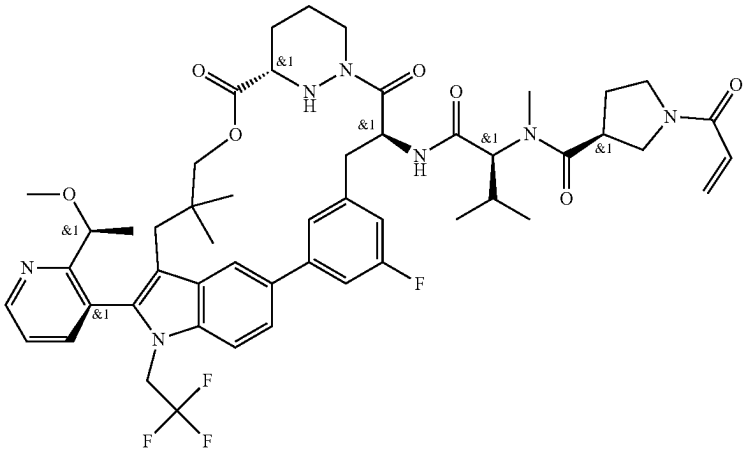
Certain Compounds of the Present Invention	
Ex #	Structure
B204	
B205	
B206	

TABLE 2-continued

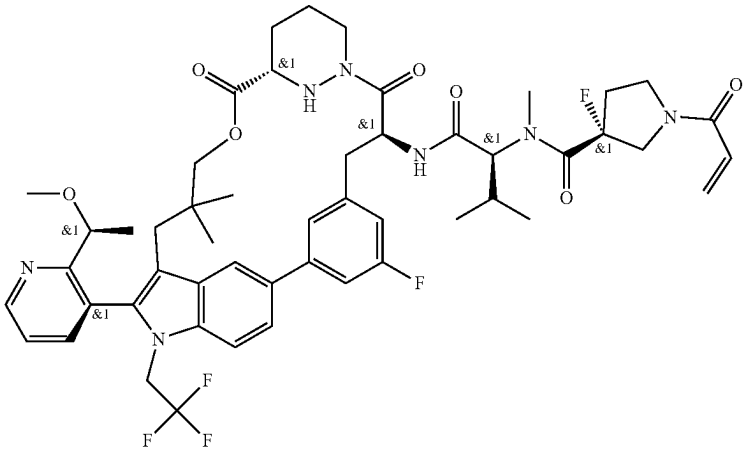
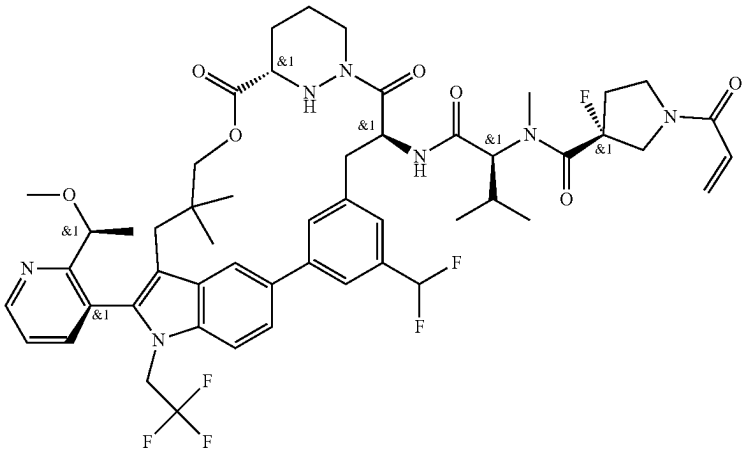
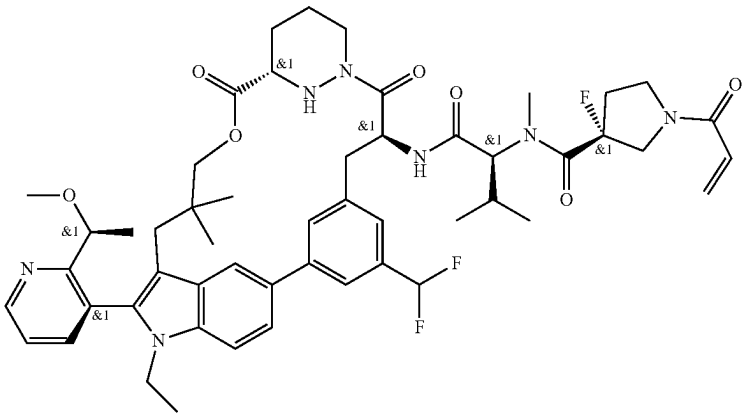
Certain Compounds of the Present Invention	
Ex #	Structure
B207	
B208	
B209	

TABLE 2-continued

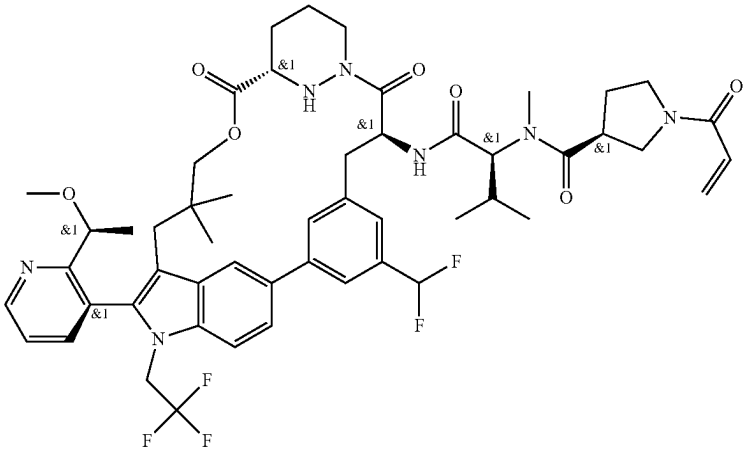
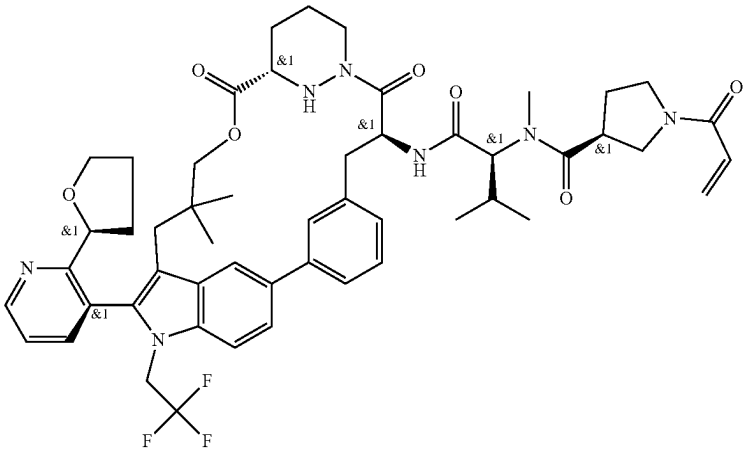
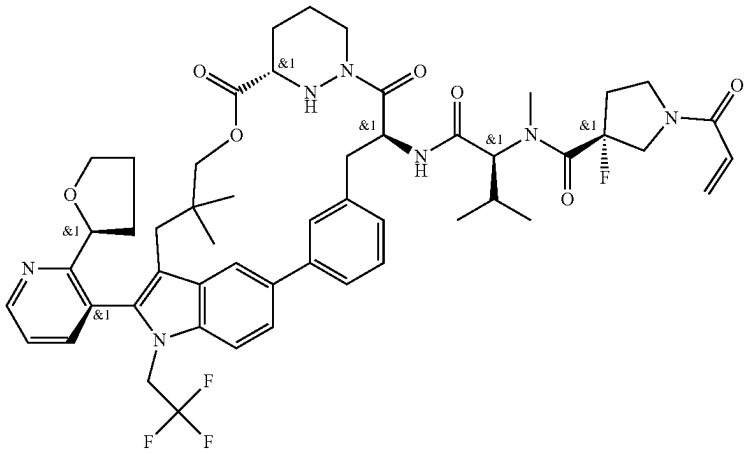
Certain Compounds of the Present Invention	
Ex #	Structure
B210	
B211	
B212	

TABLE 2-continued

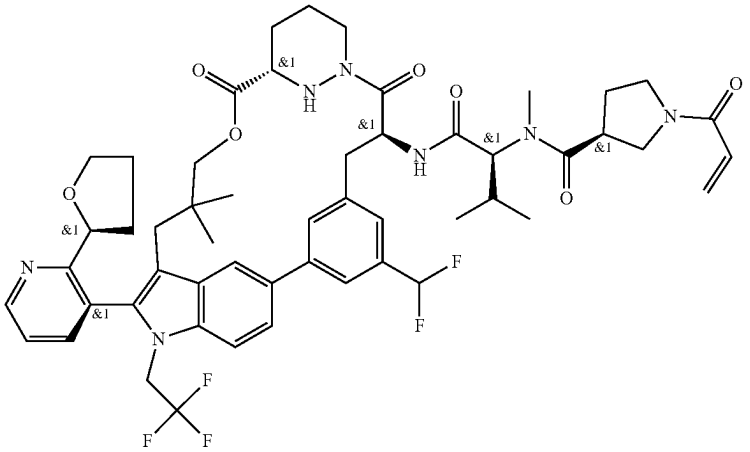
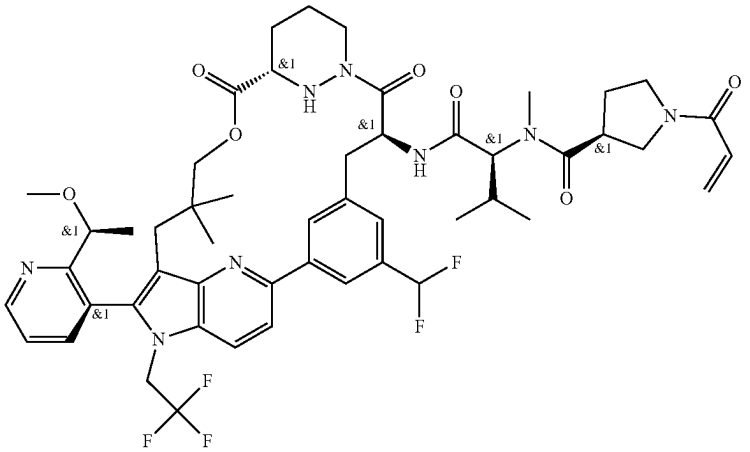
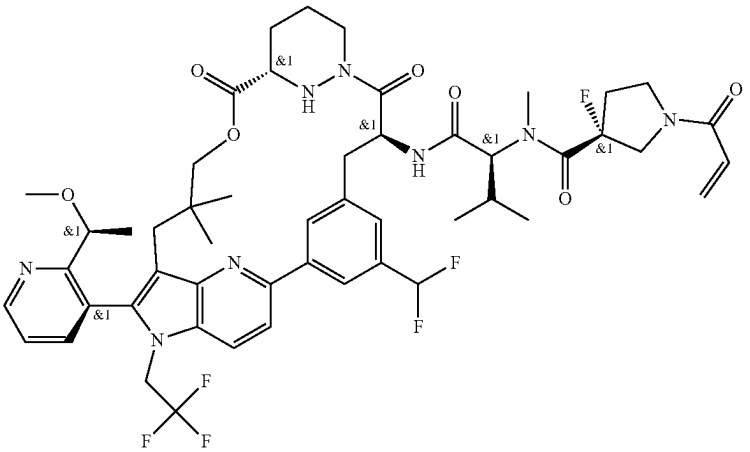
Certain Compounds of the Present Invention	
Ex #	Structure
B213	
B214	
B215	

TABLE 2-continued

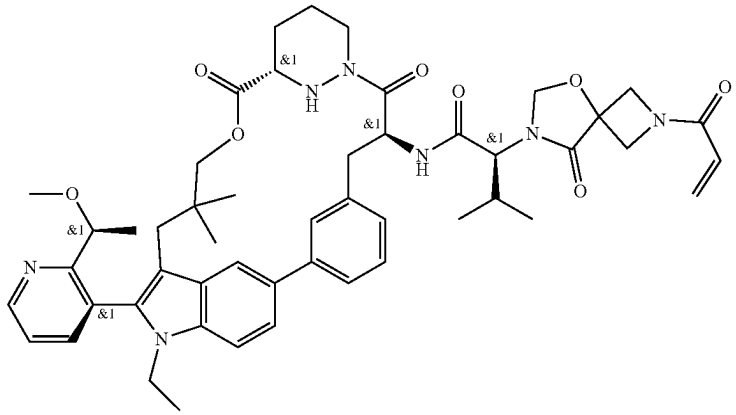
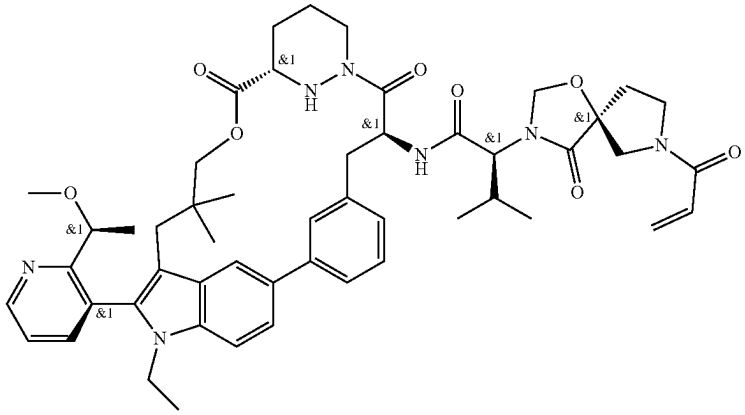
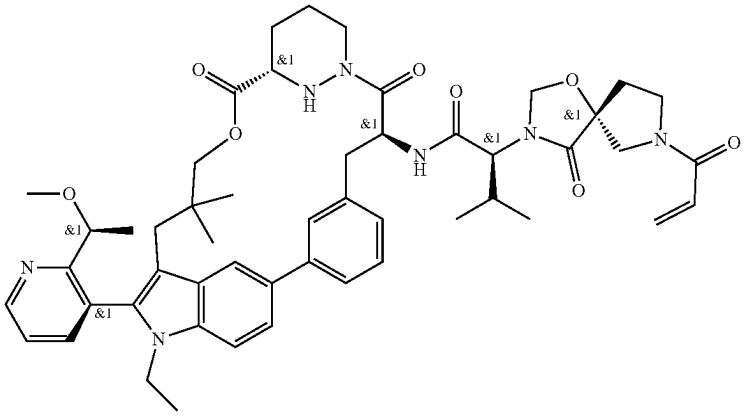
Certain Compounds of the Present Invention	
Ex #	Structure
B216	
B217	
B218	

TABLE 2-continued

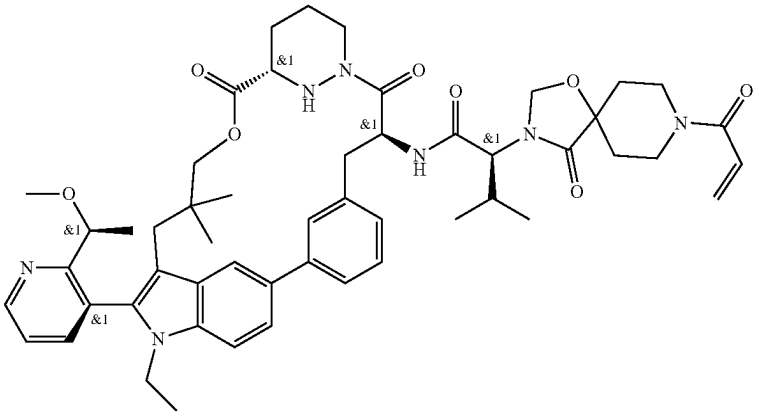
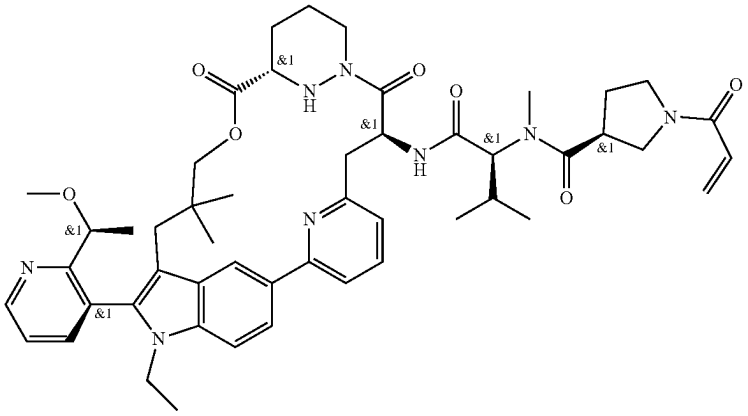
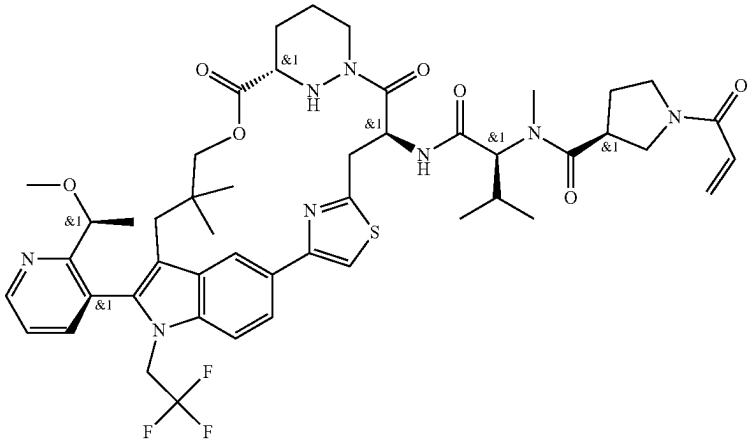
Certain Compounds of the Present Invention	
Ex #	Structure
B219	
B220	
B221	

TABLE 2-continued

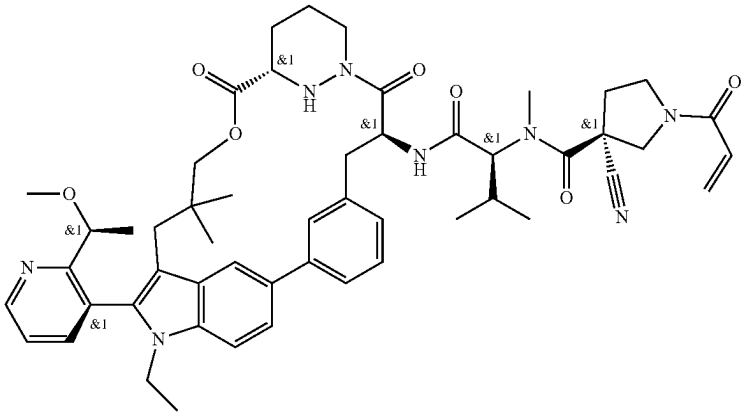
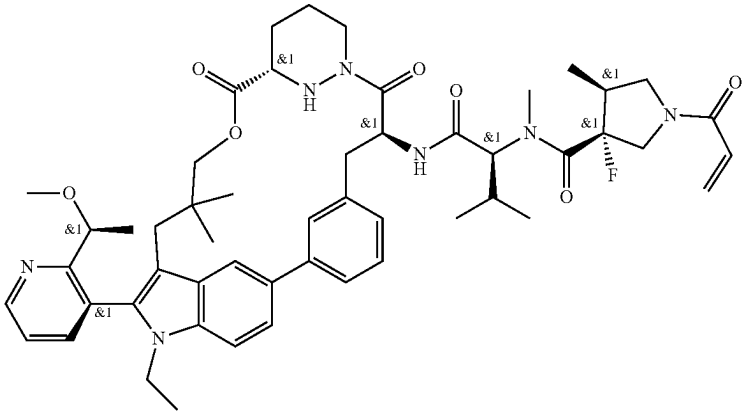
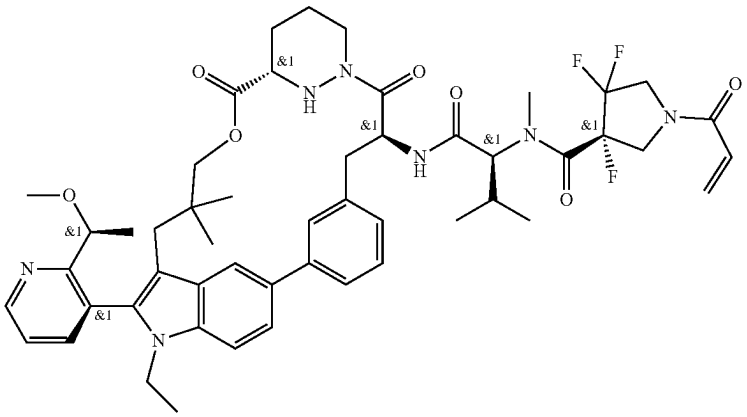
Certain Compounds of the Present Invention	
Ex #	Structure
B222	
B223	
B224	

TABLE 2-continued

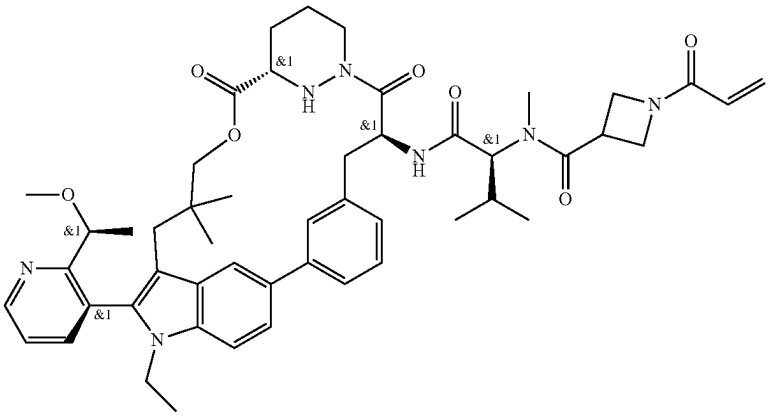
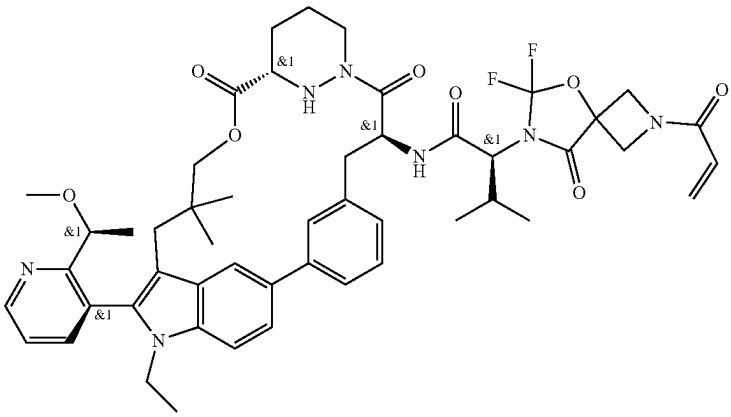
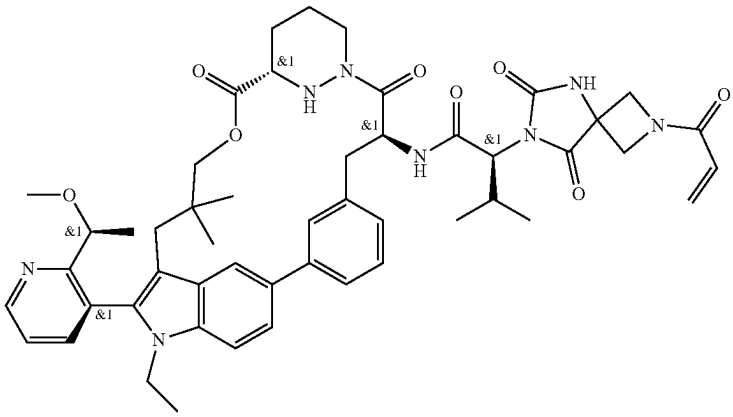
Certain Compounds of the Present Invention	
Ex #	Structure
B225	
B226	
B227	

TABLE 2-continued

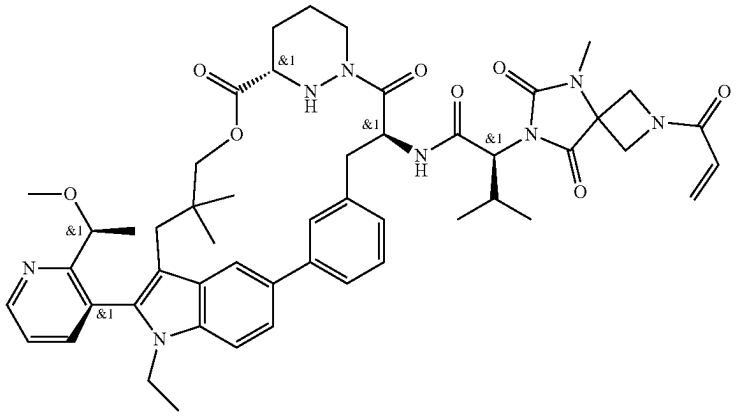
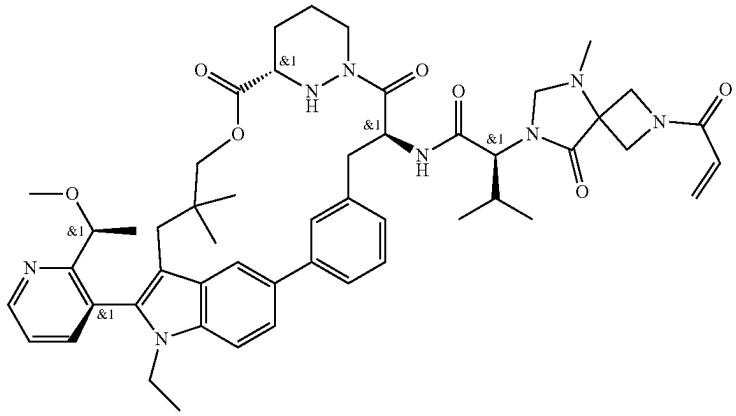
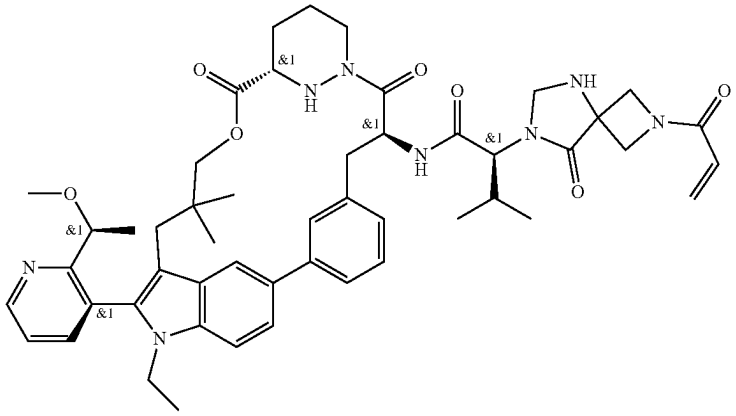
Certain Compounds of the Present Invention	
Ex #	Structure
B228	
B229	
B230	

TABLE 2-continued

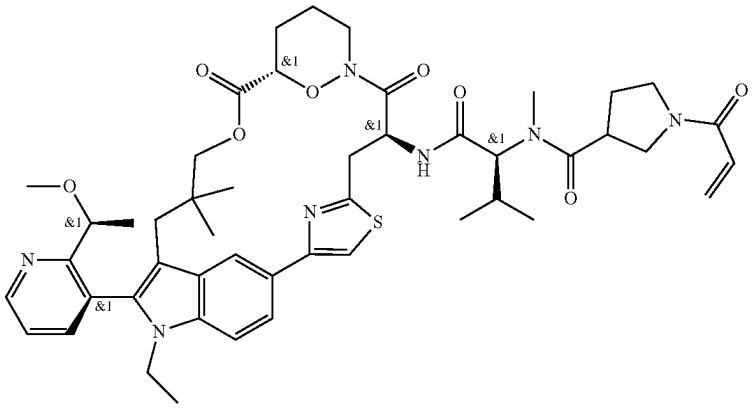
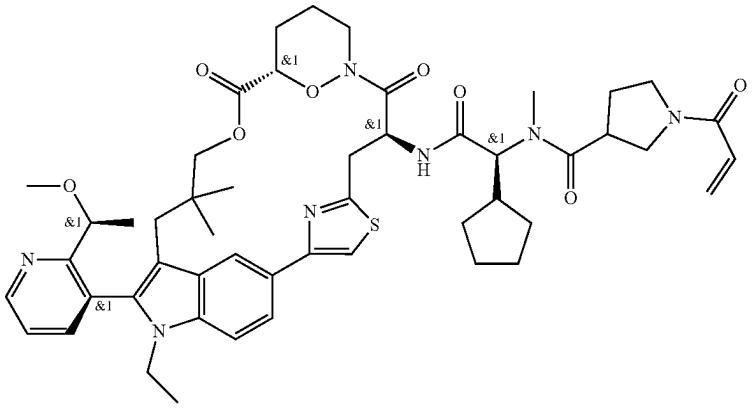
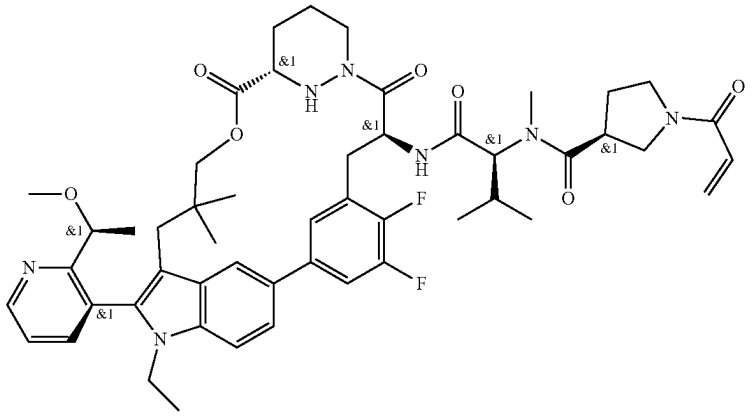
Certain Compounds of the Present Invention	
Ex #	Structure
B231	
B232	
B233	

TABLE 2-continued

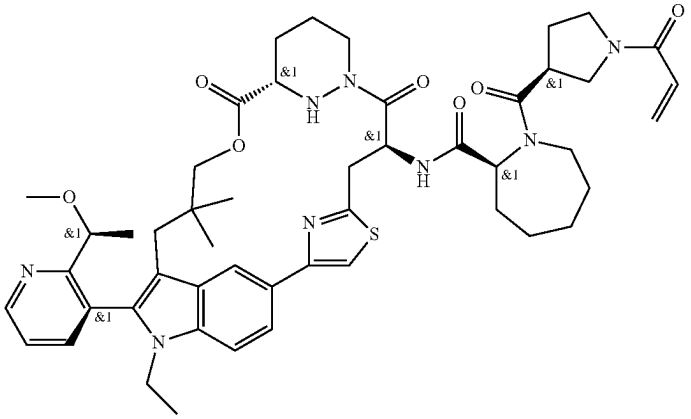
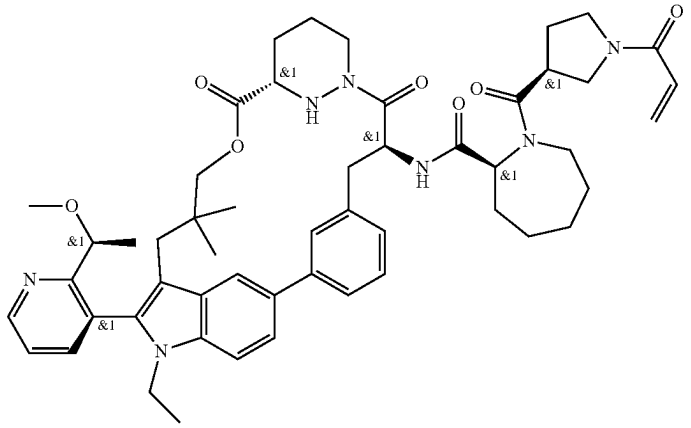
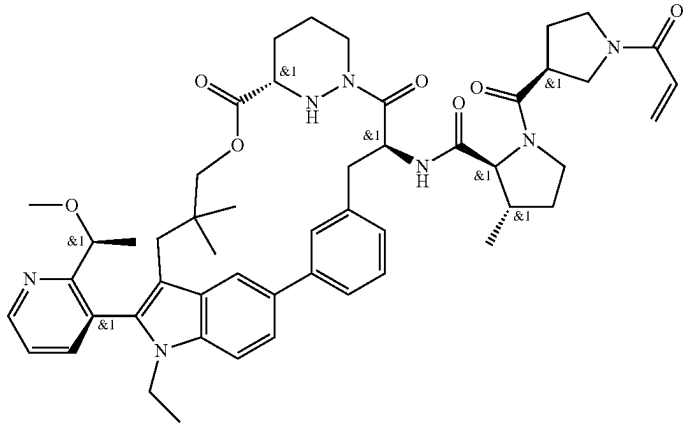
Certain Compounds of the Present Invention	
Ex #	Structure
B234	
B235	
B236	

TABLE 2-continued

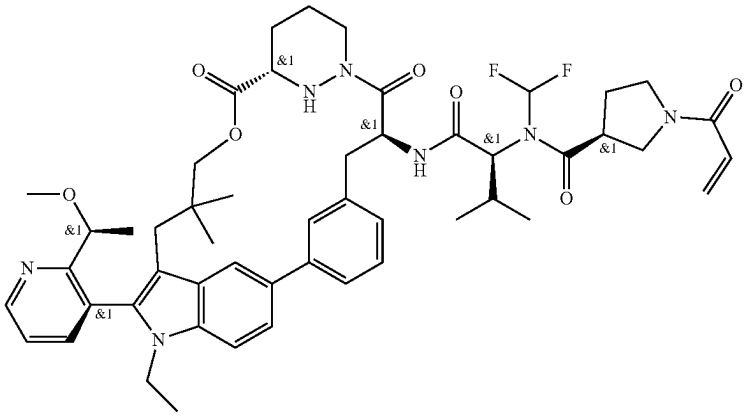
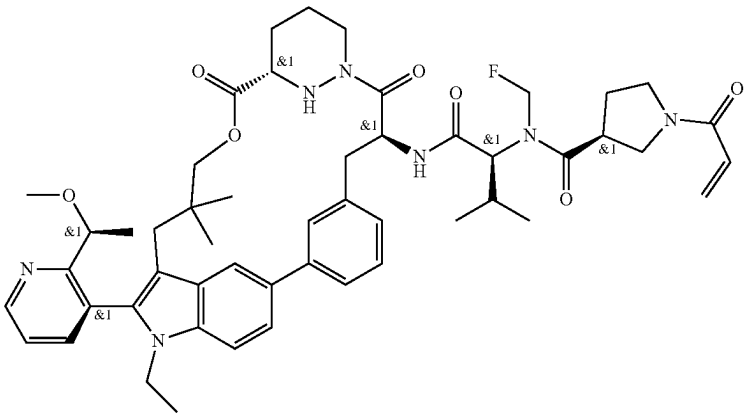
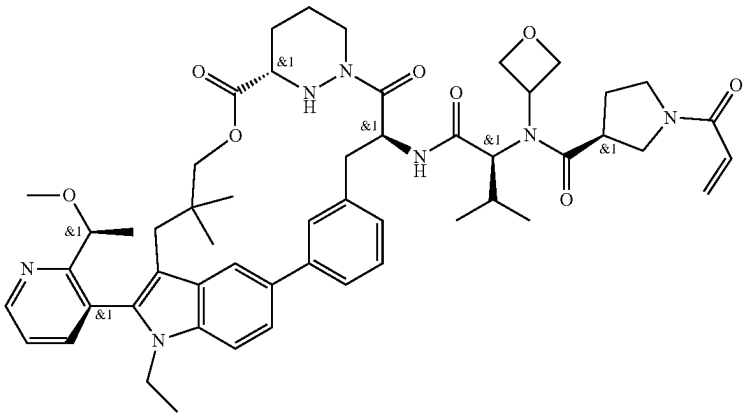
Certain Compounds of the Present Invention	
Ex #	Structure
B237	
B238	
B239	

TABLE 2-continued

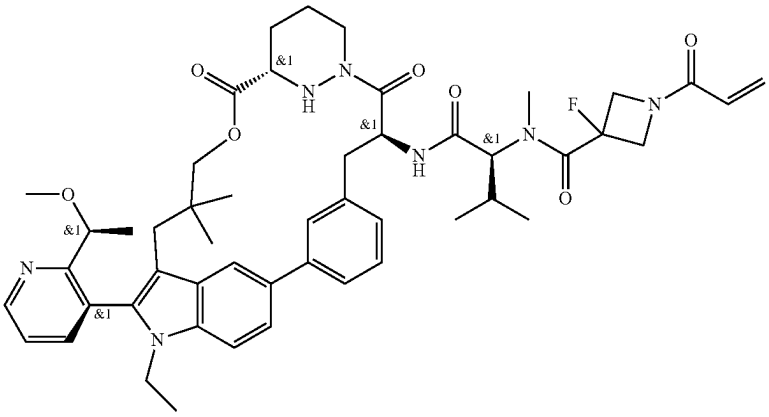
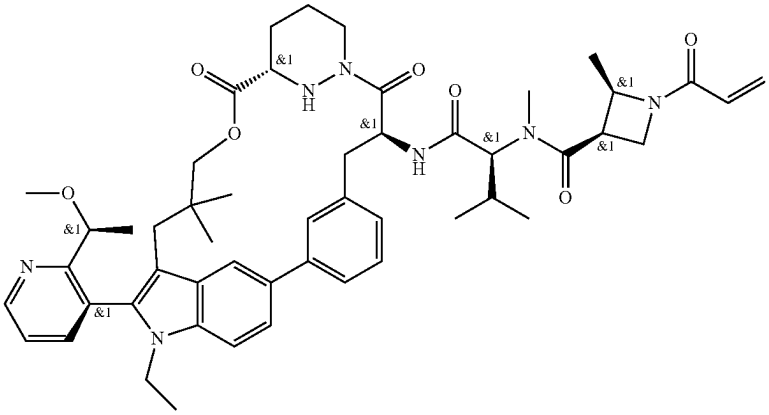
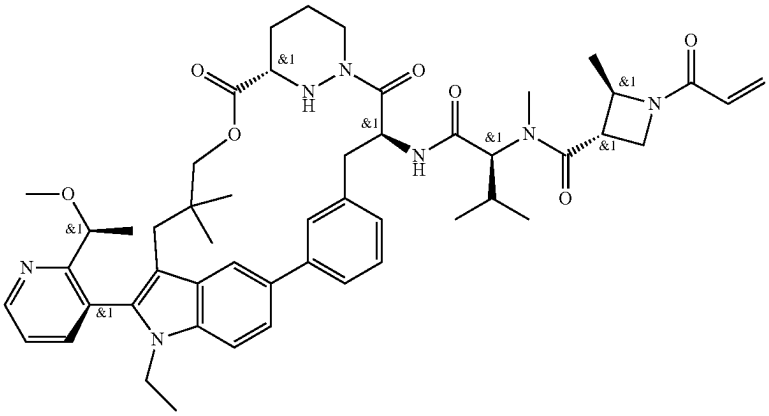
Certain Compounds of the Present Invention	
Ex #	Structure
B240	
B241	
B242	

TABLE 2-continued

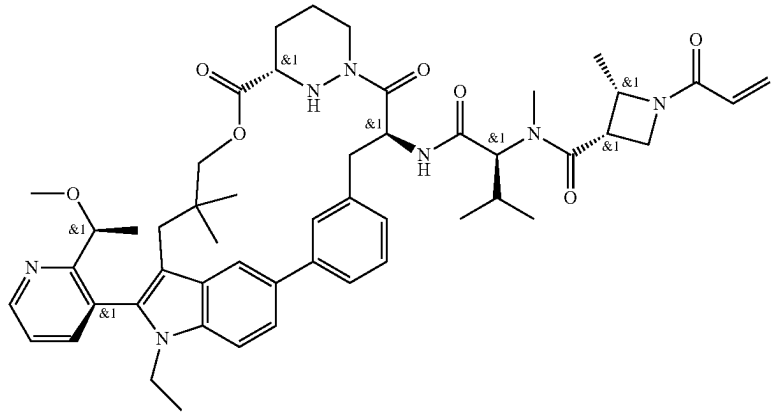
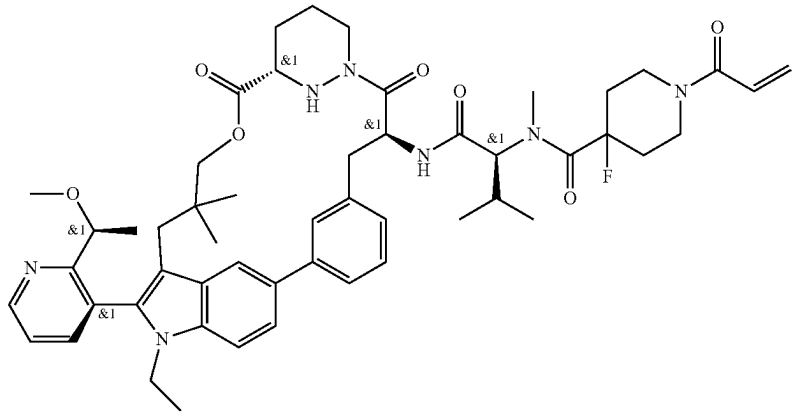
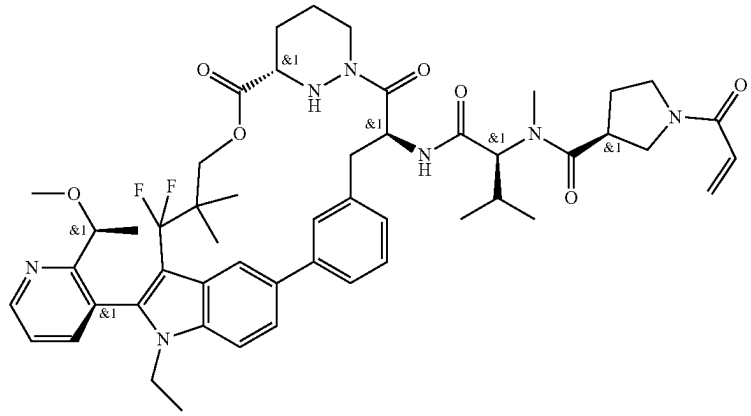
Certain Compounds of the Present Invention	
Ex #	Structure
B243	
B244	
B245	

TABLE 2-continued

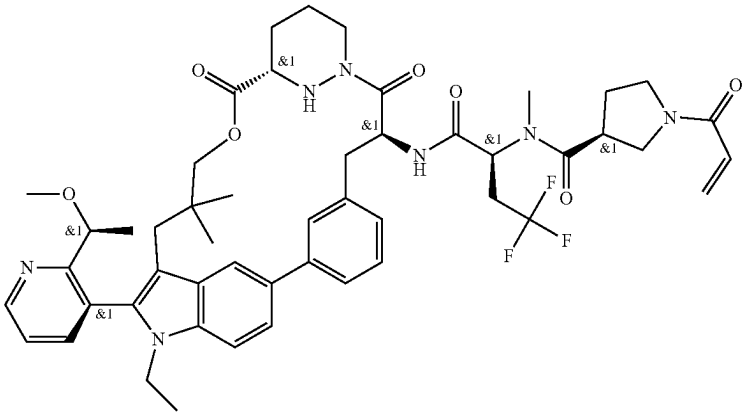
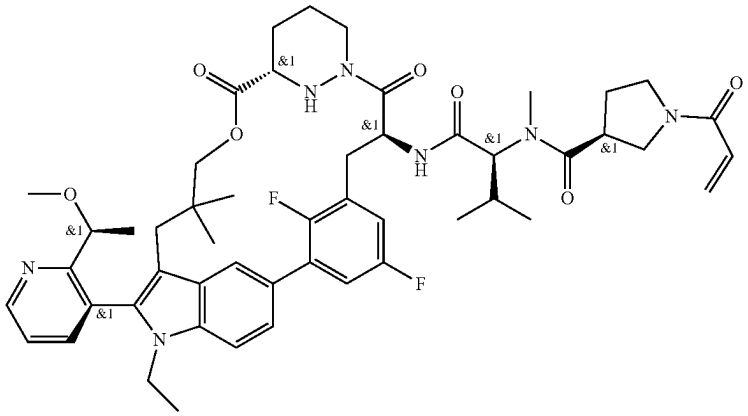
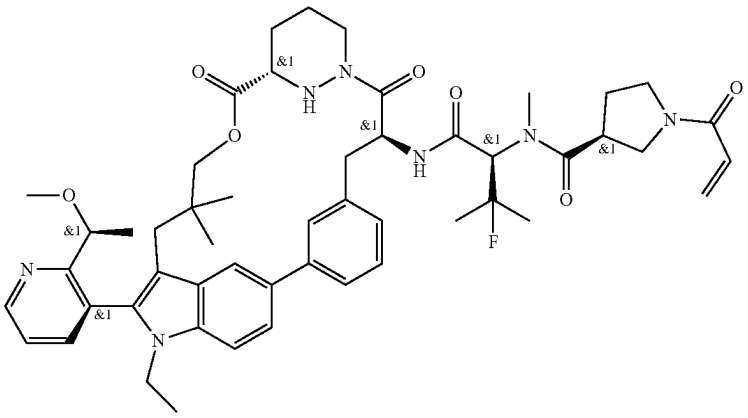
Certain Compounds of the Present Invention	
Ex #	Structure
B246	
B247	
B248	

TABLE 2-continued

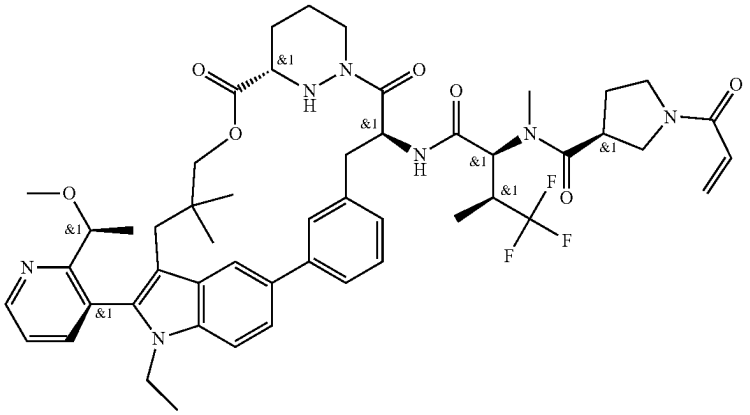
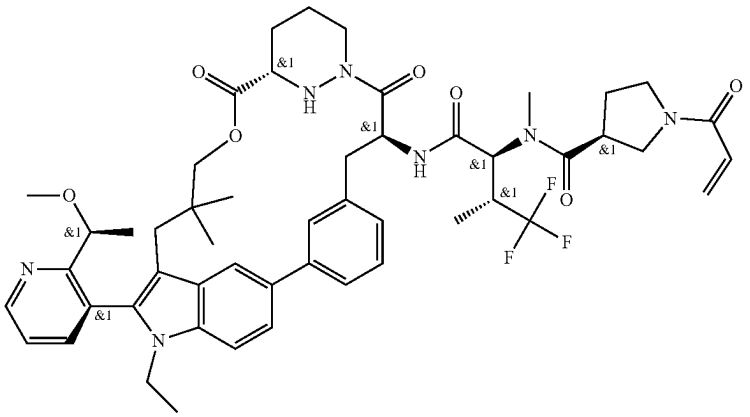
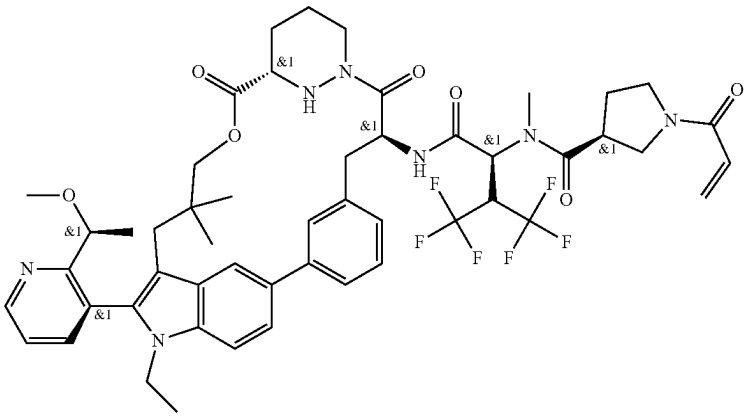
Certain Compounds of the Present Invention	
Ex #	Structure
B249	
B250	
B251	

TABLE 2-continued

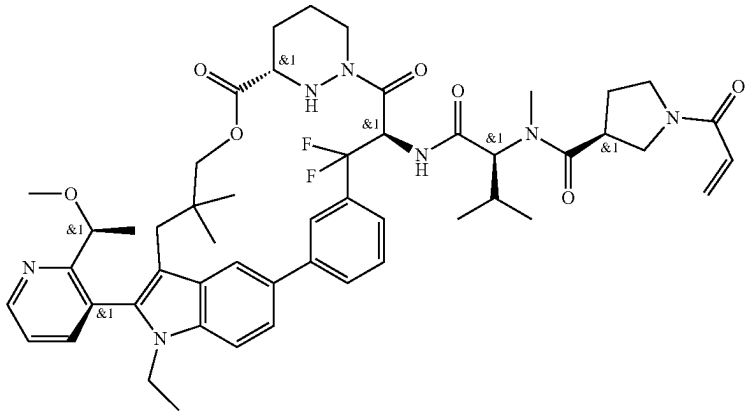
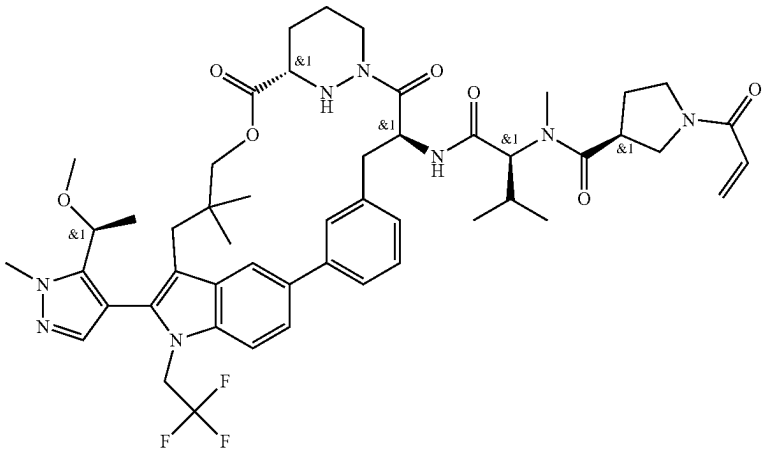
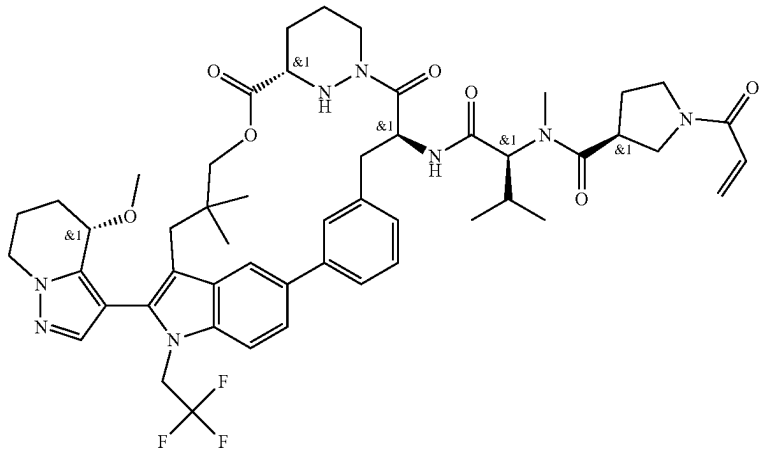
Certain Compounds of the Present Invention	
Ex #	Structure
B252	
B253	
B254	

TABLE 2-continued

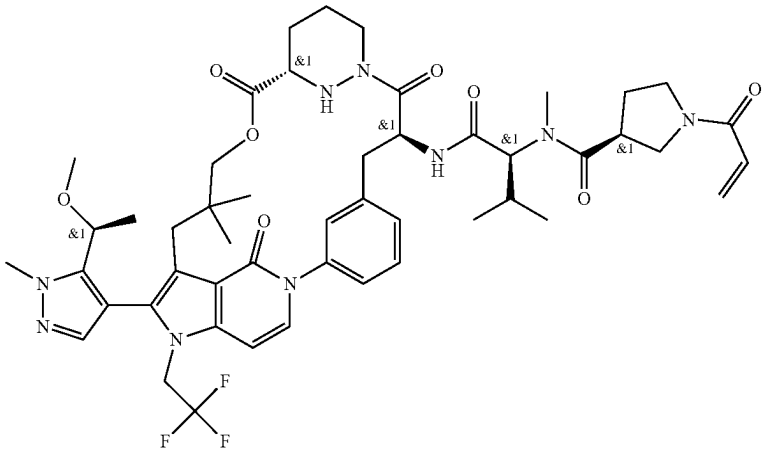
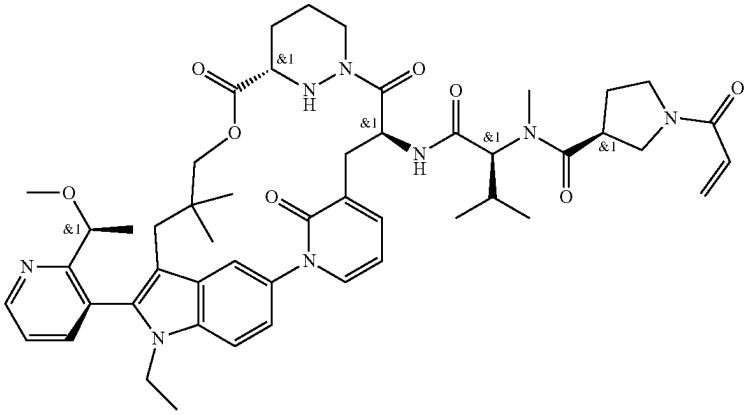
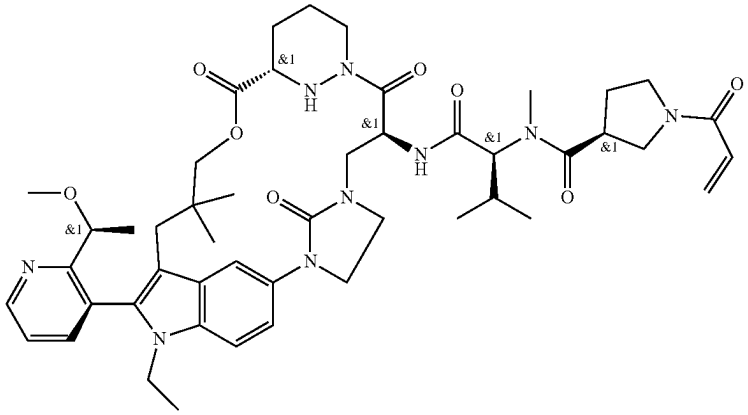
Certain Compounds of the Present Invention	
Ex #	Structure
B255	
B256	
B257	

TABLE 2-continued

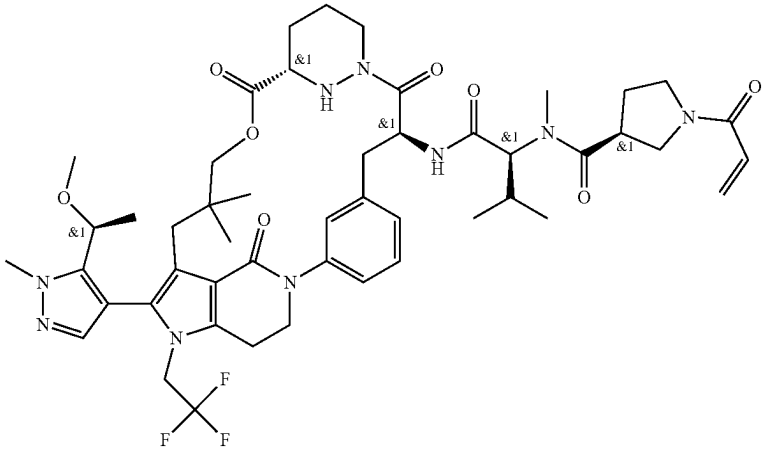
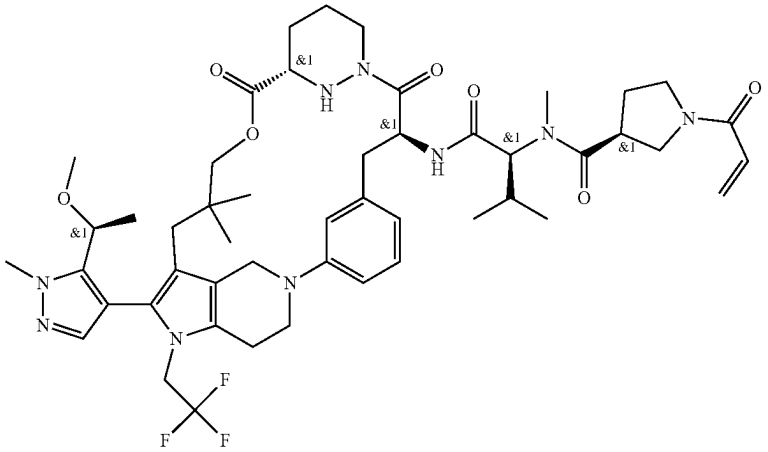
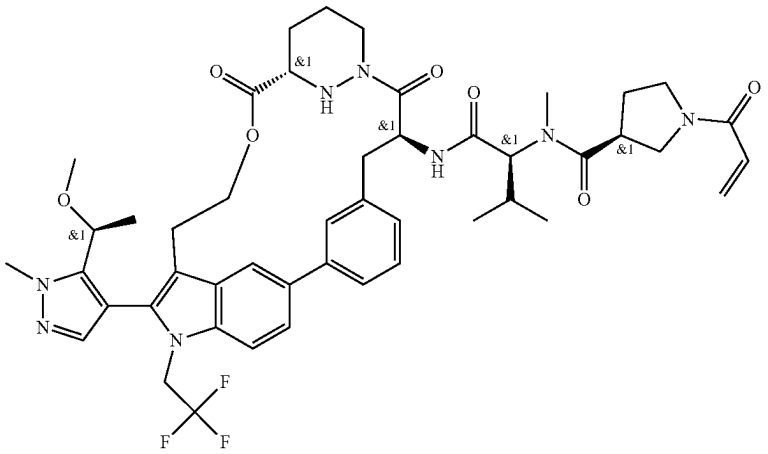
Certain Compounds of the Present Invention	
Ex #	Structure
B258	
B259	
B260	

TABLE 2-continued

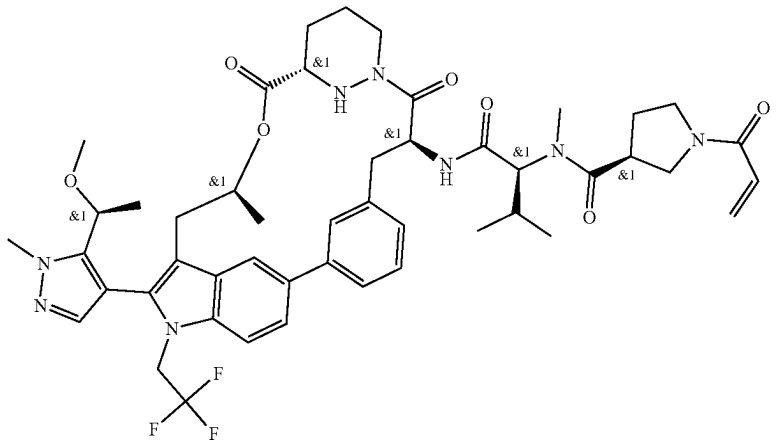
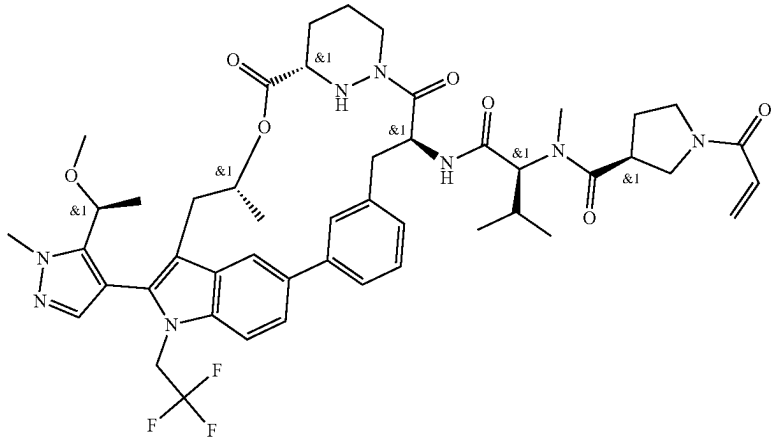
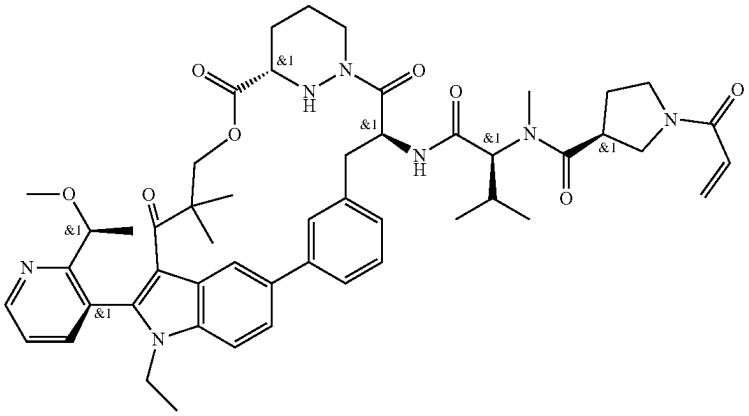
Certain Compounds of the Present Invention	
Ex #	Structure
B261	
B262	
B263	

TABLE 2-continued

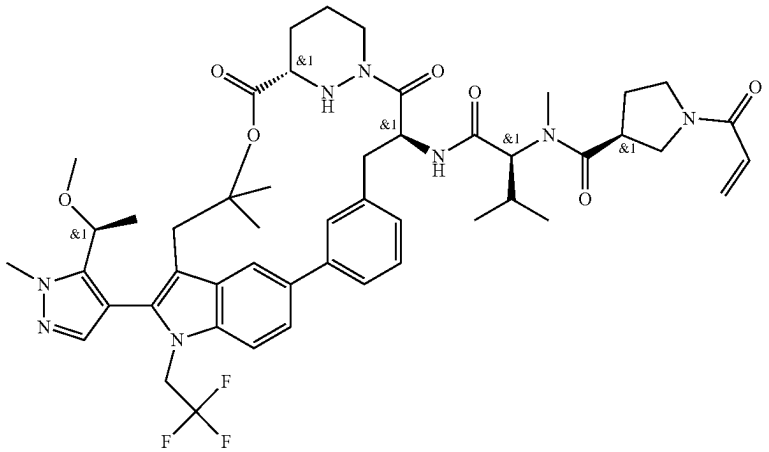
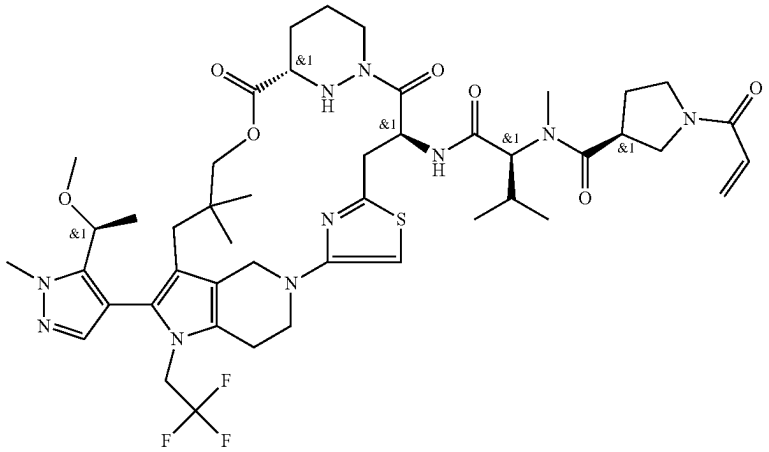
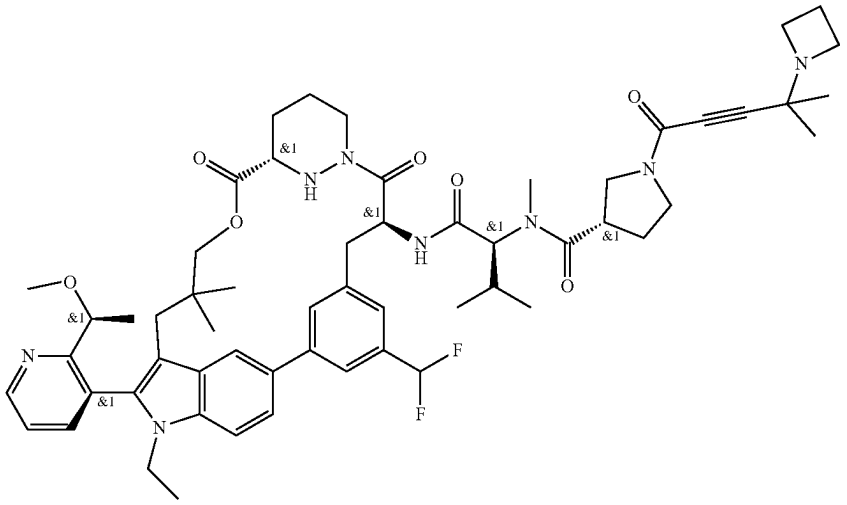
Certain Compounds of the Present Invention	
Ex #	Structure
B264	
B265	
B266	

TABLE 2-continued

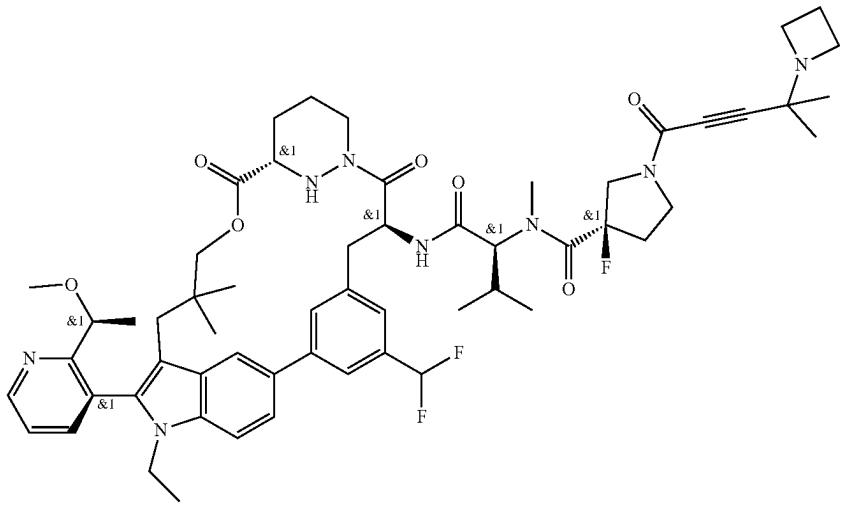
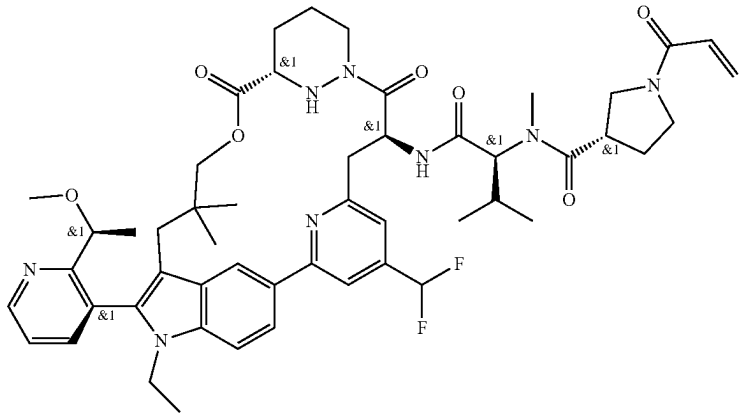
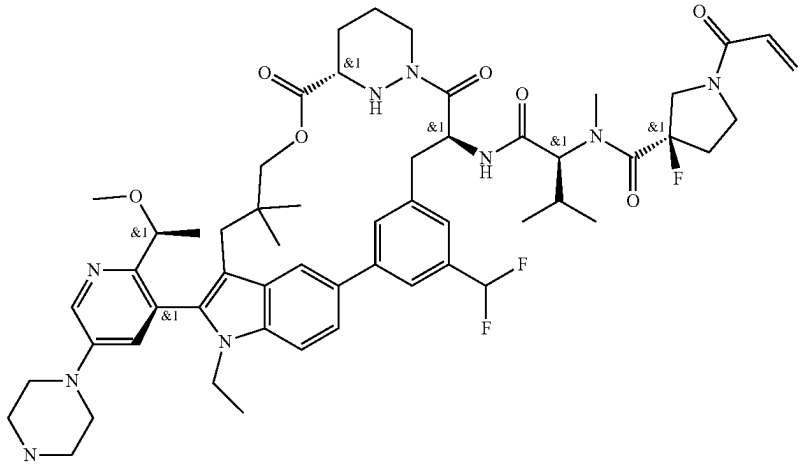
Certain Compounds of the Present Invention	
Ex #	Structure
B267	
B268	
B269	

TABLE 2-continued

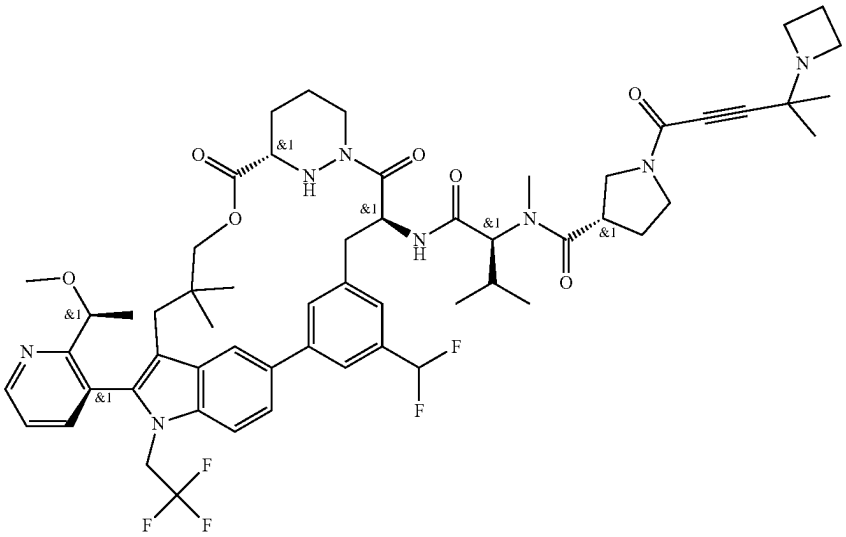
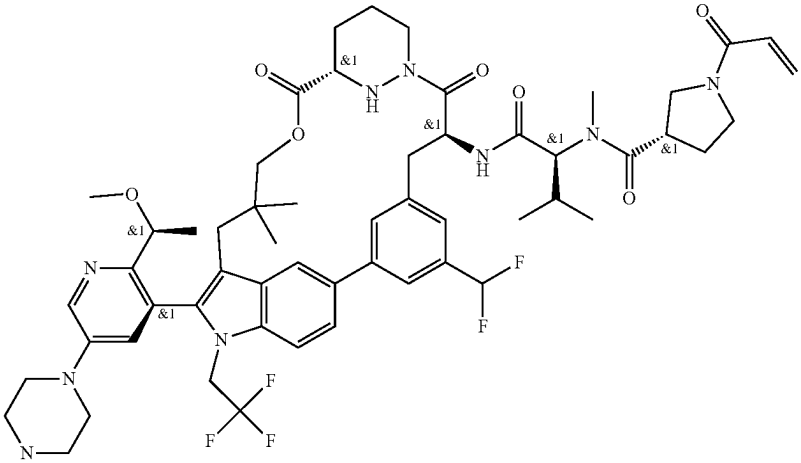
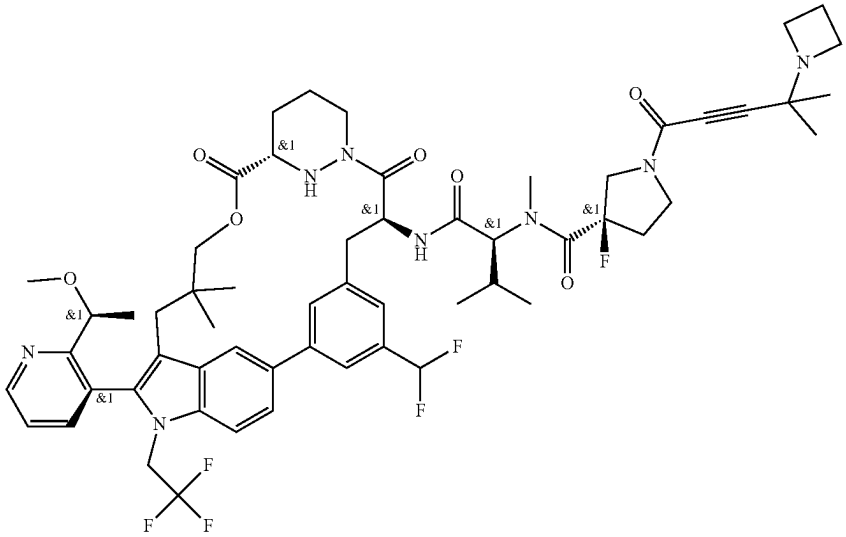
Certain Compounds of the Present Invention	
Ex #	Structure
B270	
B271	
B272	

TABLE 2-continued

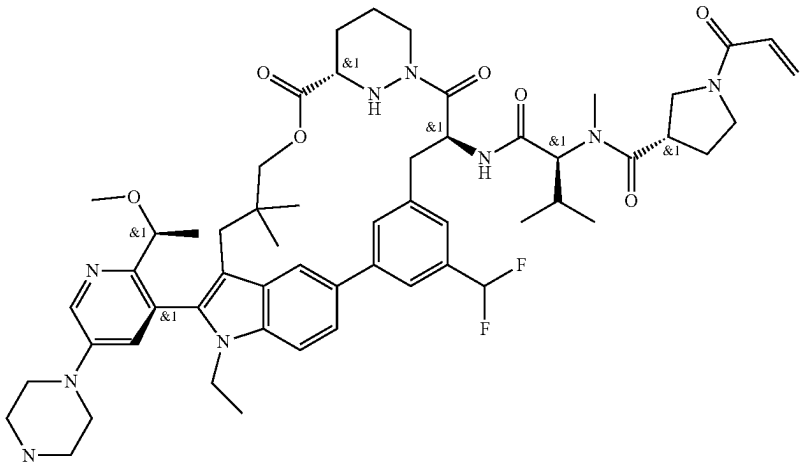
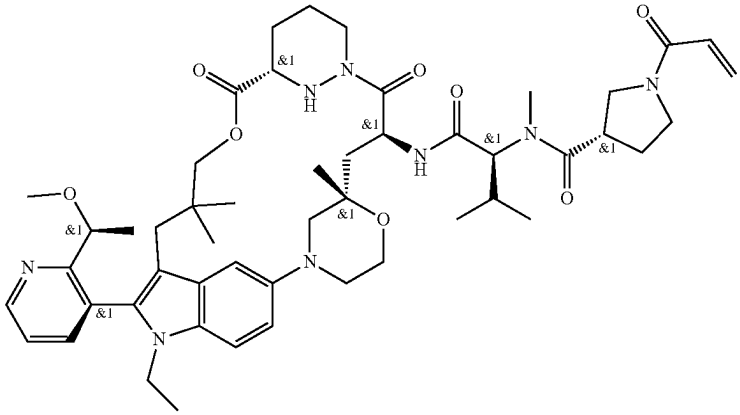
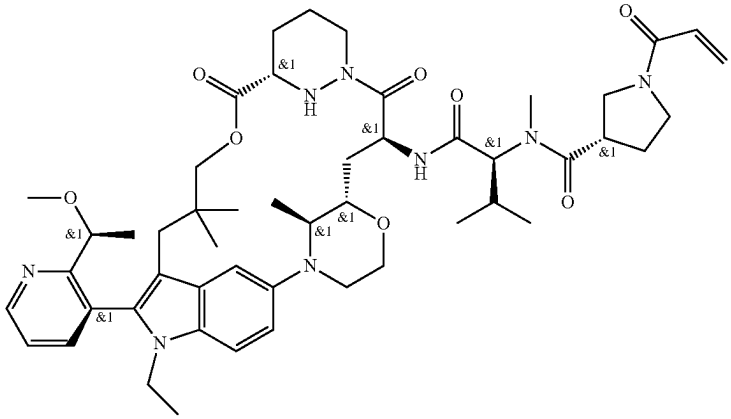
Certain Compounds of the Present Invention	
Ex #	Structure
B273	
B274	
B275	

TABLE 2-continued

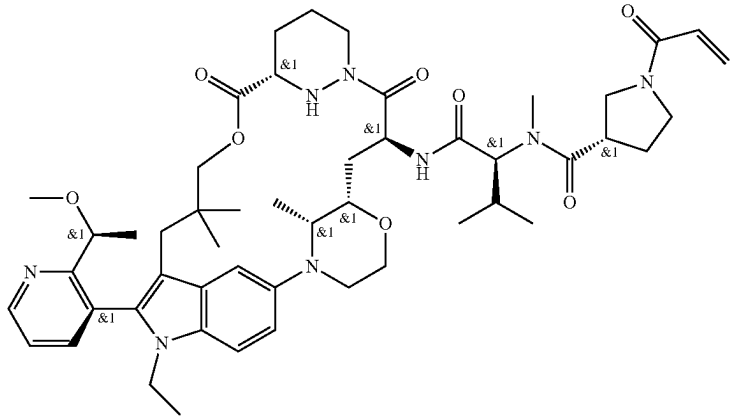
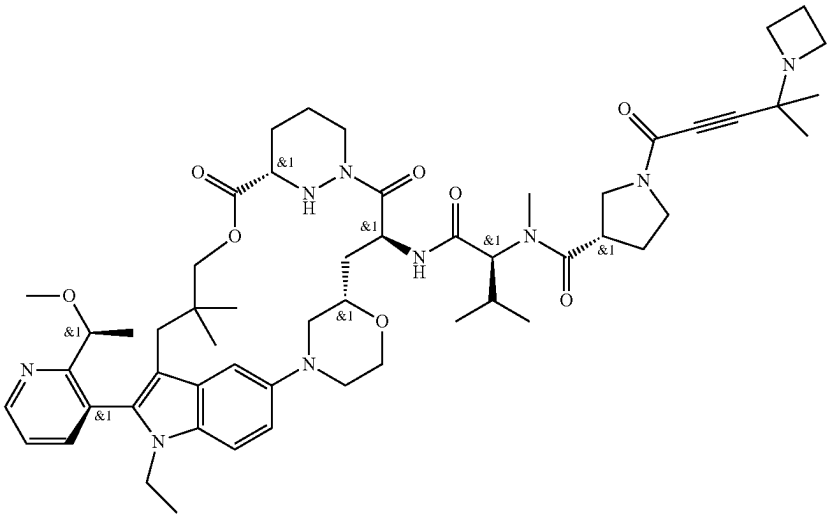
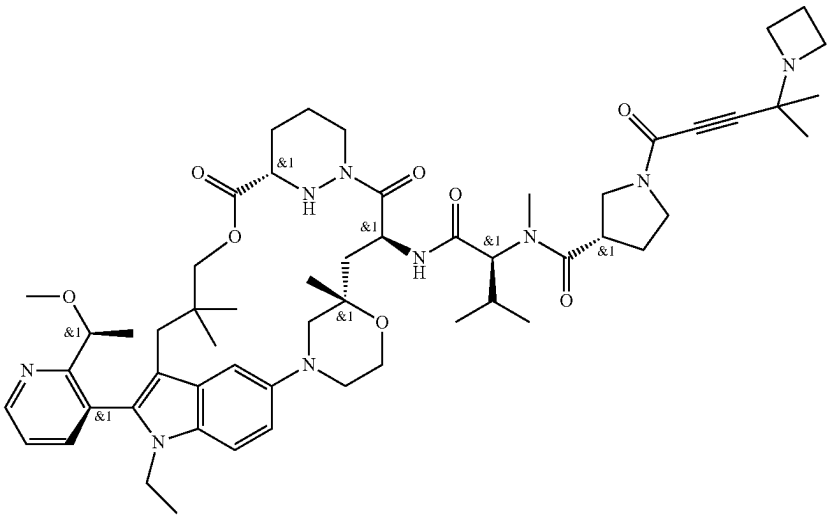
Certain Compounds of the Present Invention	
Ex #	Structure
B276	
B277	
B278	

TABLE 2-continued

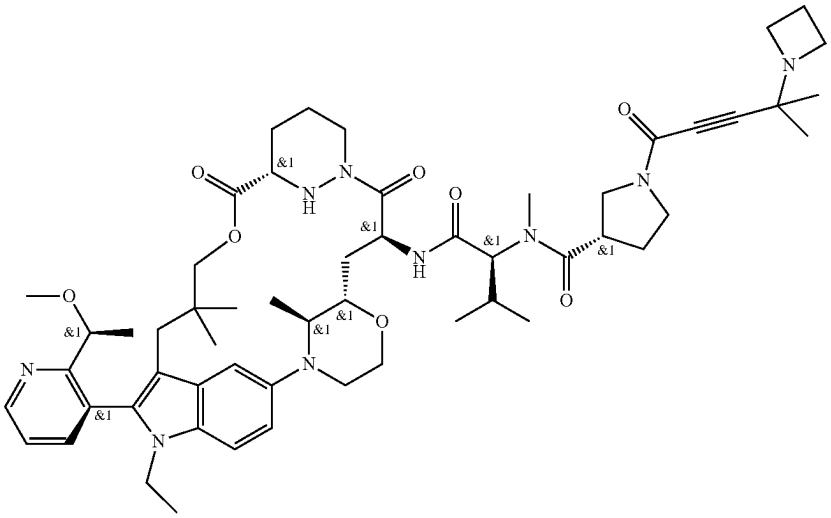
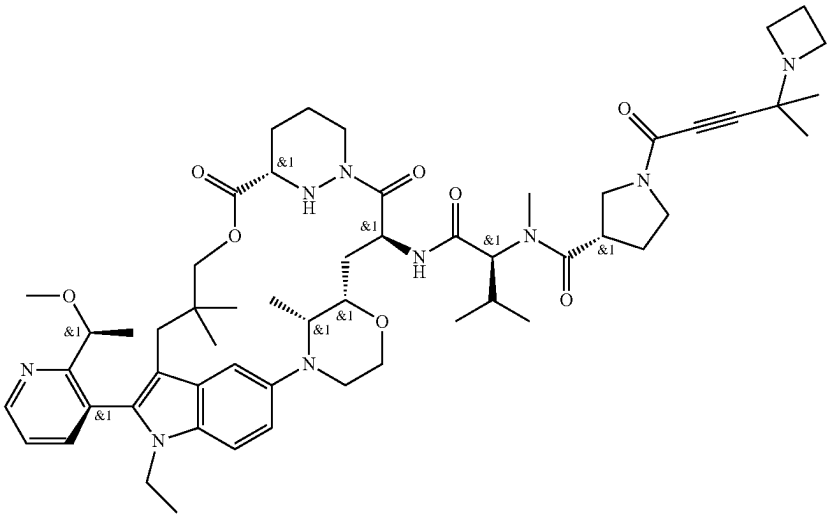
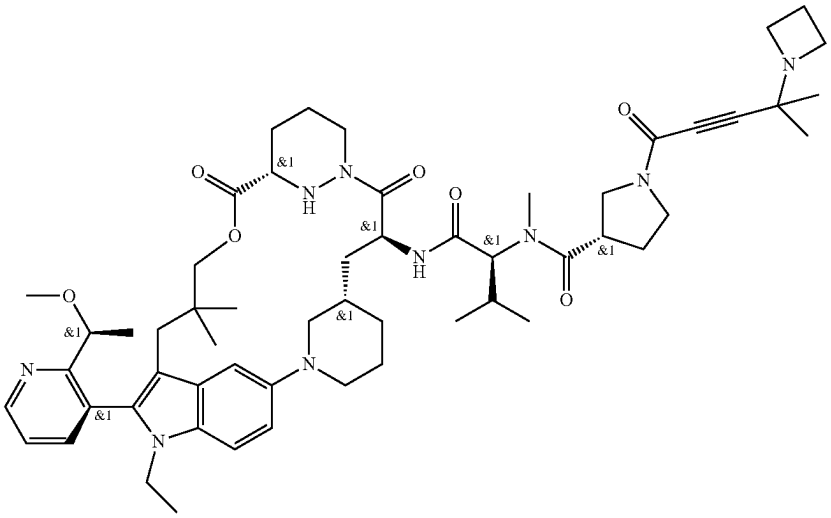
Certain Compounds of the Present Invention	
Ex #	Structure
B279	
B280	
B282	

TABLE 2-continued

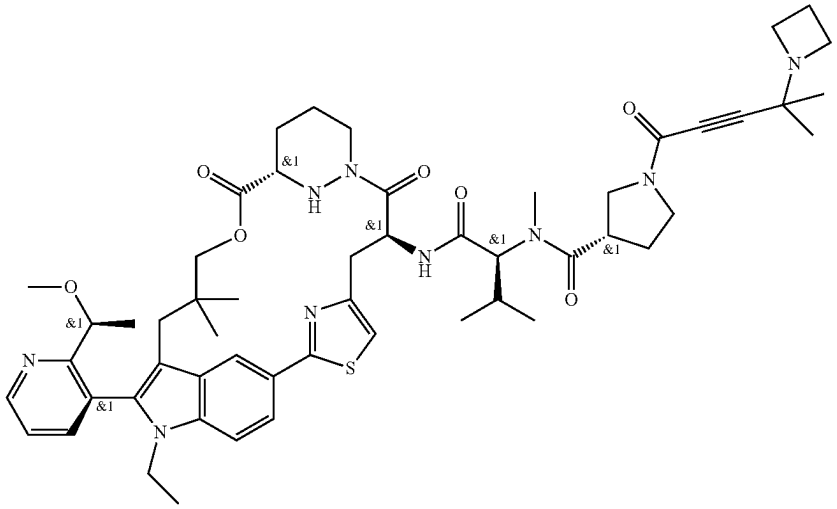
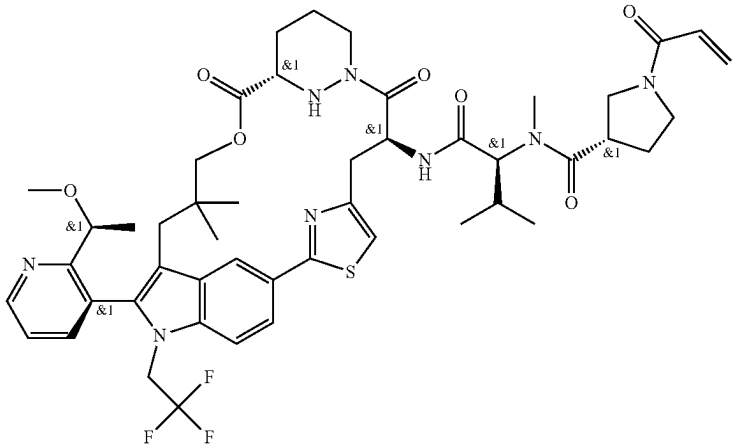
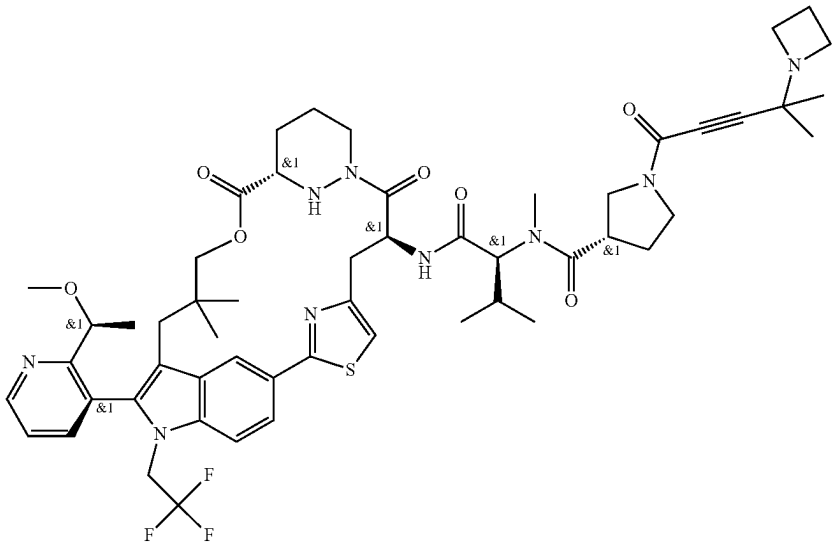
Certain Compounds of the Present Invention	
Ex #	Structure
B283	
B284	
B285	

TABLE 2-continued

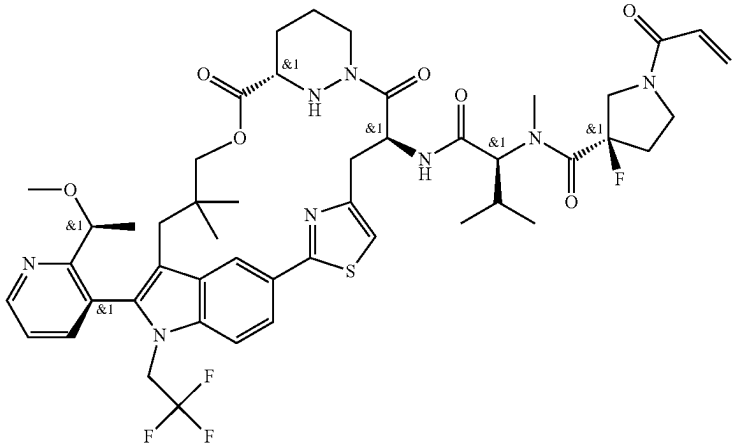
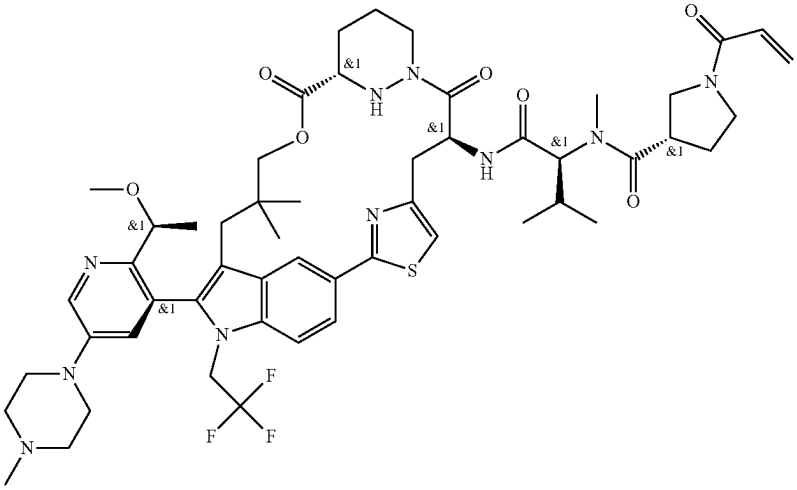
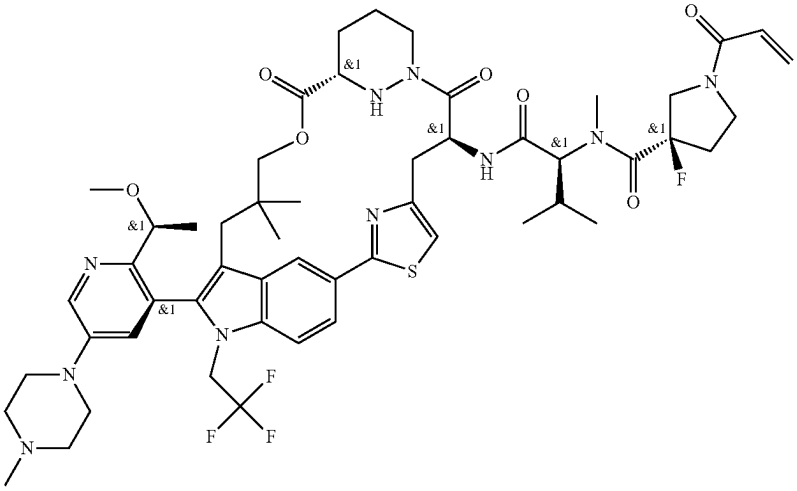
Certain Compounds of the Present Invention	
Ex #	Structure
B286	
B287	
B288	

TABLE 2-continued

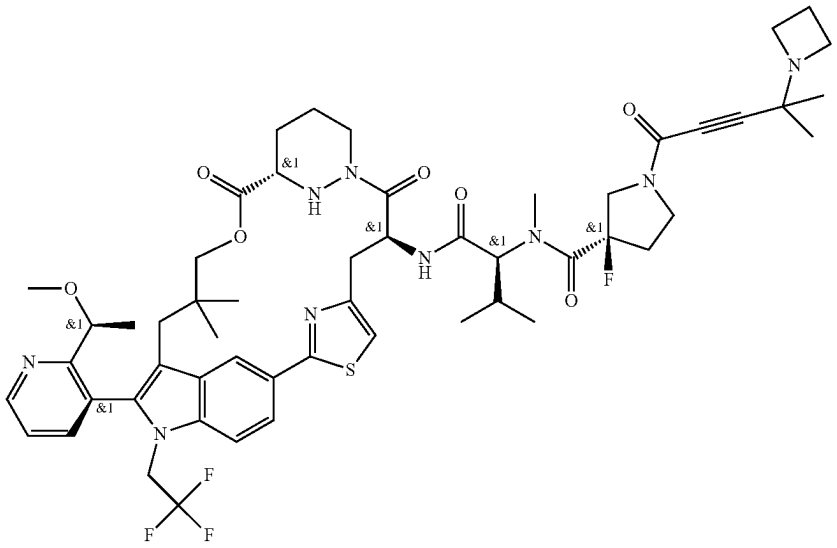
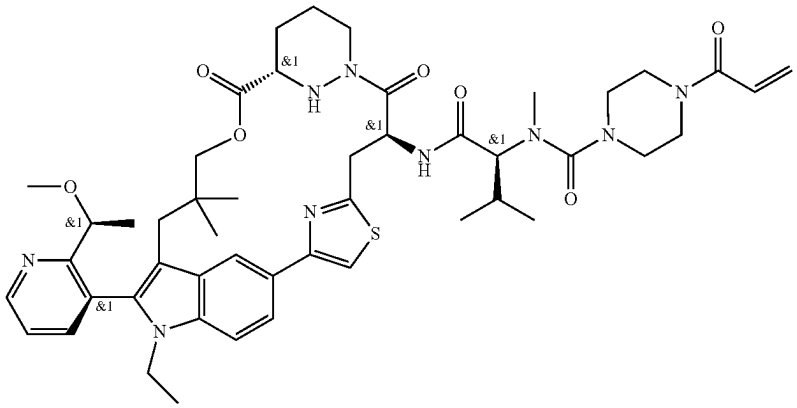
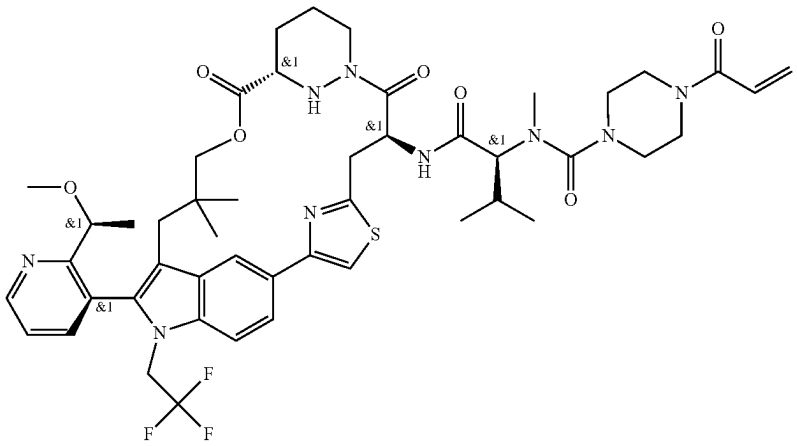
Certain Compounds of the Present Invention	
Ex #	Structure
B289	
B290	
B291	

TABLE 2-continued

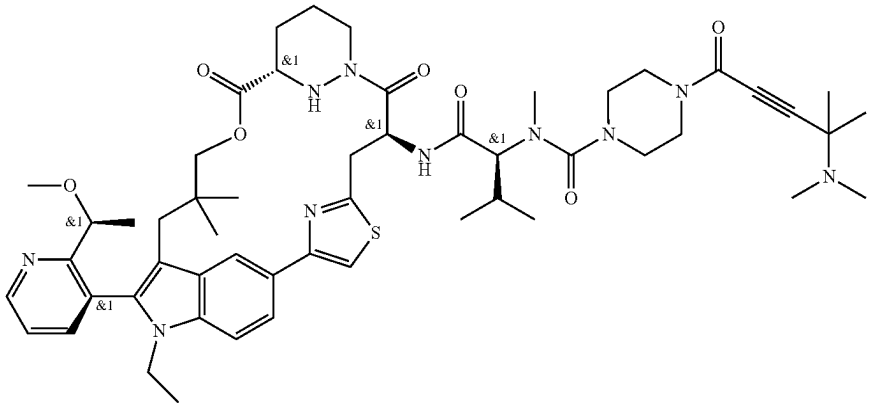
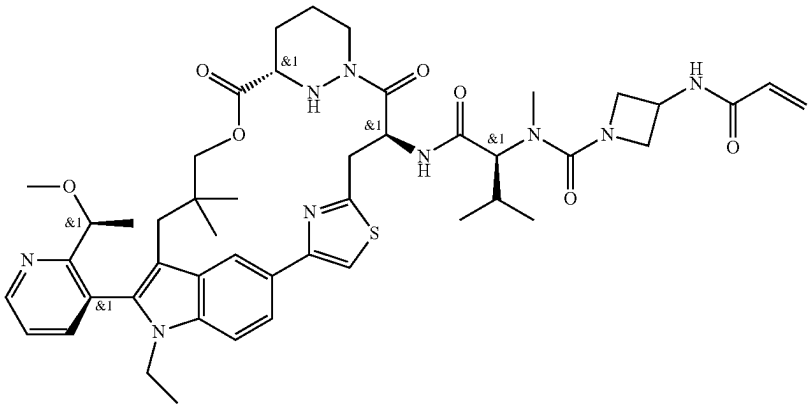
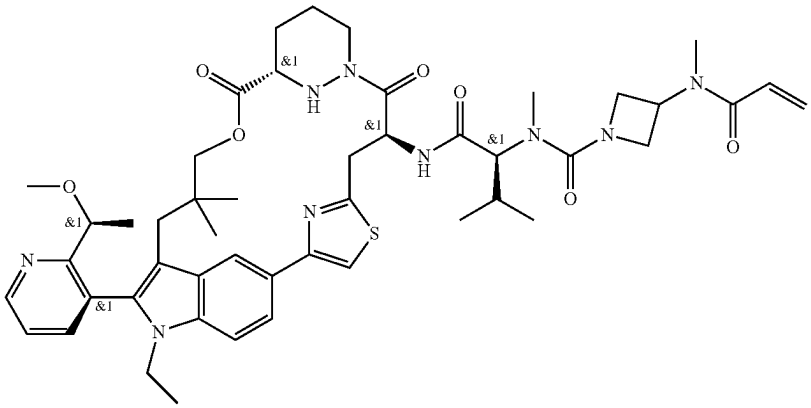
Certain Compounds of the Present Invention	
Ex #	Structure
B292	
B293	
B294	

TABLE 2-continued

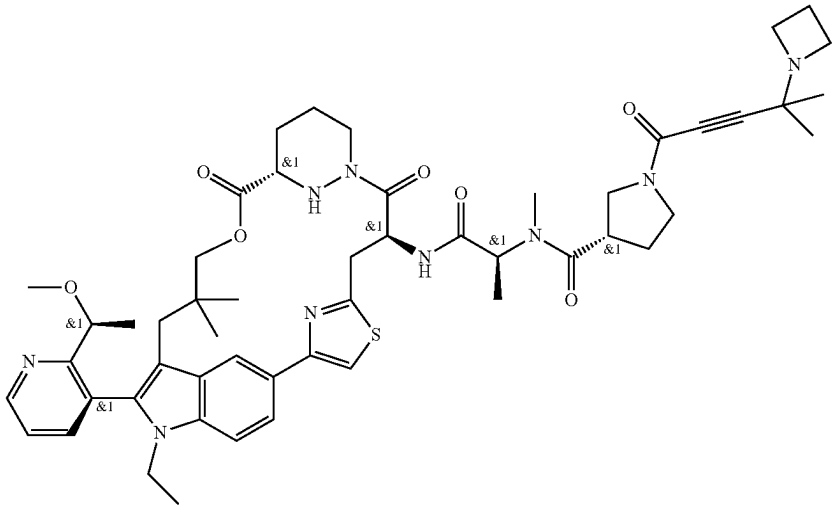
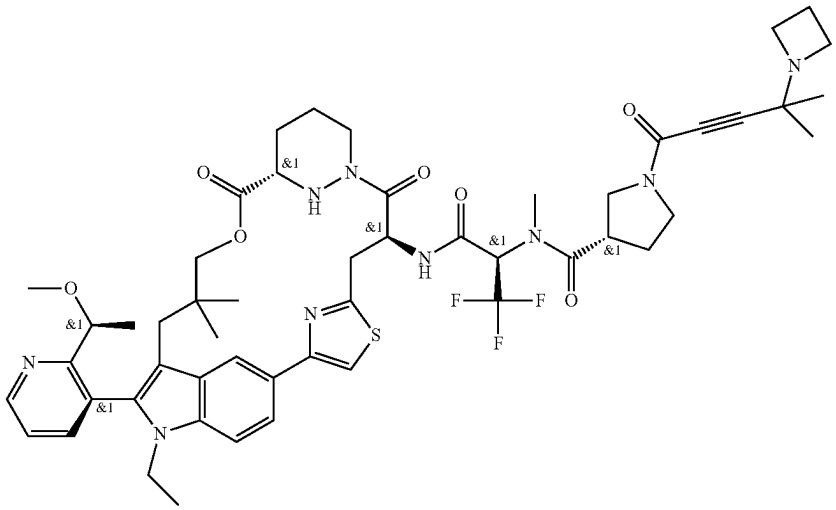
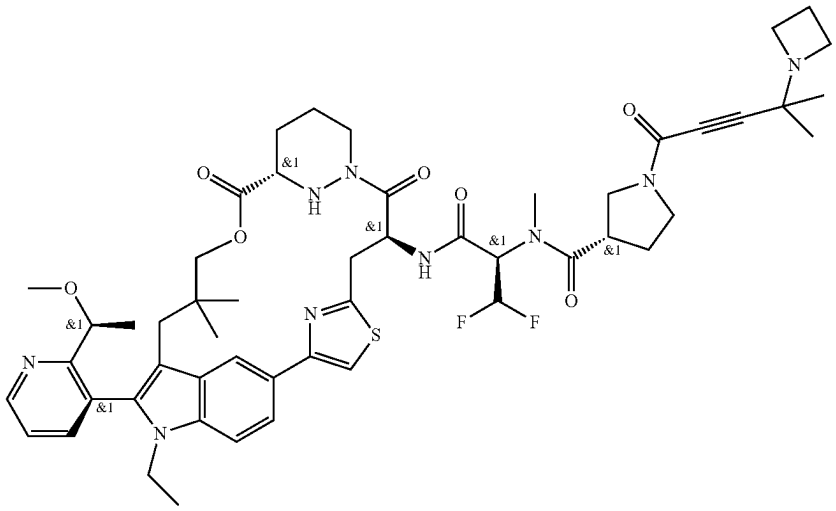
Certain Compounds of the Present Invention	
Ex #	Structure
B295	
B296	
B297	

TABLE 2-continued

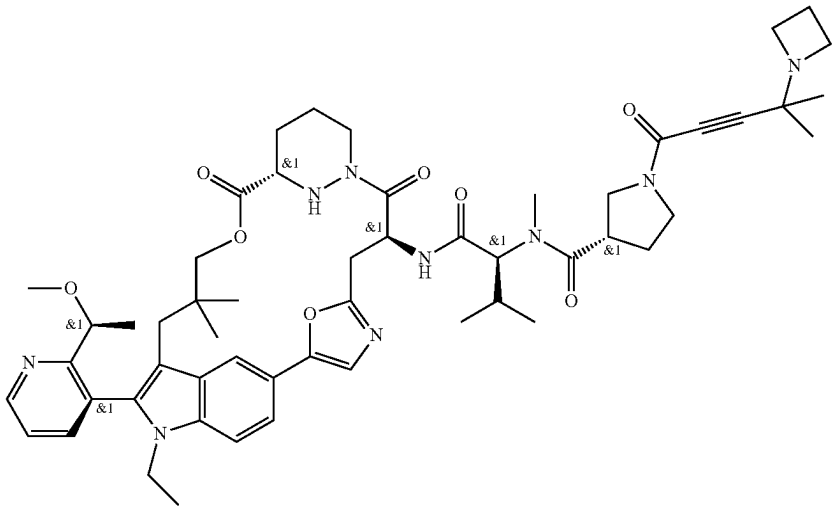
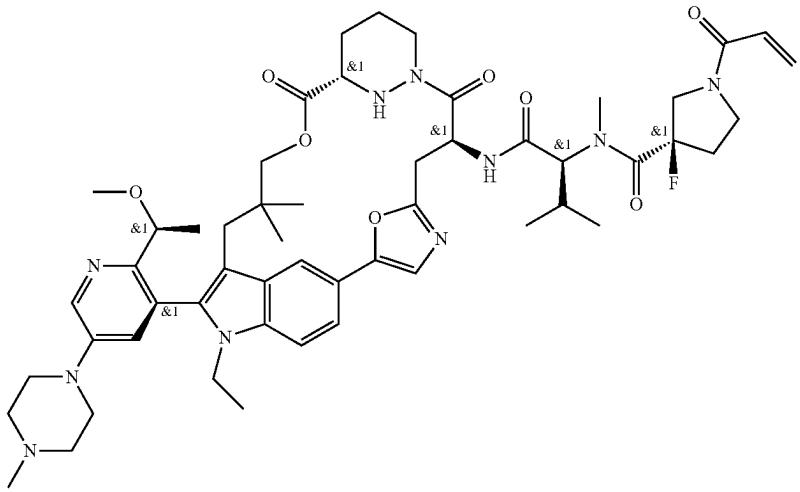
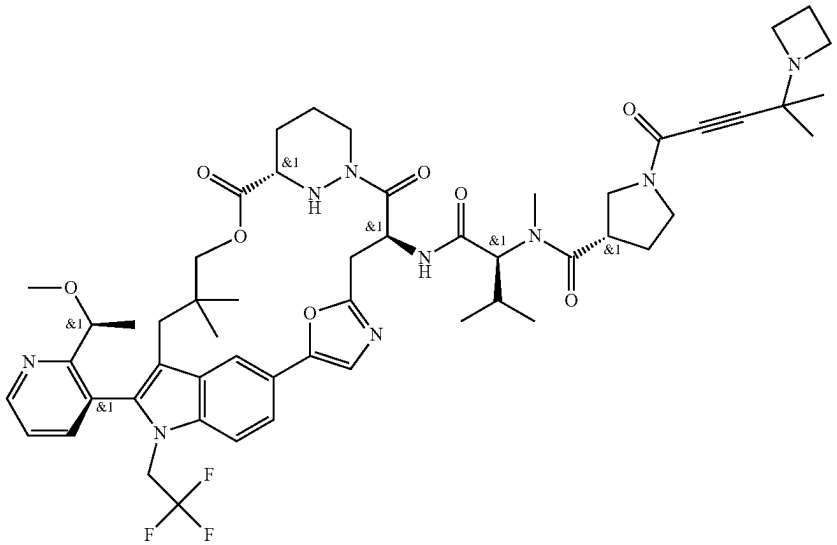
Certain Compounds of the Present Invention	
Ex #	Structure
B298	
B299	
B300	

TABLE 2-continued

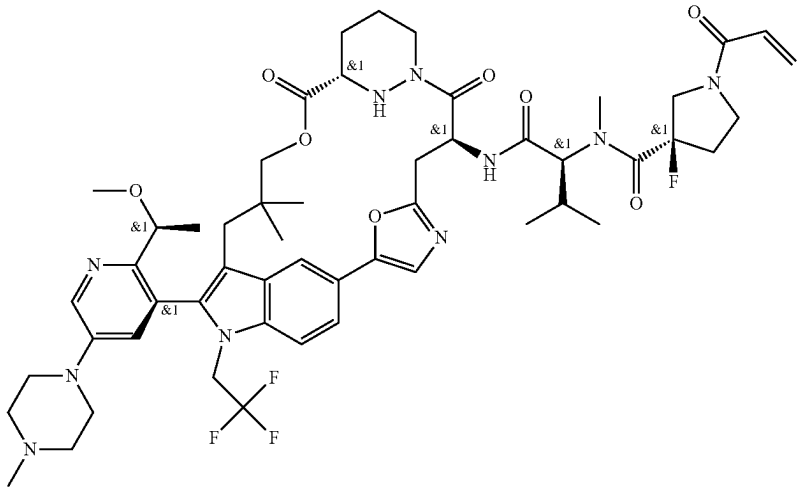
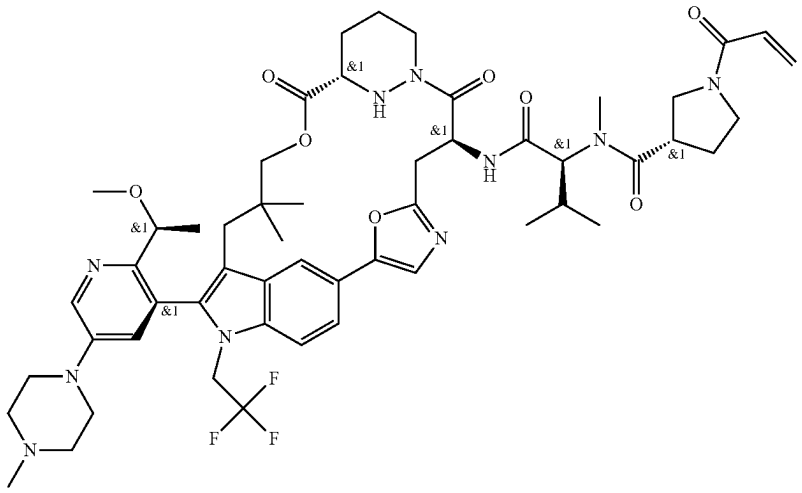
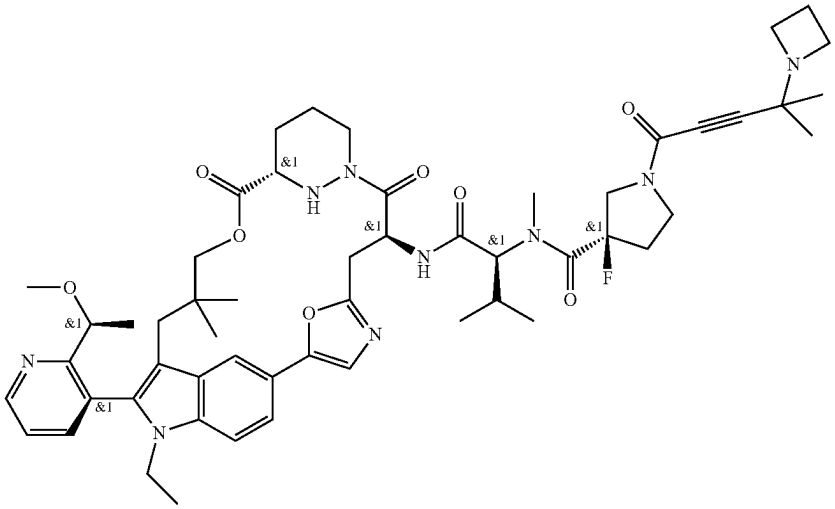
Certain Compounds of the Present Invention	
Ex #	Structure
B301	
B302	
B303	

TABLE 2-continued

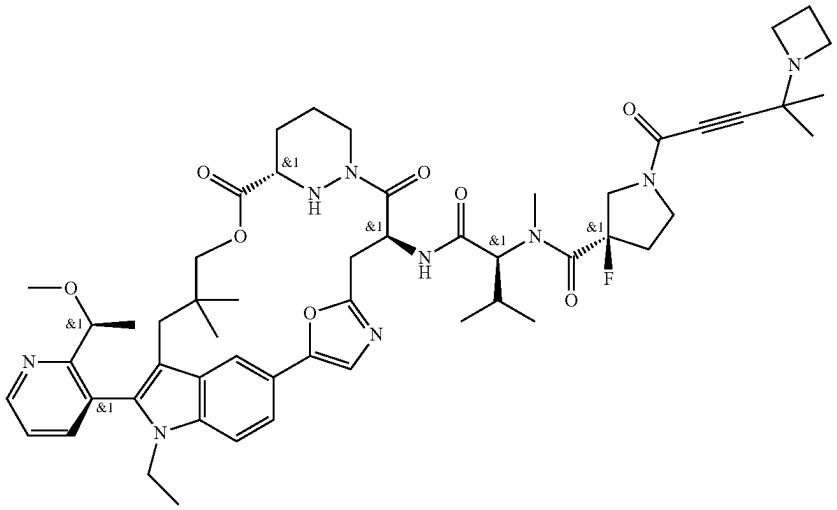
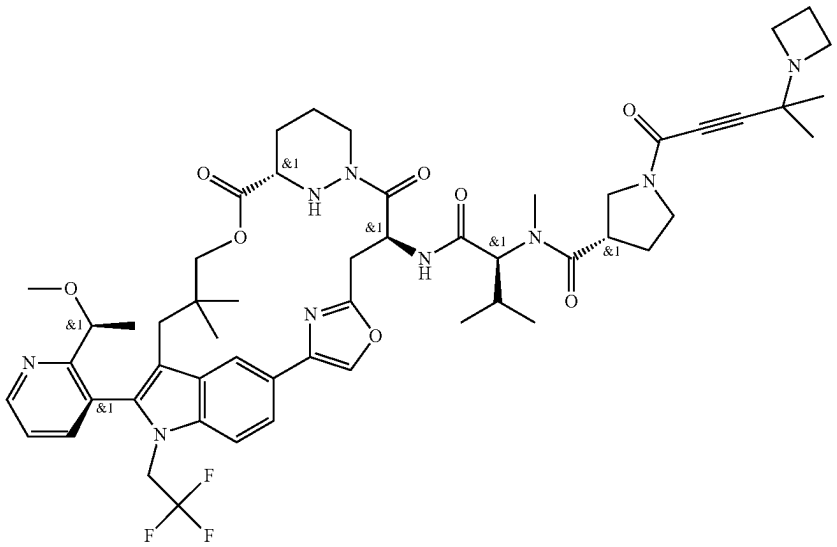
Certain Compounds of the Present Invention	
Ex #	Structure
B304	
B305	

TABLE 2-continued

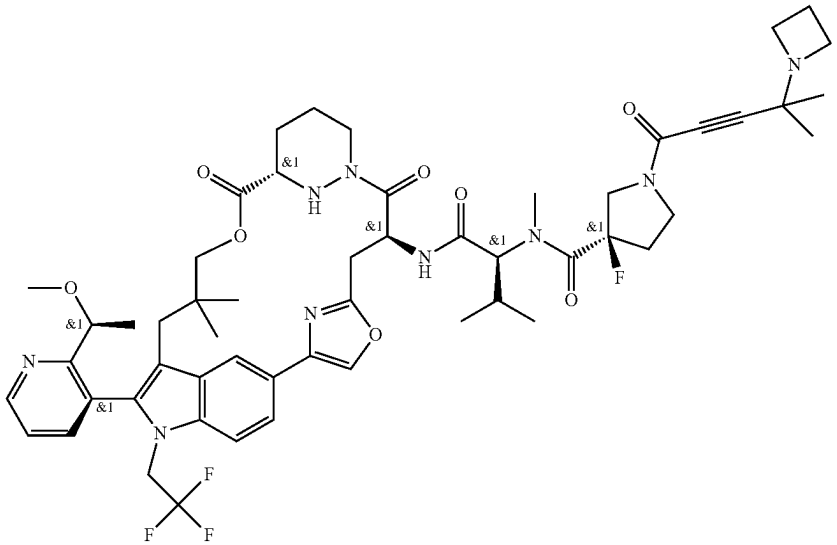
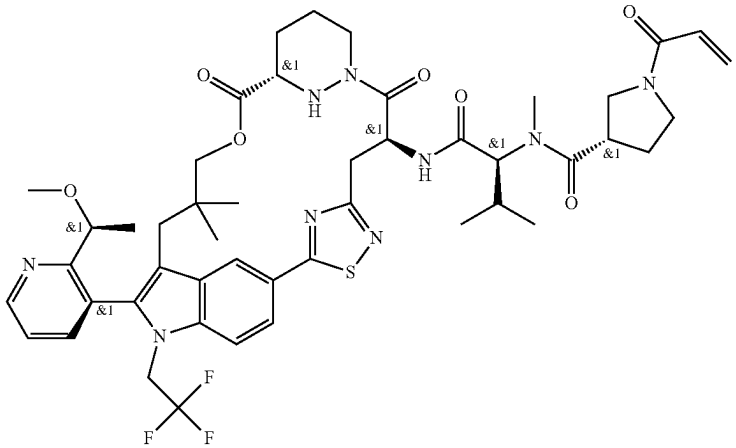
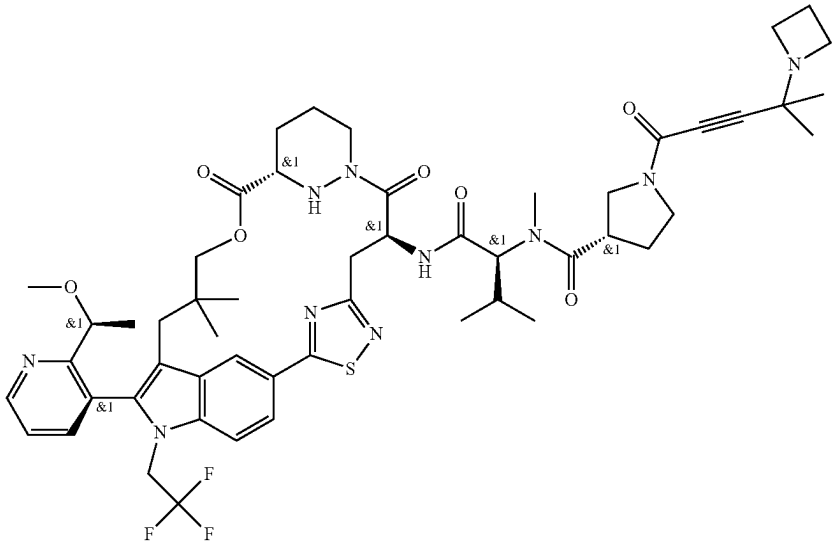
Certain Compounds of the Present Invention	
Ex #	Structure
B306	
B307	
B308	

TABLE 2-continued

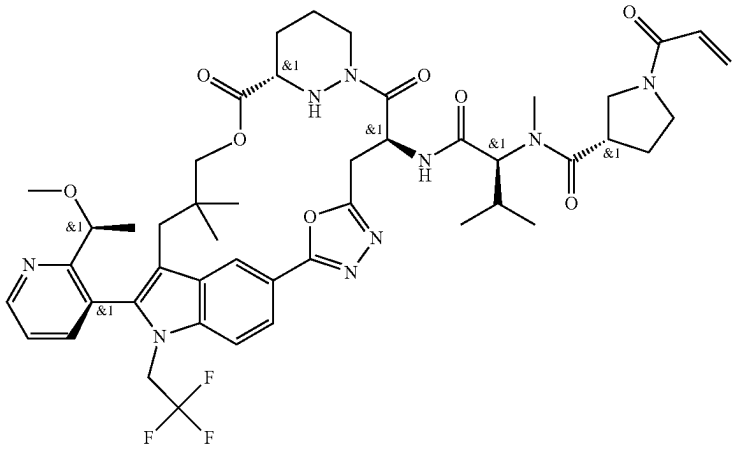
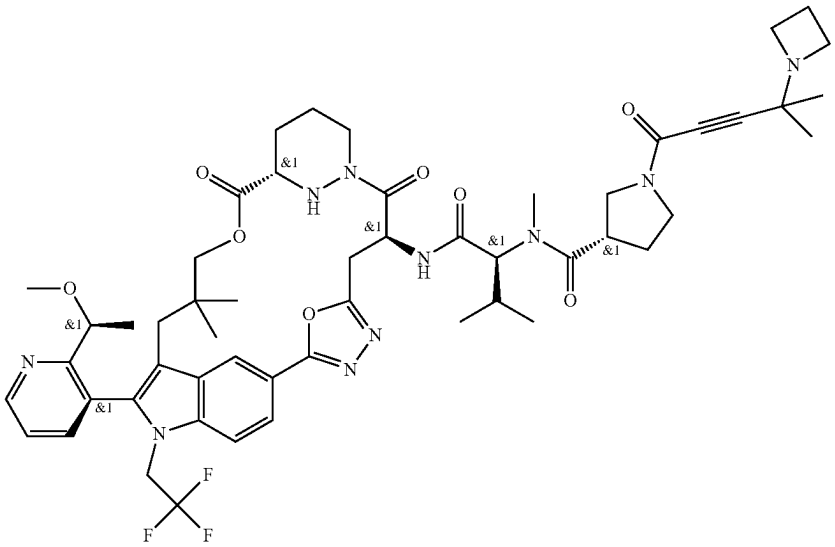
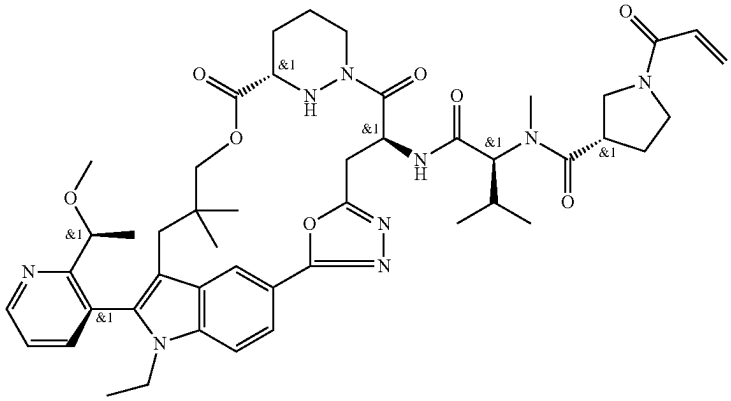
Certain Compounds of the Present Invention	
Ex #	Structure
B309	
B310	
B311	

TABLE 2-continued

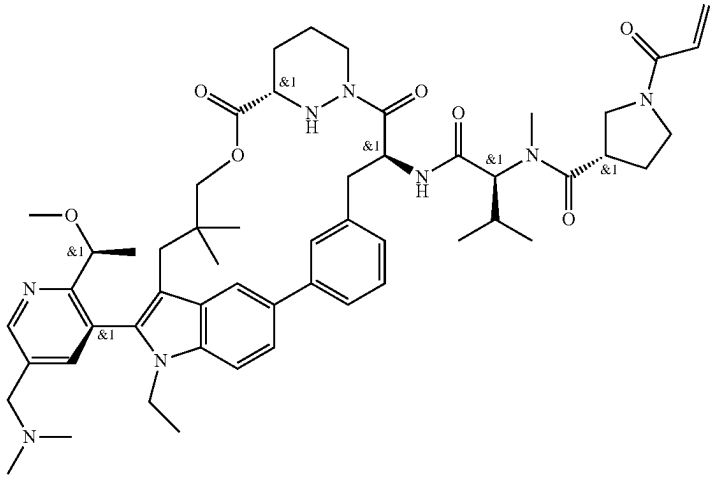
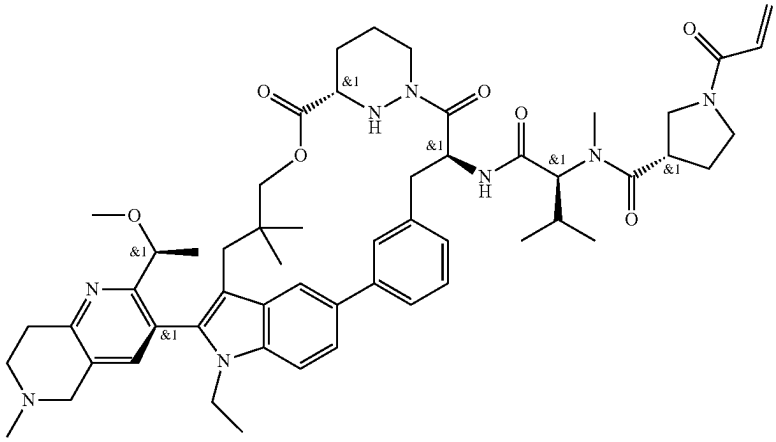
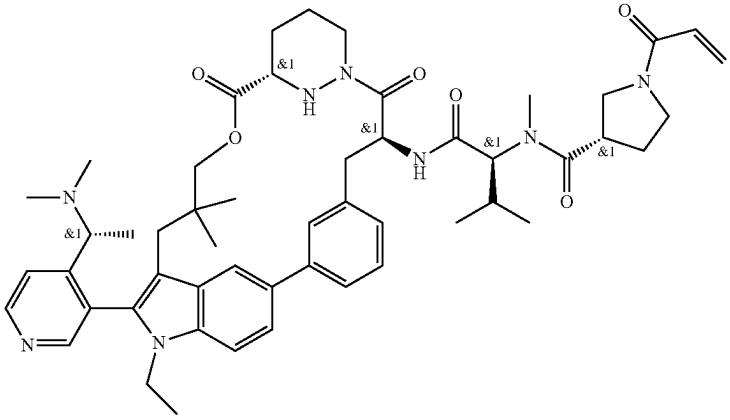
Certain Compounds of the Present Invention	
Ex #	Structure
B312	
B313	
B314	

TABLE 2-continued

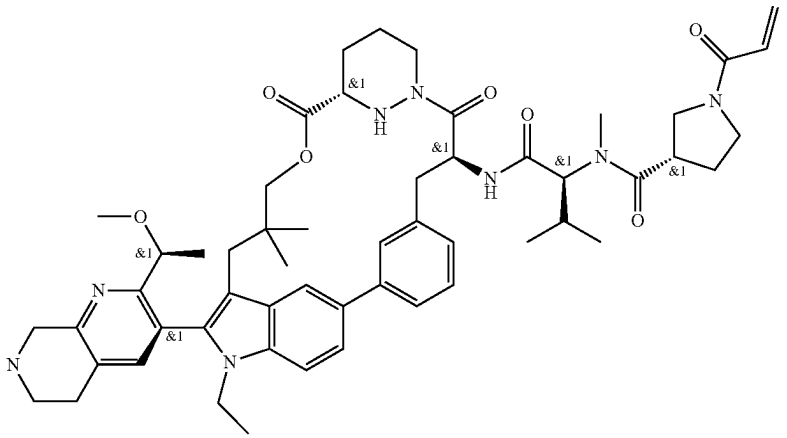
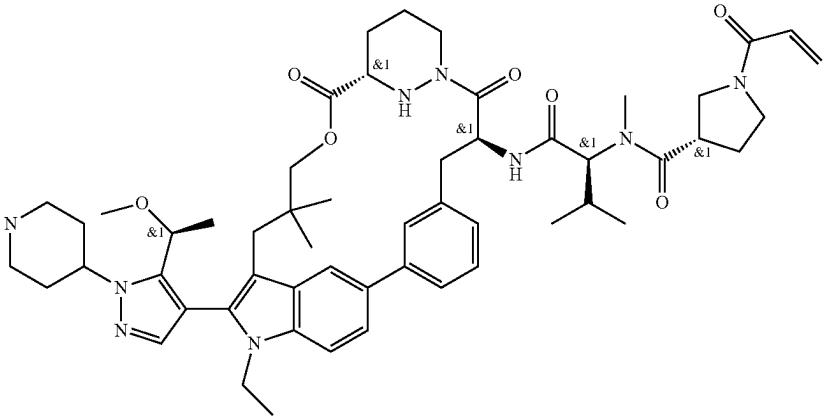
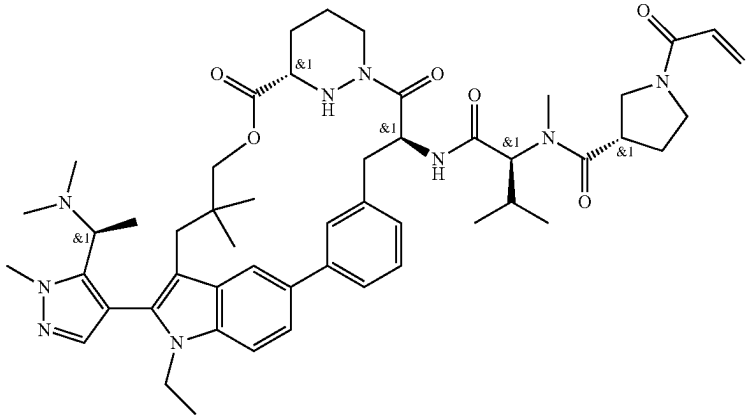
Certain Compounds of the Present Invention	
Ex #	Structure
B315	
B316	
B317	

TABLE 2-continued

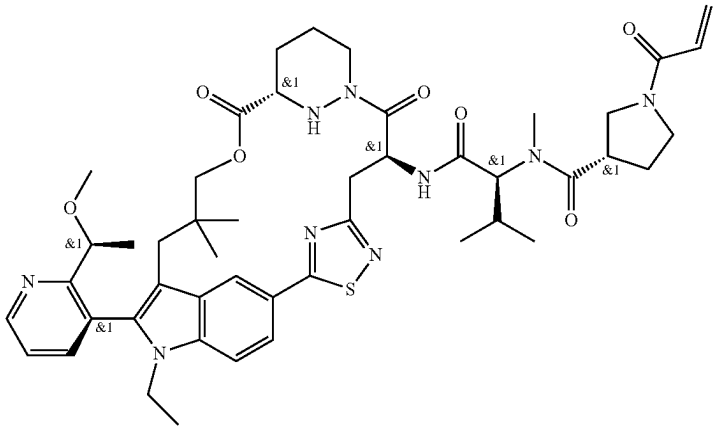
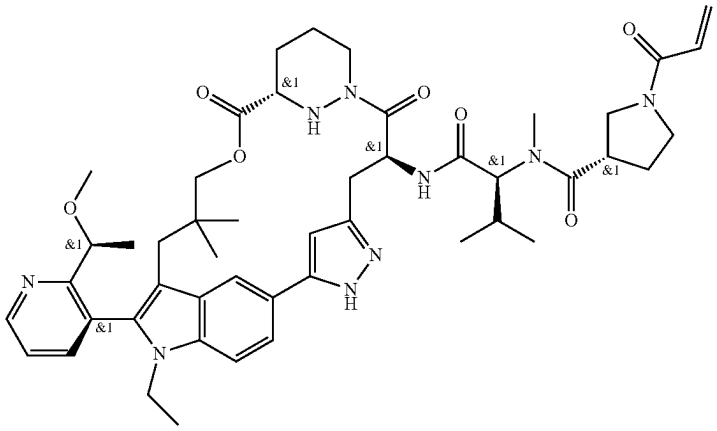
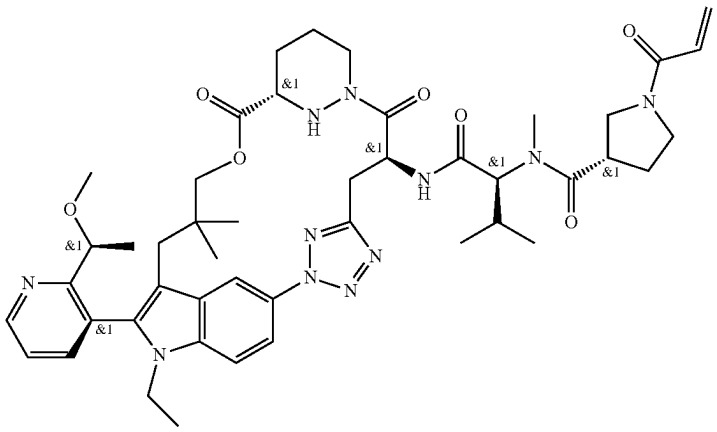
Certain Compounds of the Present Invention	
Ex #	Structure
B318	
B319	
B320	

TABLE 2-continued

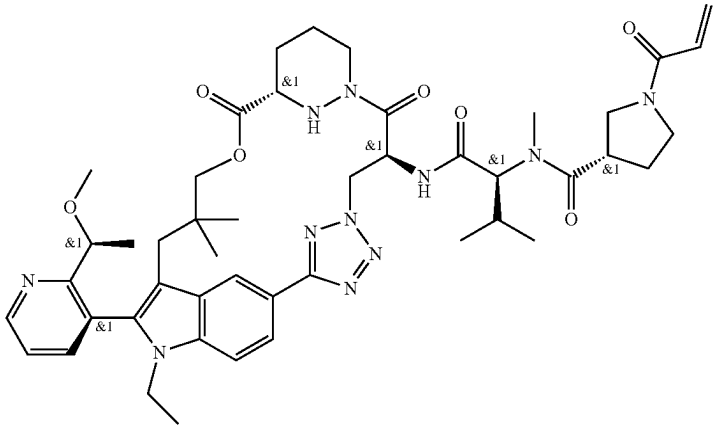
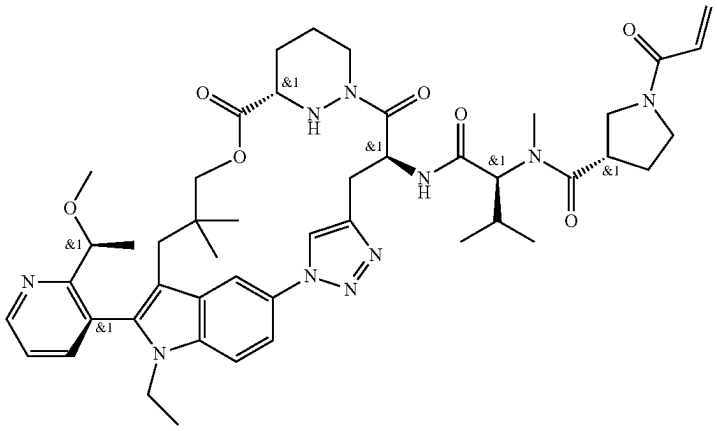
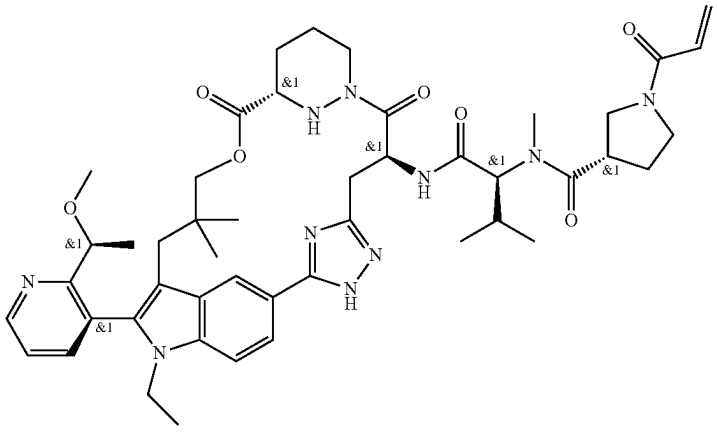
Certain Compounds of the Present Invention	
Ex #	Structure
B321	
B322	
B323	

TABLE 2-continued

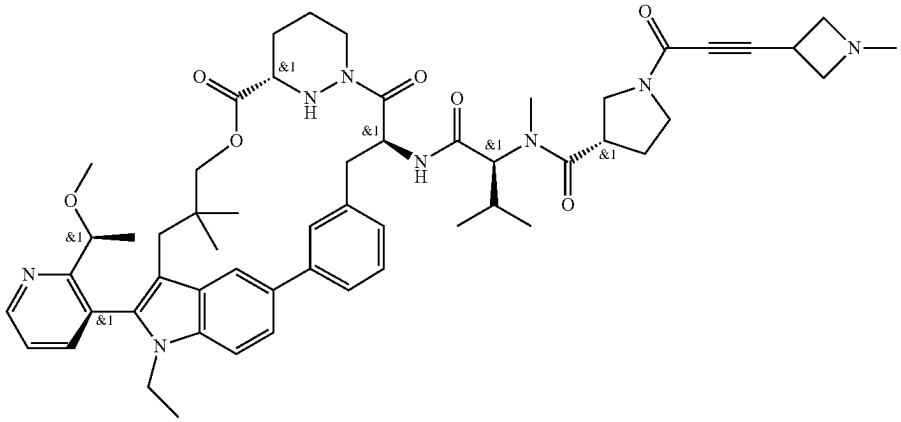
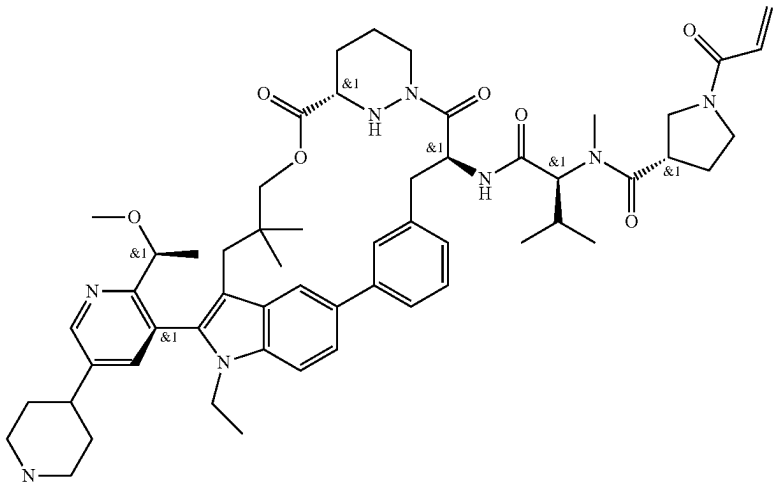
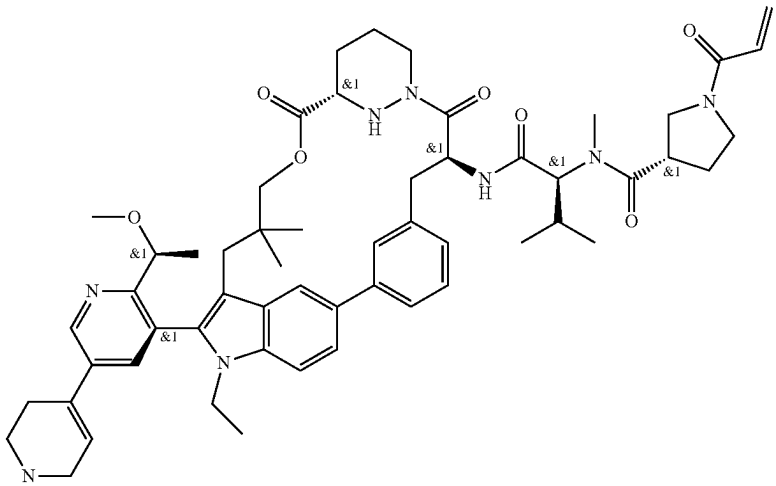
Certain Compounds of the Present Invention	
Ex #	Structure
B324	
B325	
B326	

TABLE 2-continued

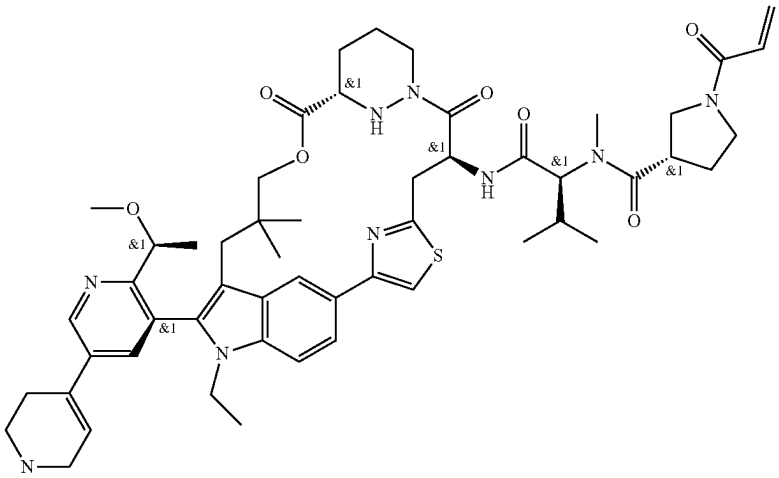
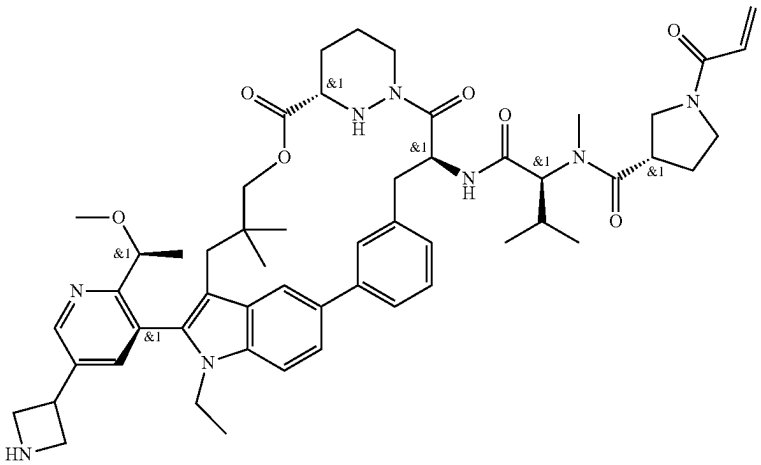
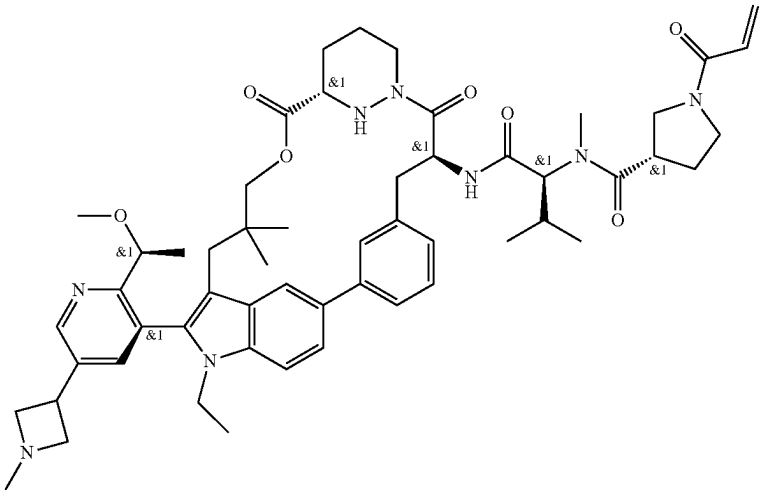
Certain Compounds of the Present Invention	
Ex #	Structure
B327	
B328	
B329	

TABLE 2-continued

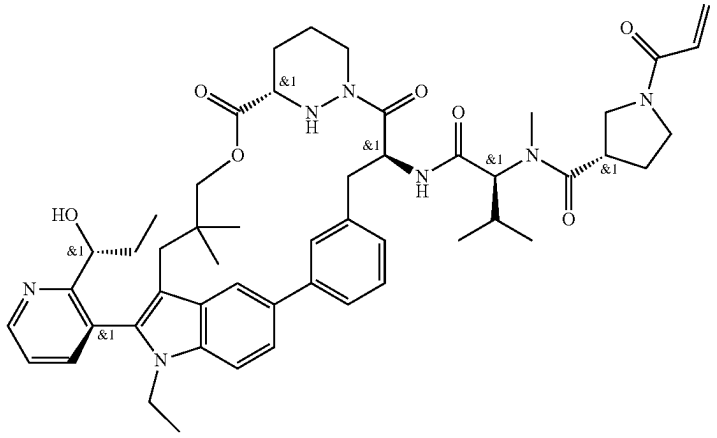
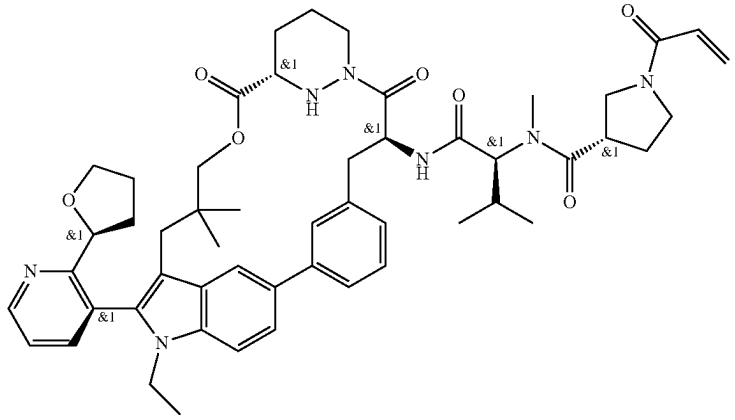
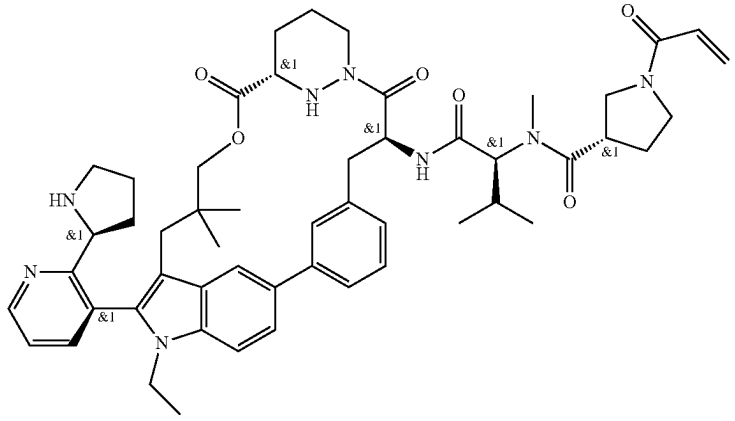
Certain Compounds of the Present Invention	
Ex #	Structure
B330	
B331	
B332	

TABLE 2-continued

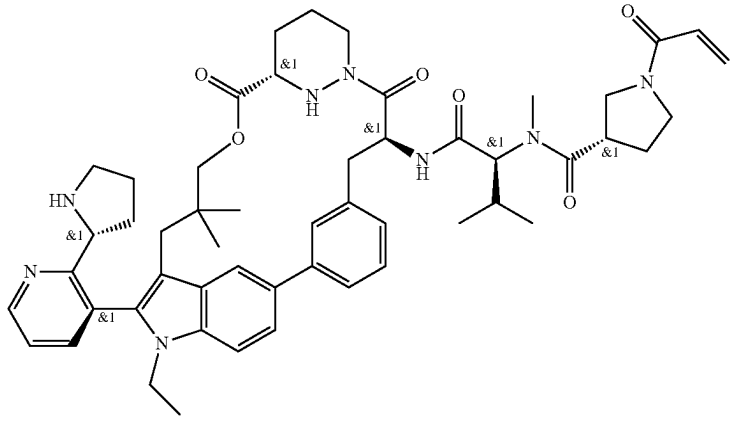
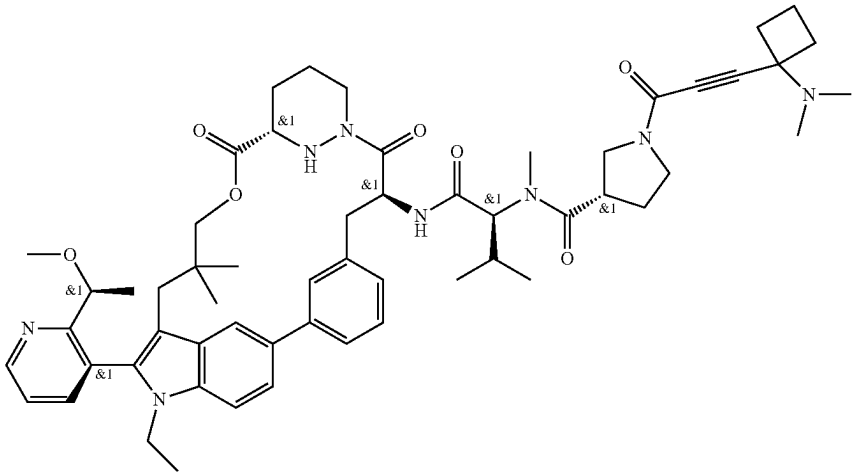
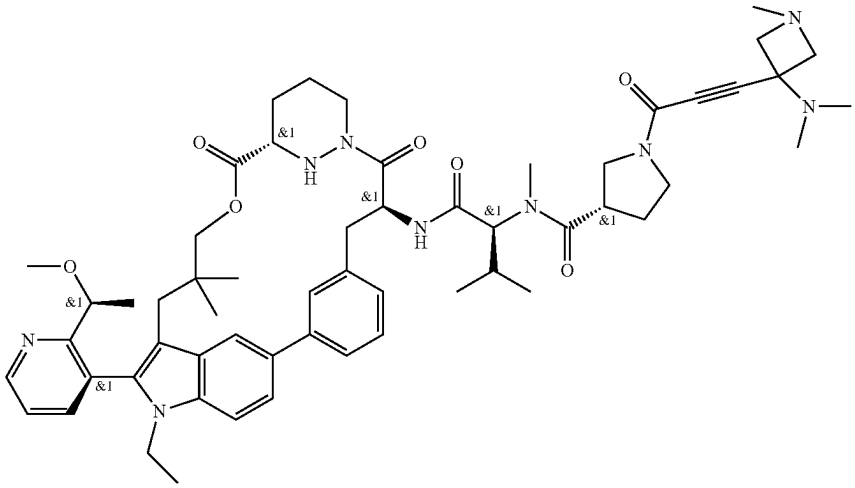
Certain Compounds of the Present Invention	
Ex #	Structure
B333	
B334	
B335	

TABLE 2-continued

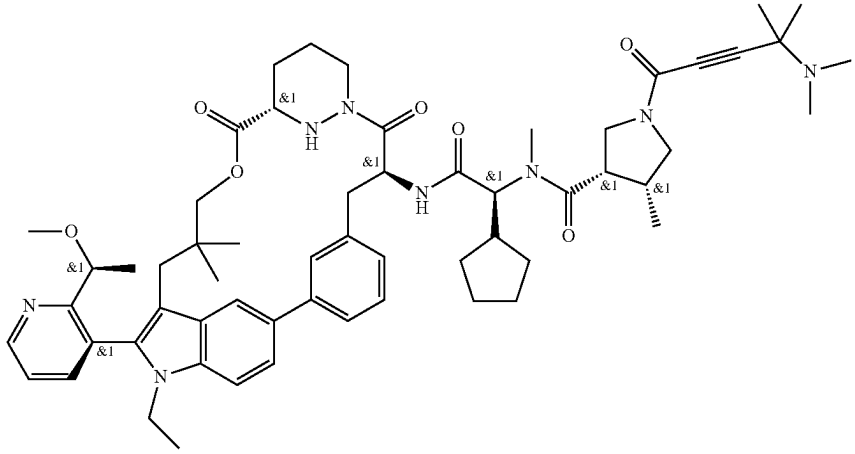
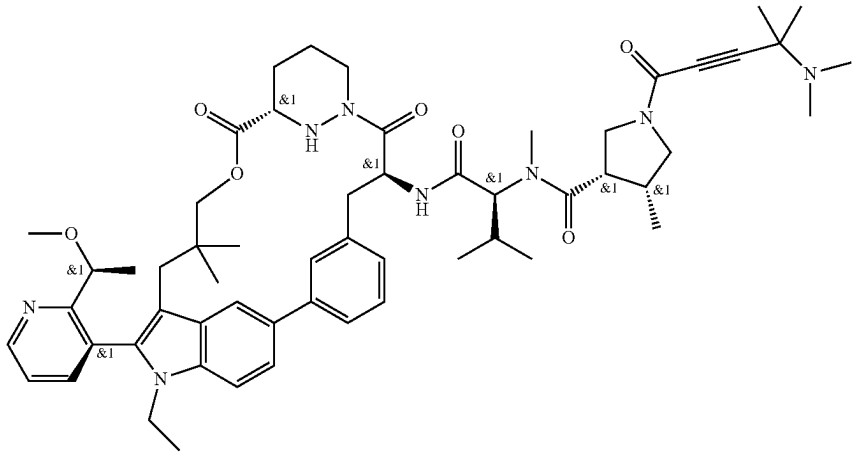
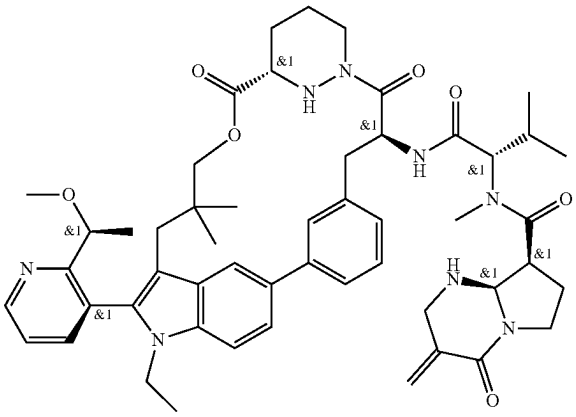
Certain Compounds of the Present Invention	
Ex #	Structure
B336	
B337	
B338	

TABLE 2-continued

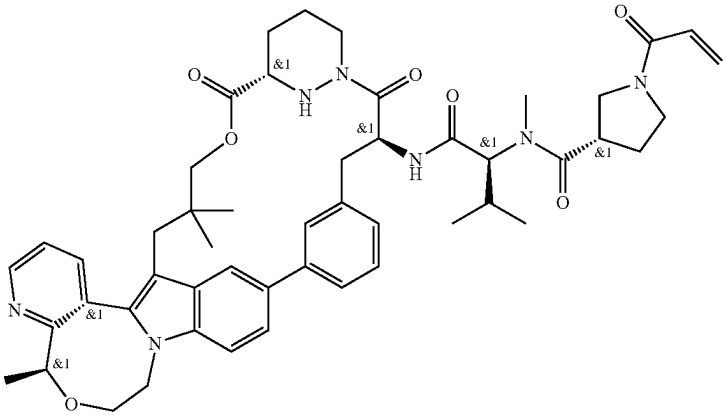
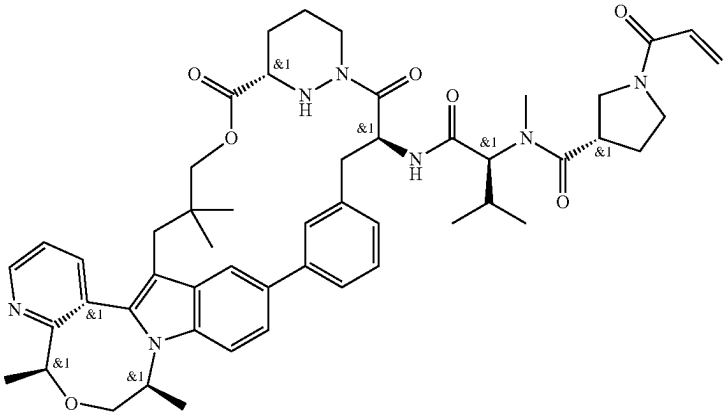
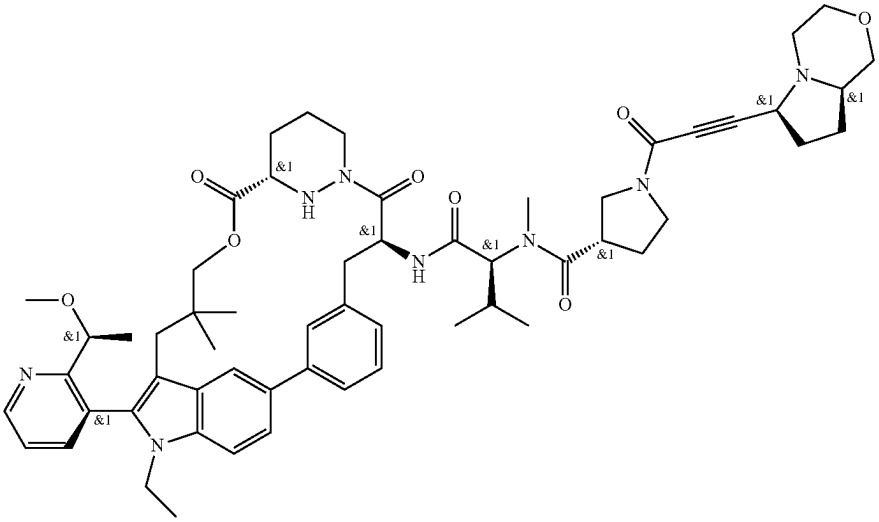
Certain Compounds of the Present Invention	
Ex #	Structure
B339	
B340	
B341	

TABLE 2-continued

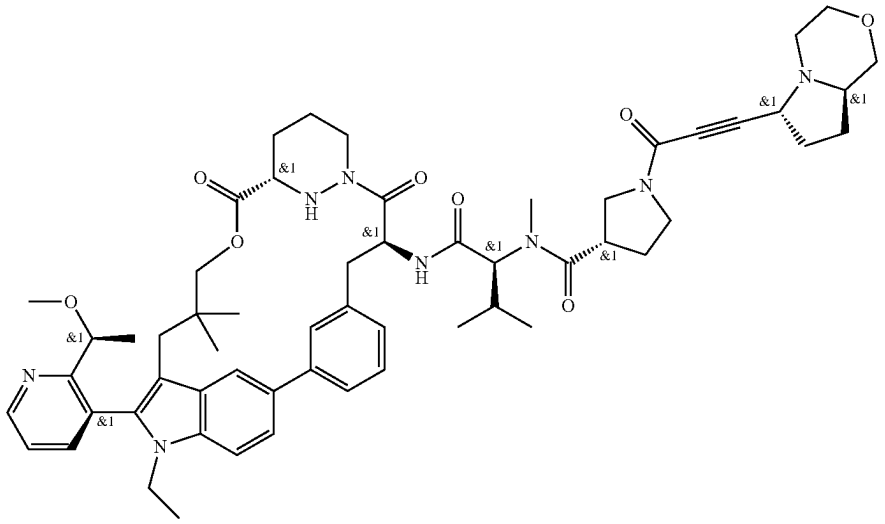
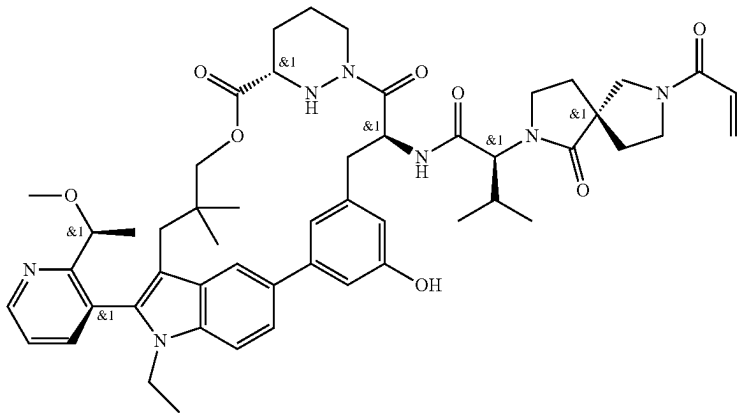
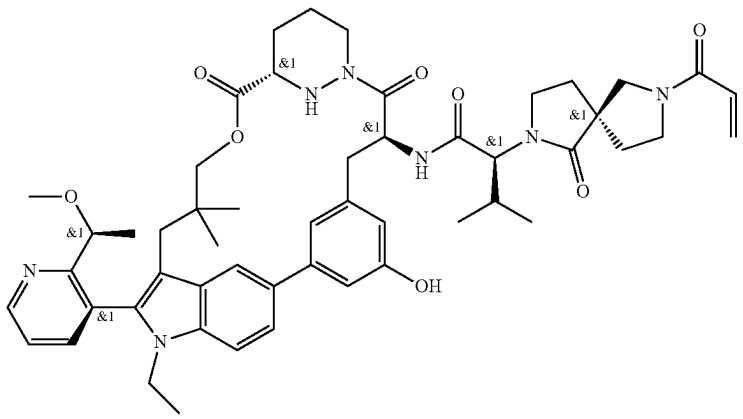
Certain Compounds of the Present Invention	
Ex #	Structure
B342	
B343	
B344	

TABLE 2-continued

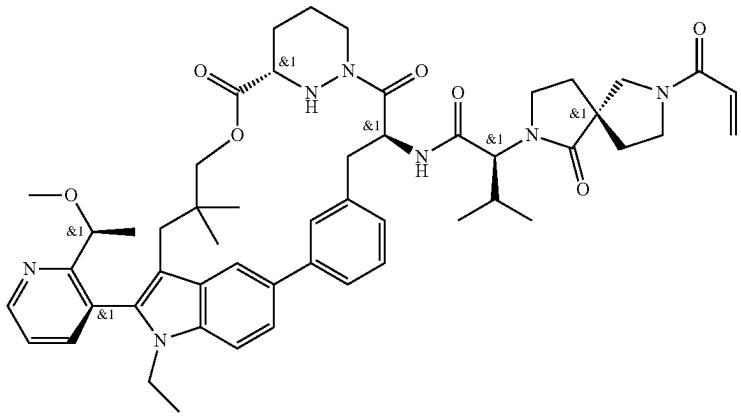
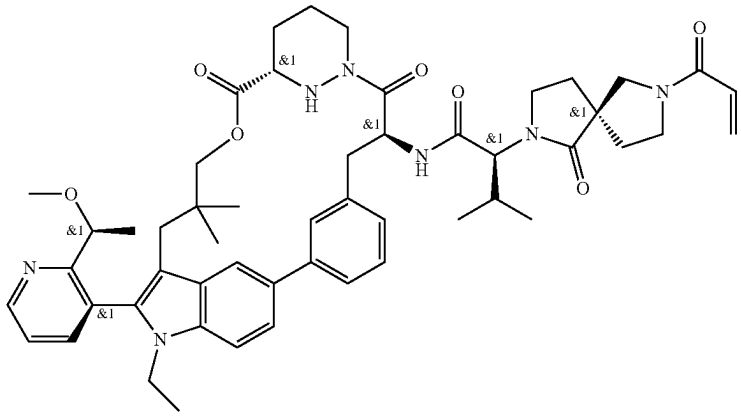
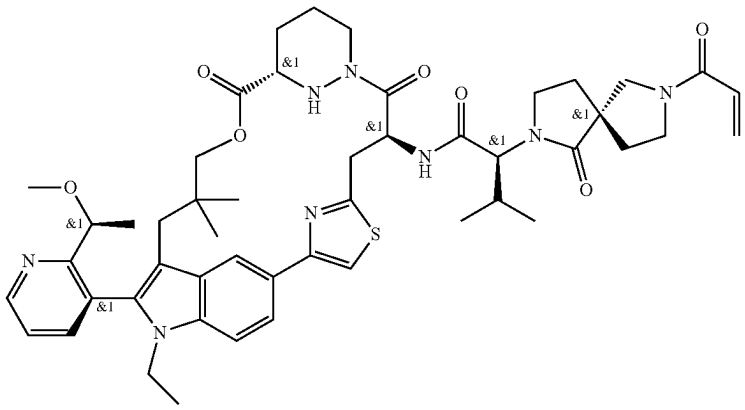
Certain Compounds of the Present Invention	
Ex #	Structure
B345	
B346	
B347	

TABLE 2-continued

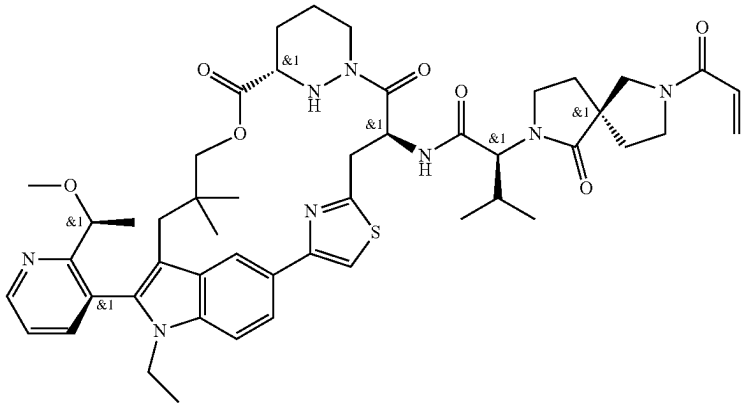
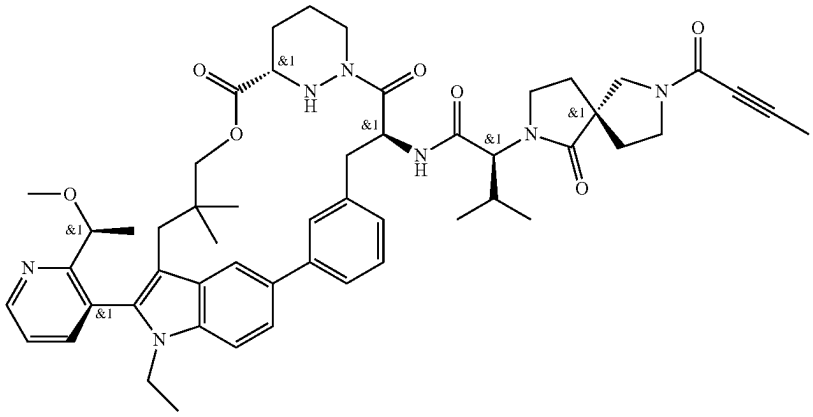
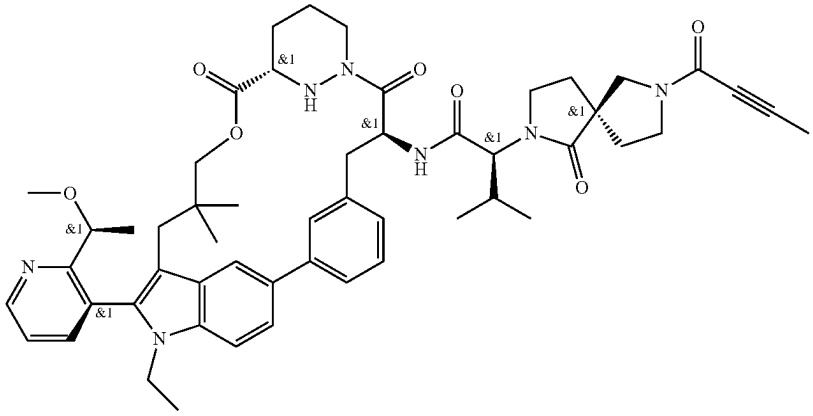
Certain Compounds of the Present Invention	
Ex #	Structure
B348	
B349	
B350	

TABLE 2-continued

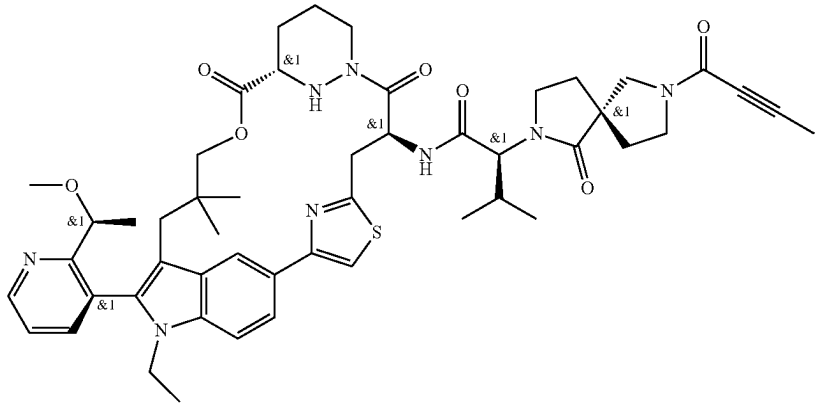
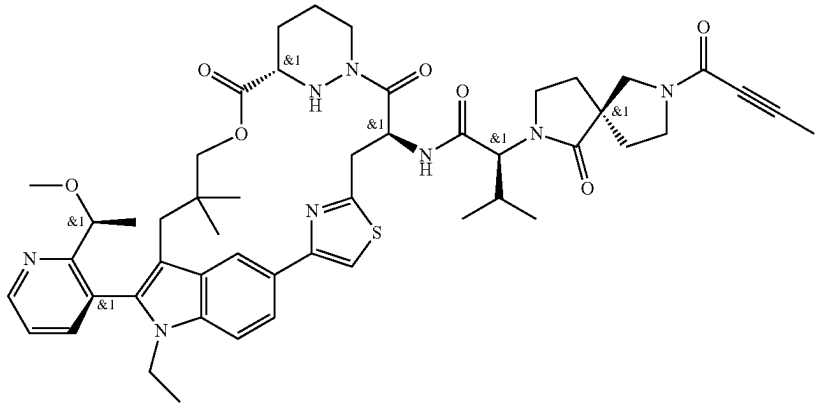
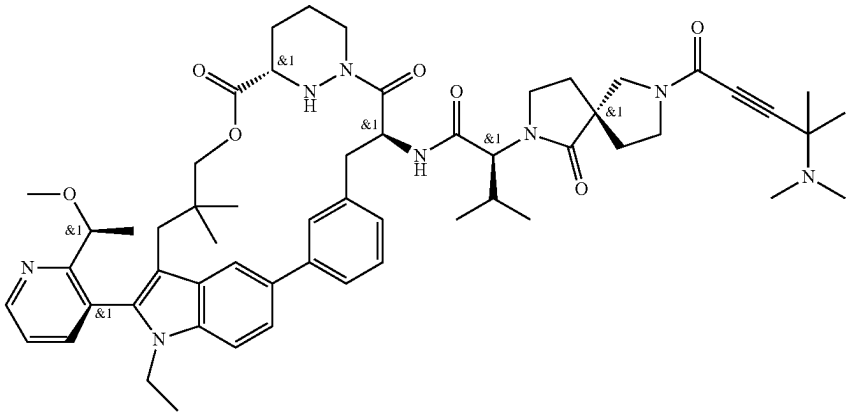
Certain Compounds of the Present Invention	
Ex #	Structure
B351	
B352	
B353	

TABLE 2-continued

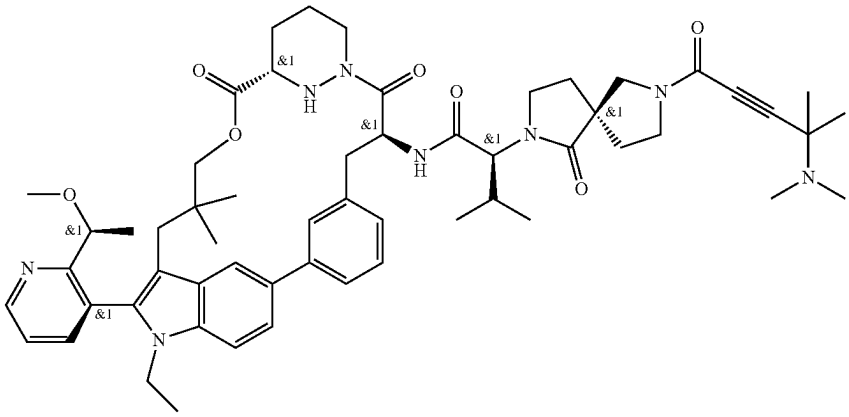
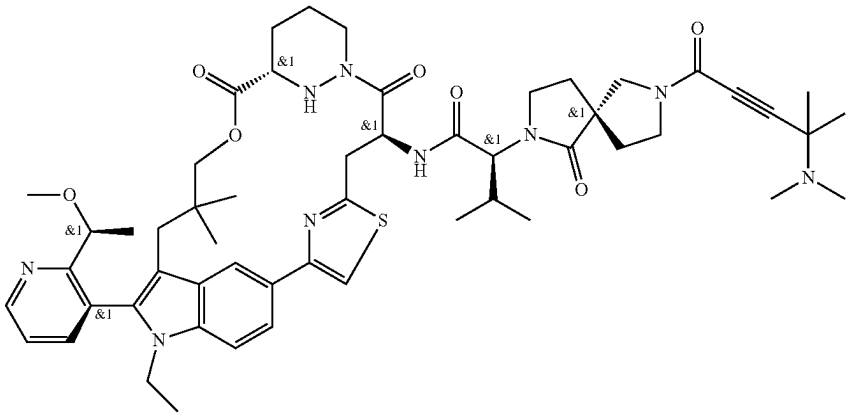
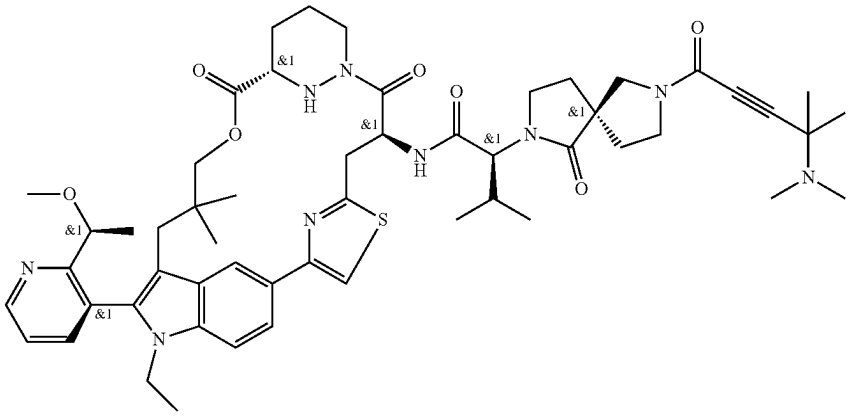
Certain Compounds of the Present Invention	
Ex #	Structure
B354	
B355	
B356	

TABLE 2-continued

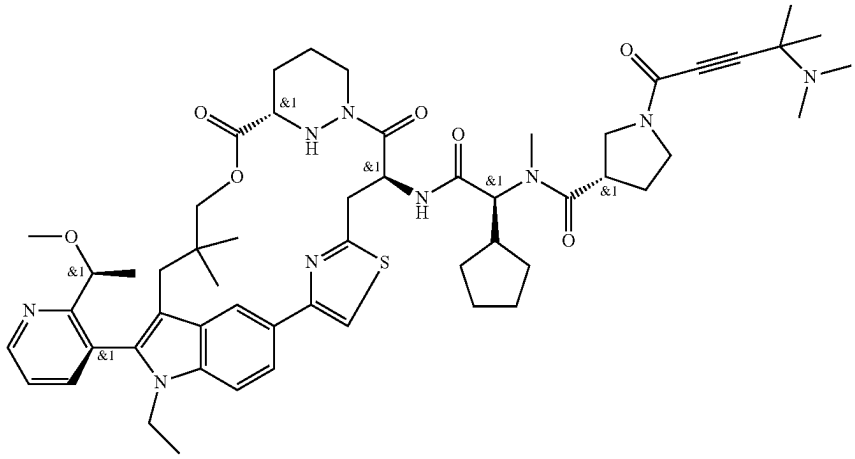
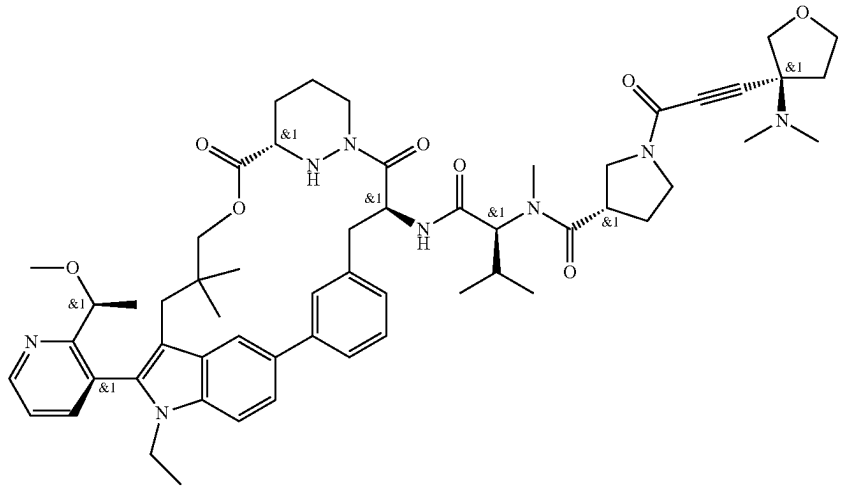
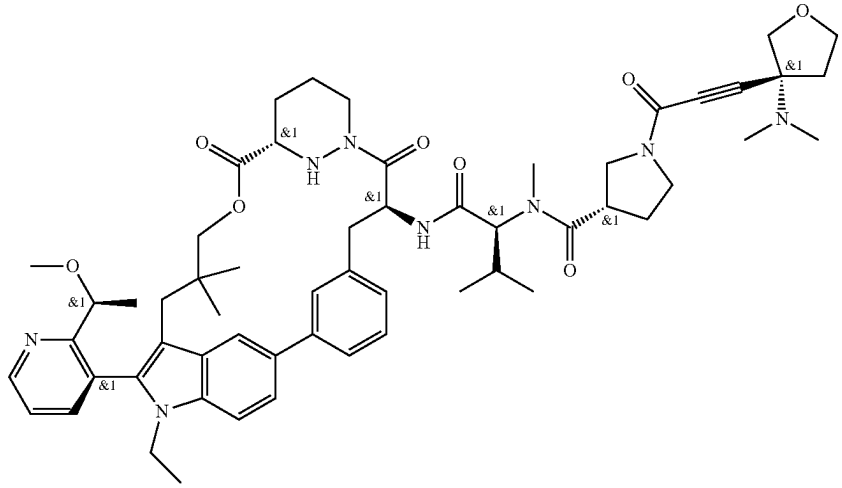
Certain Compounds of the Present Invention	
Ex #	Structure
B357	
B358	
B359	

TABLE 2-continued

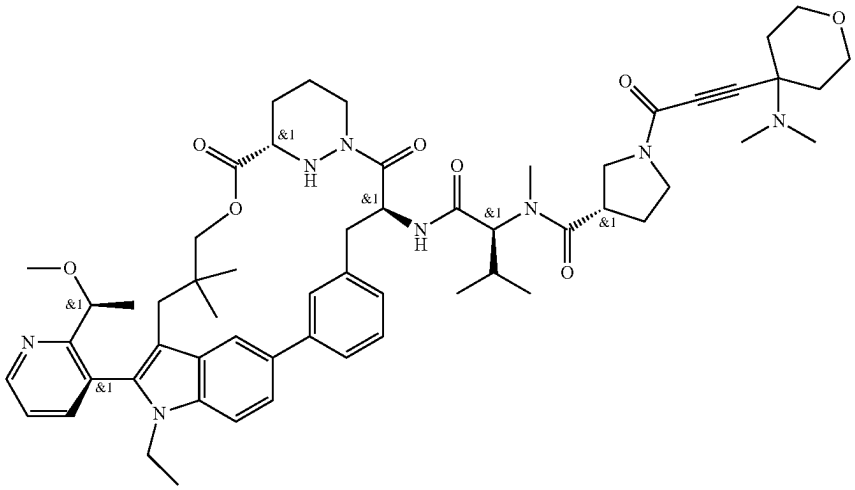
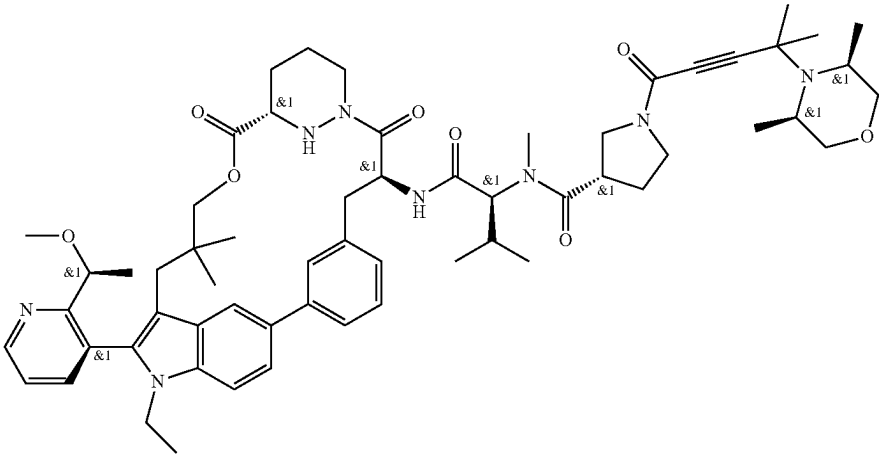
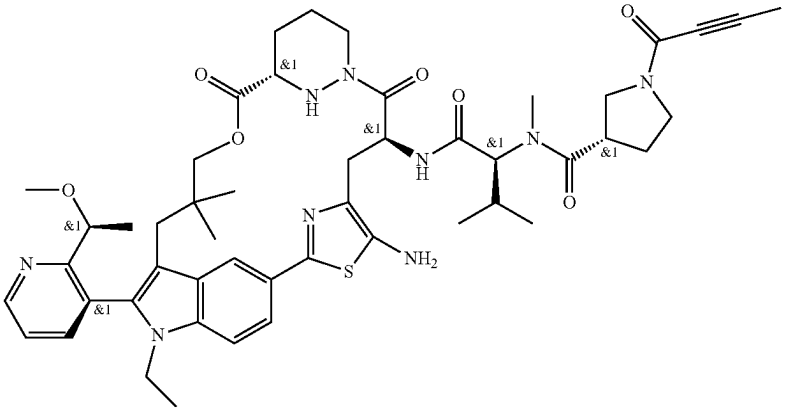
Certain Compounds of the Present Invention	
Ex #	Structure
B360	
B361	
B362	

TABLE 2-continued

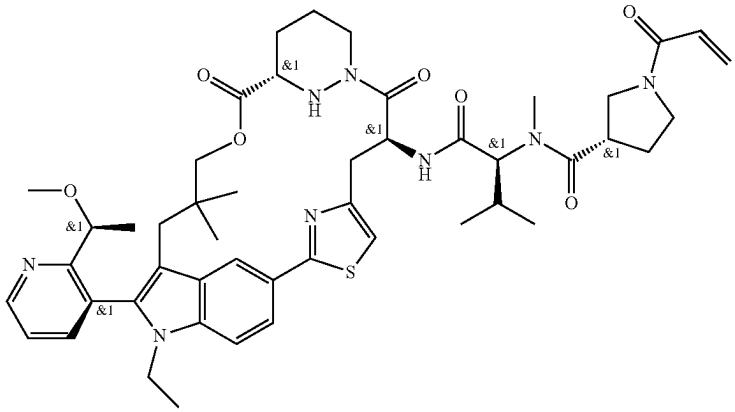
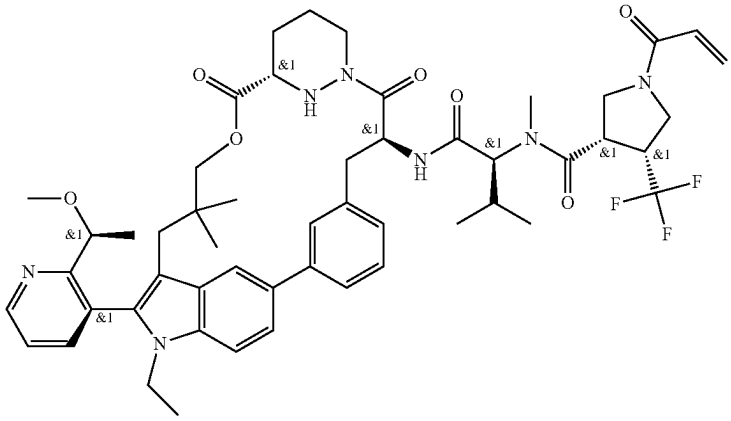
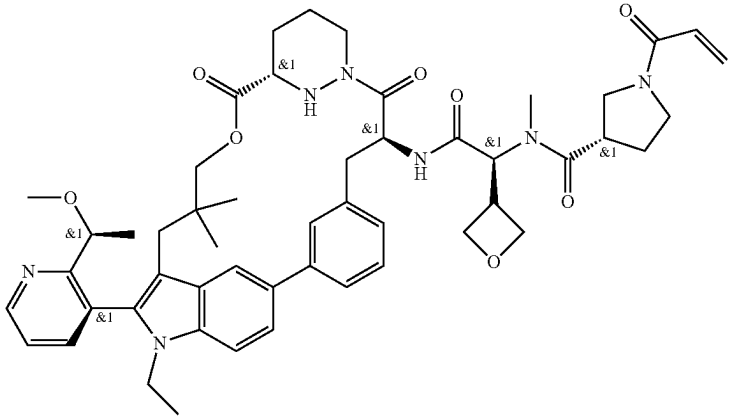
Certain Compounds of the Present Invention	
Ex #	Structure
B363	
B364	
B365	

TABLE 2-continued

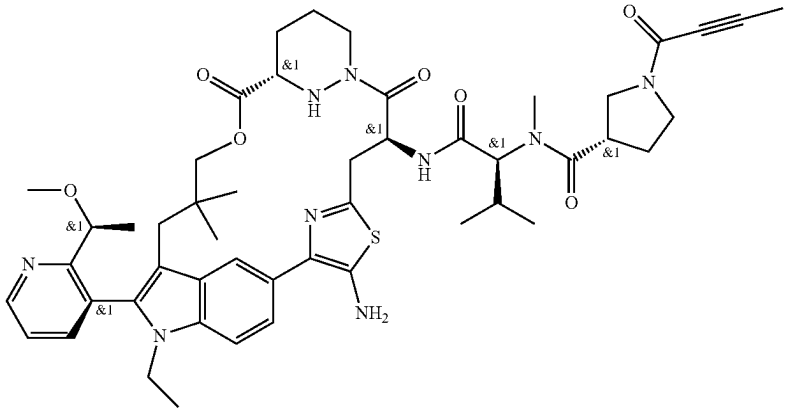
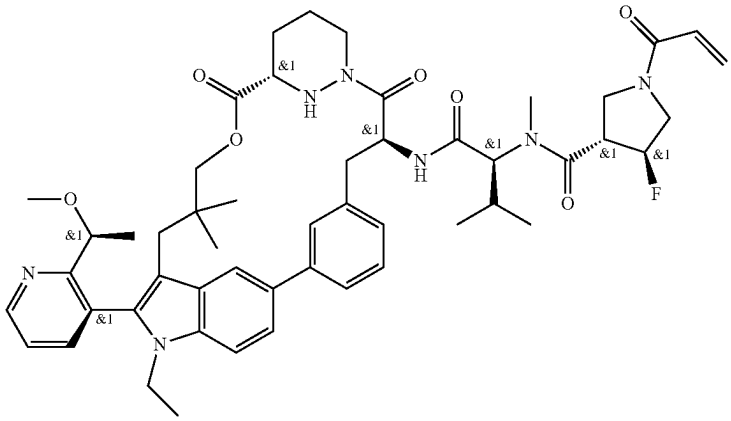
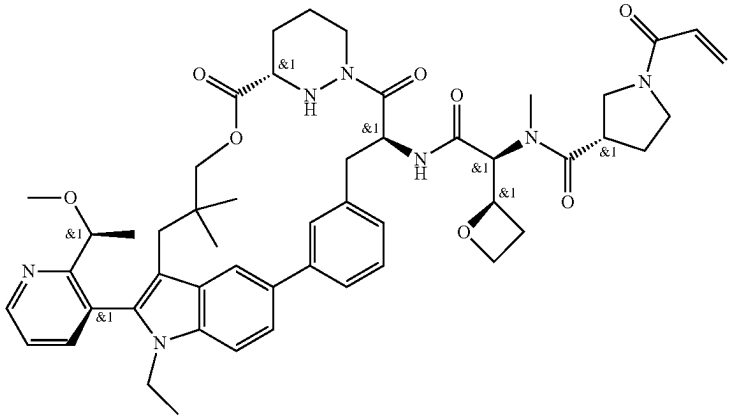
Certain Compounds of the Present Invention	
Ex #	Structure
B366	
B367	
B368	

TABLE 2-continued

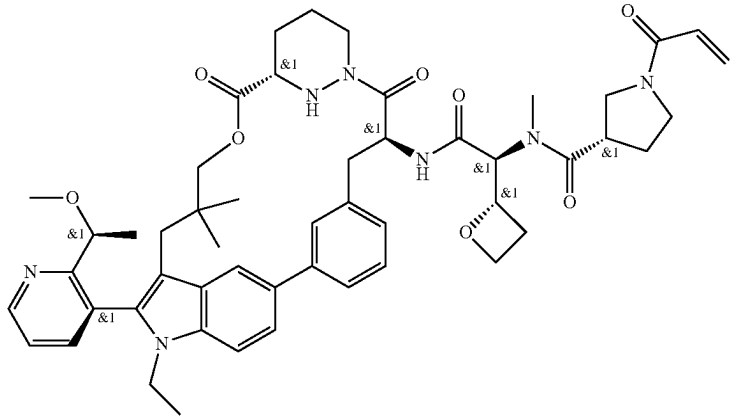
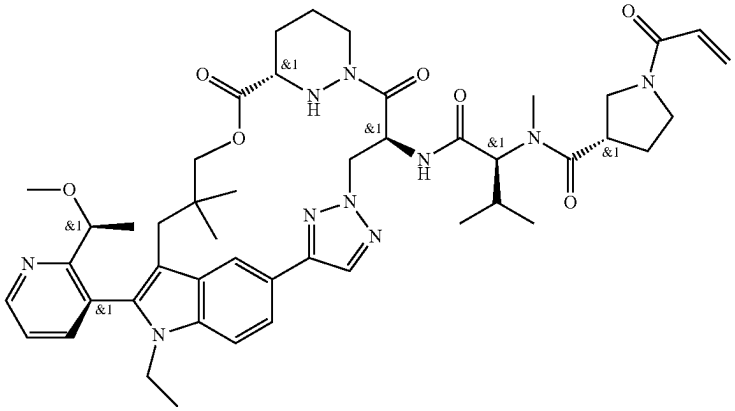
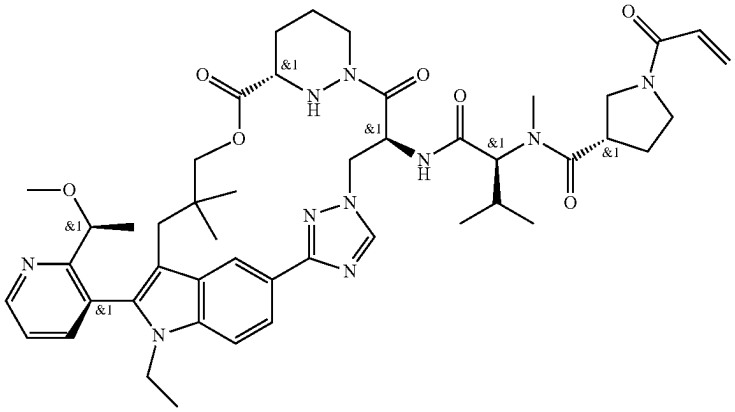
Certain Compounds of the Present Invention	
Ex #	Structure
B369	
B370	
B371	

TABLE 2-continued

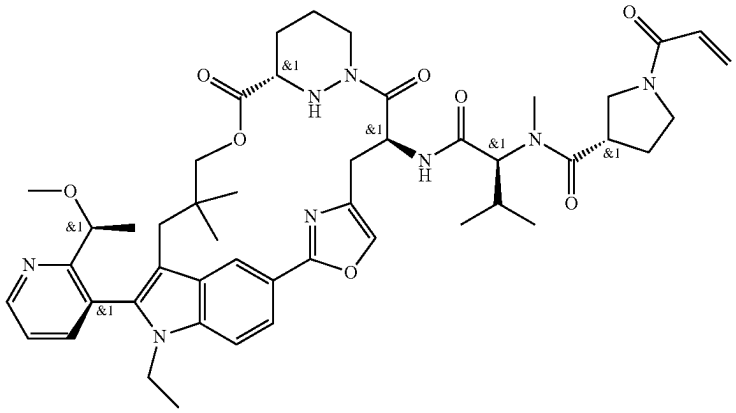
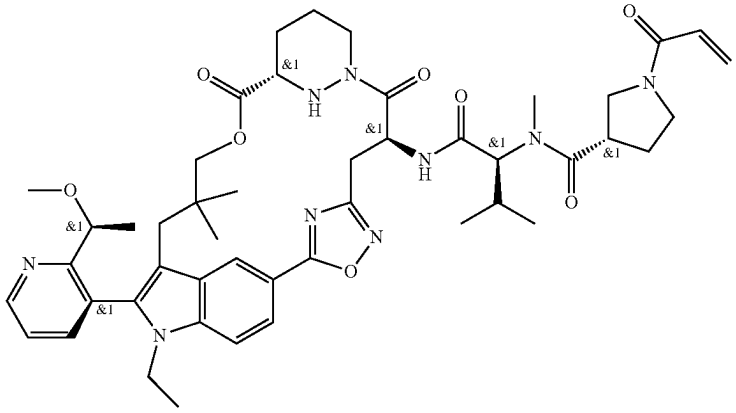
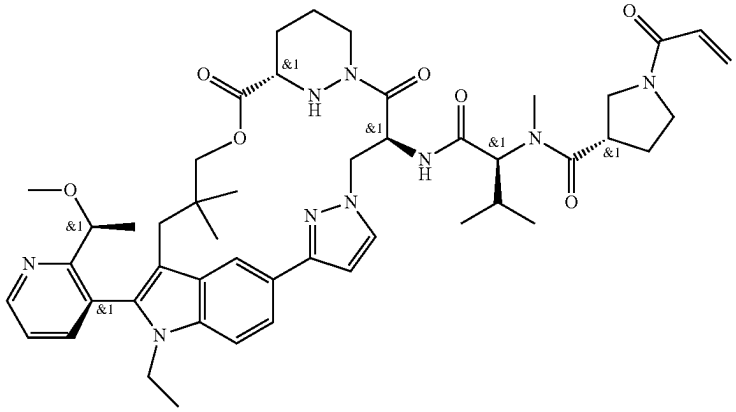
Certain Compounds of the Present Invention	
Ex #	Structure
B372	
B373	
B374	

TABLE 2-continued

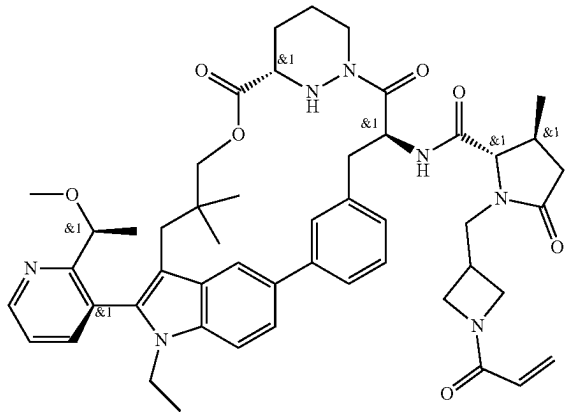
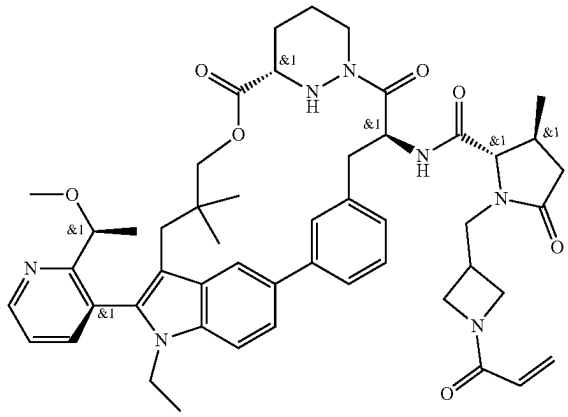
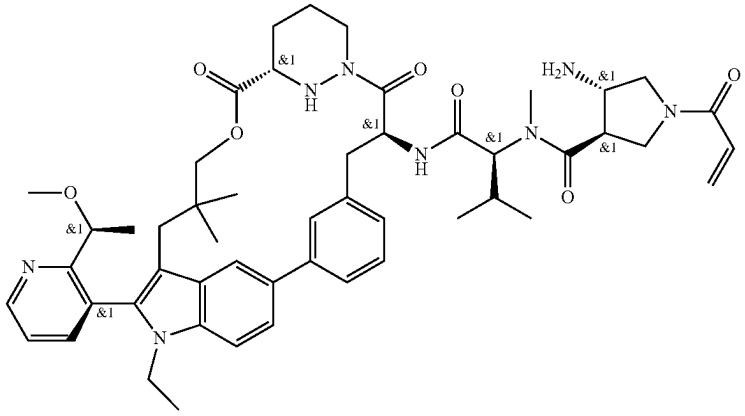
Certain Compounds of the Present Invention	
Ex #	Structure
B375	
B376	
B377	

TABLE 2-continued

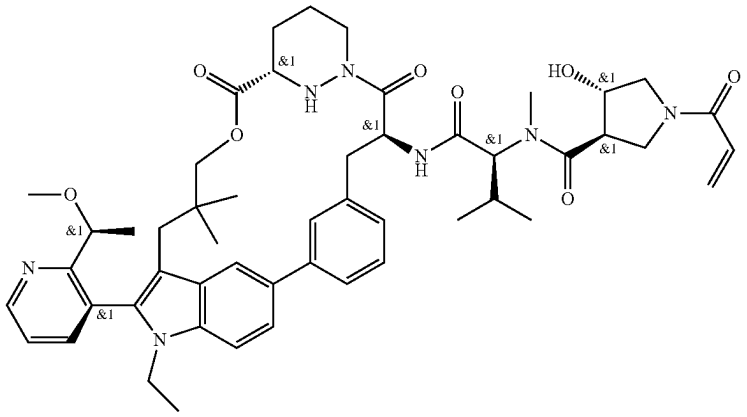
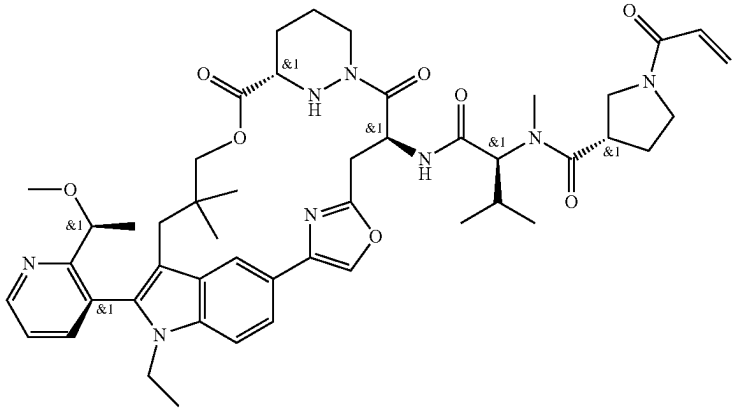
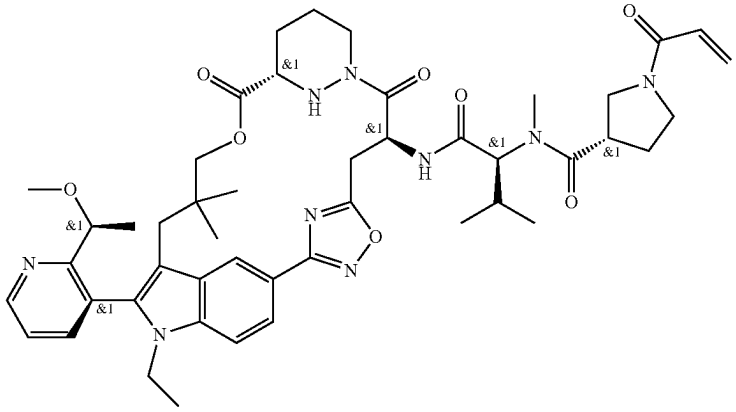
Certain Compounds of the Present Invention	
Ex #	Structure
B378	
B379	
B380	

TABLE 2-continued

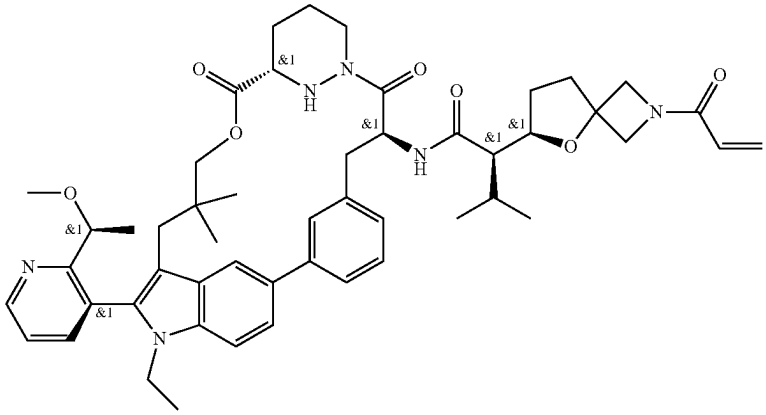
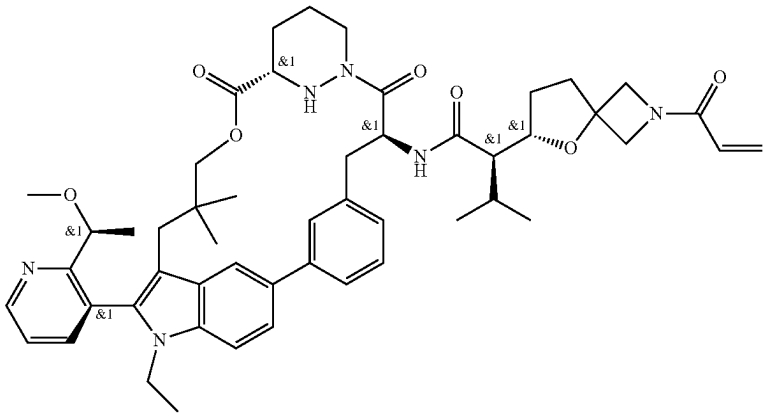
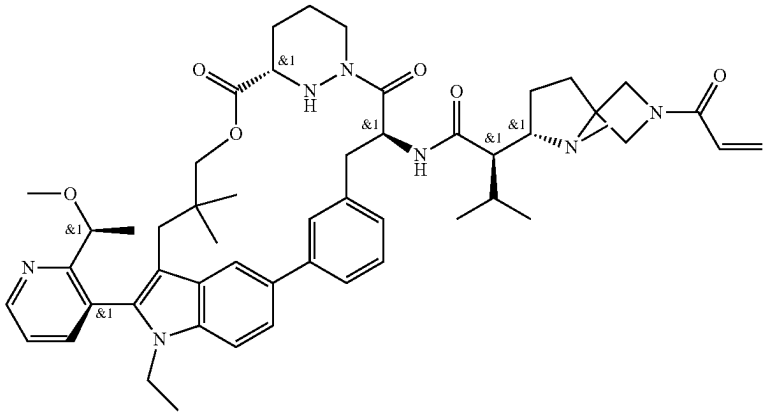
Certain Compounds of the Present Invention	
Ex #	Structure
B381	
B382	
B383	

TABLE 2-continued

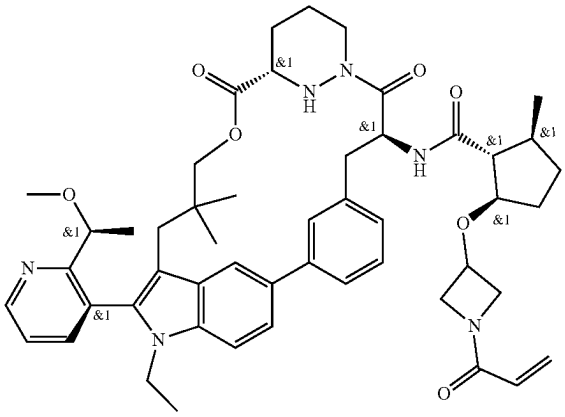
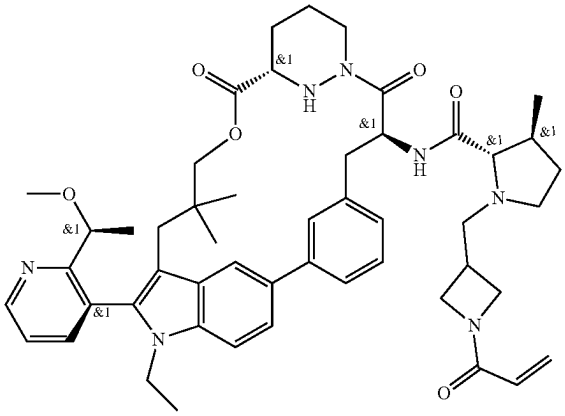
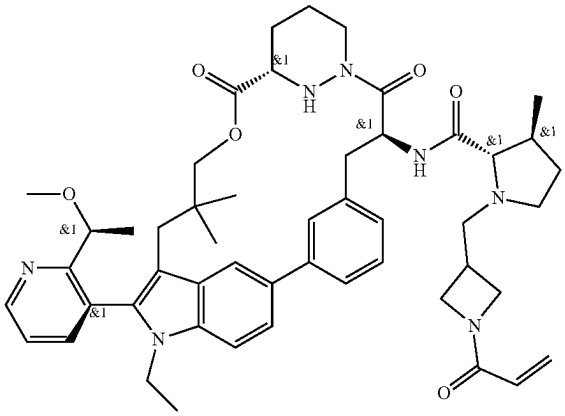
Certain Compounds of the Present Invention	
Ex #	Structure
B384	
B385	
B386	

TABLE 2-continued

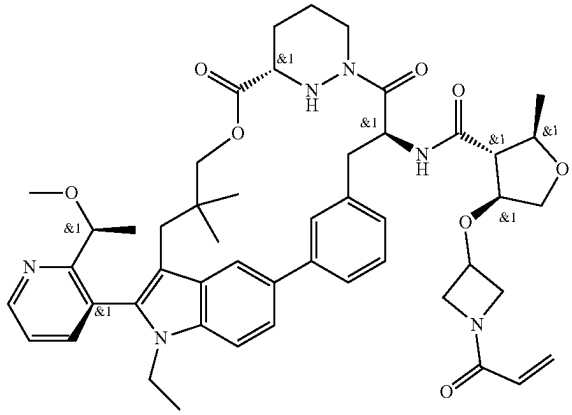
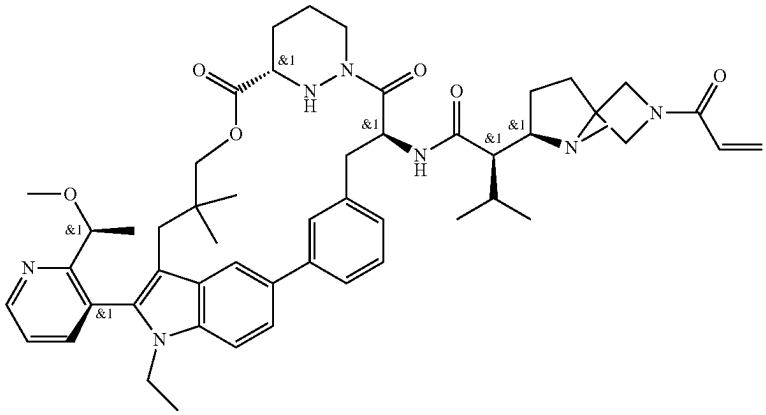
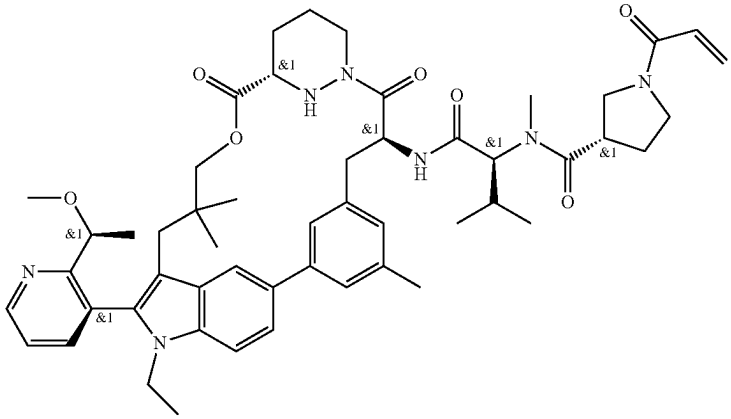
Certain Compounds of the Present Invention	
Ex #	Structure
B387	
B388	
B389	

TABLE 2-continued

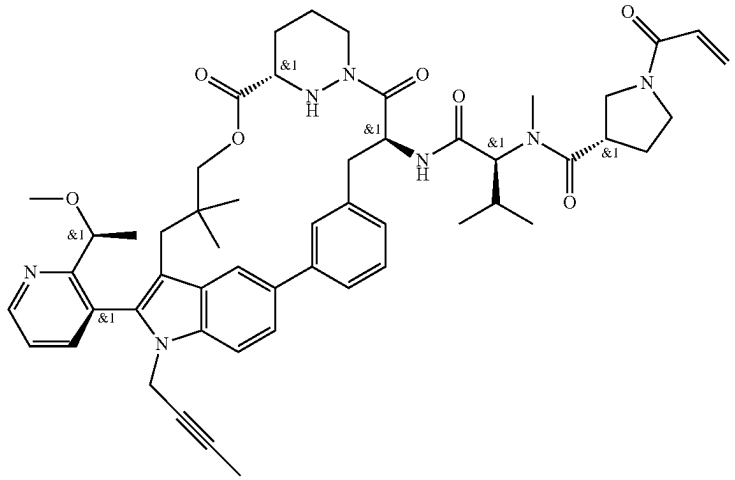
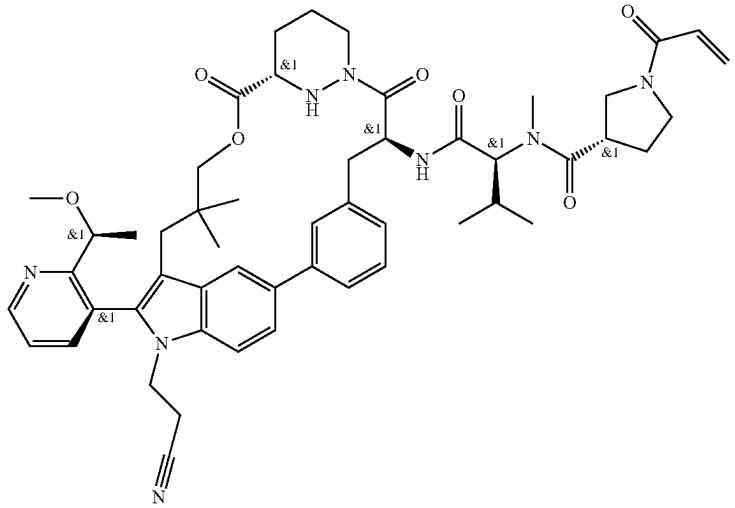
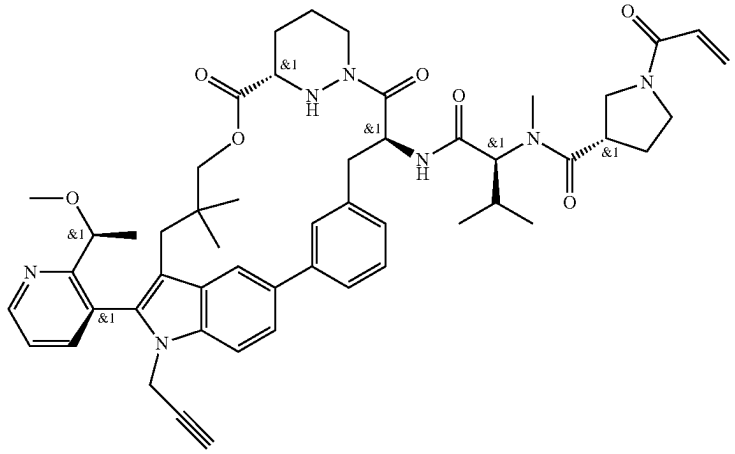
Certain Compounds of the Present Invention	
Ex #	Structure
B390	
B391	
B392	

TABLE 2-continued

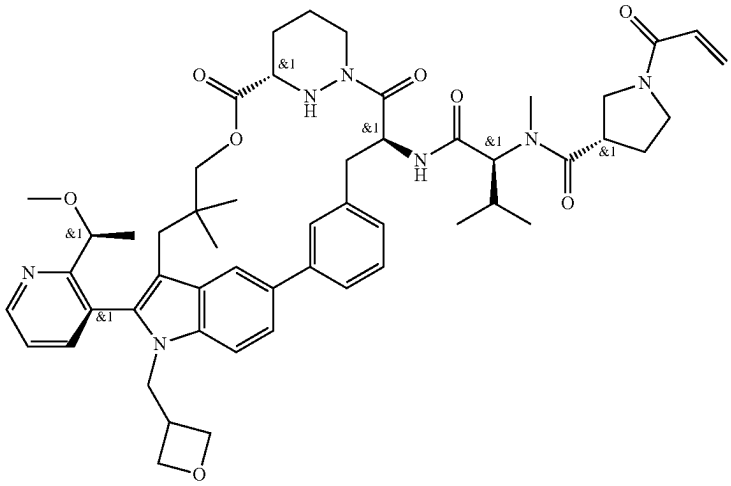
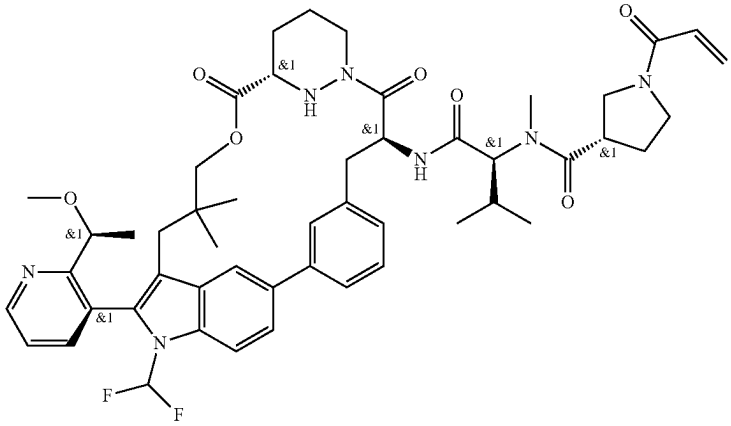
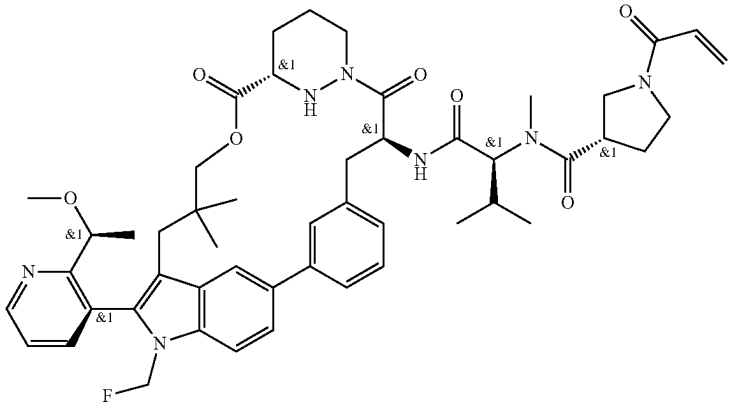
Certain Compounds of the Present Invention	
Ex #	Structure
B393	
B394	
B395	

TABLE 2-continued

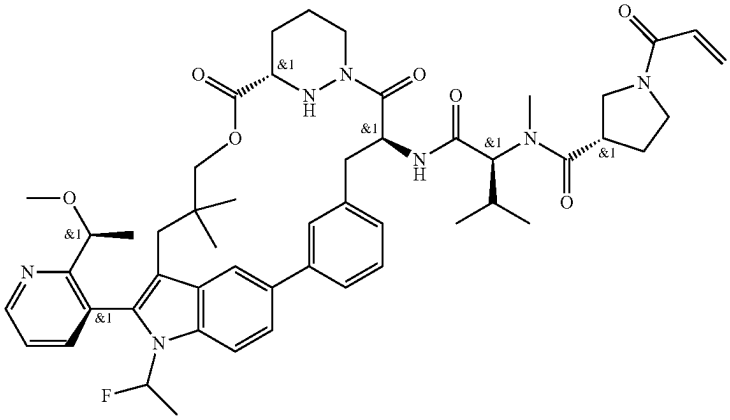
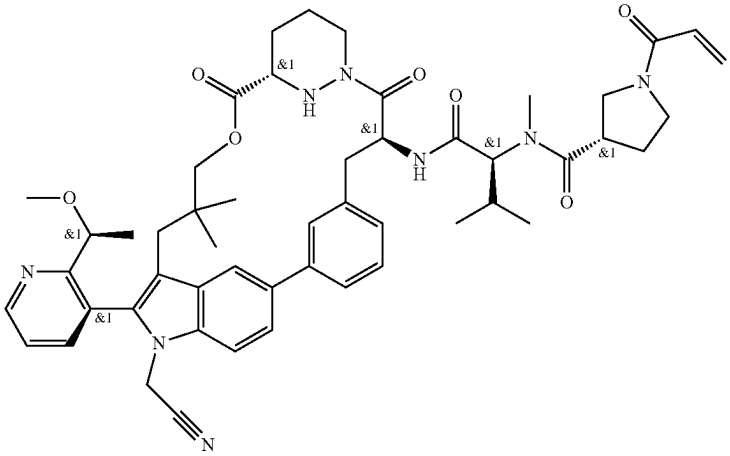
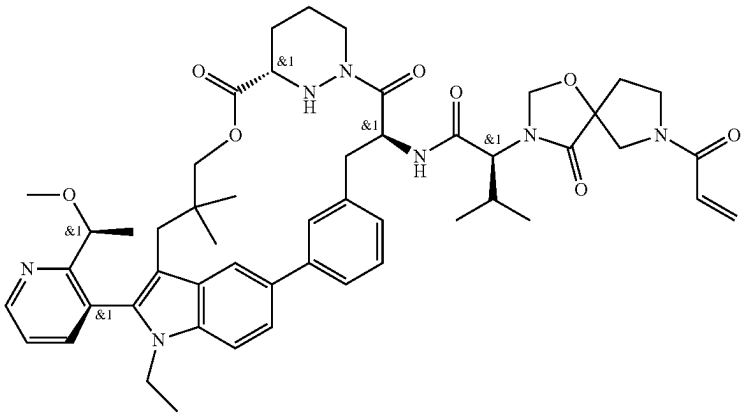
Certain Compounds of the Present Invention	
Ex #	Structure
B396	
B397	
B398	

TABLE 2-continued

Certain Compounds of the Present Invention	
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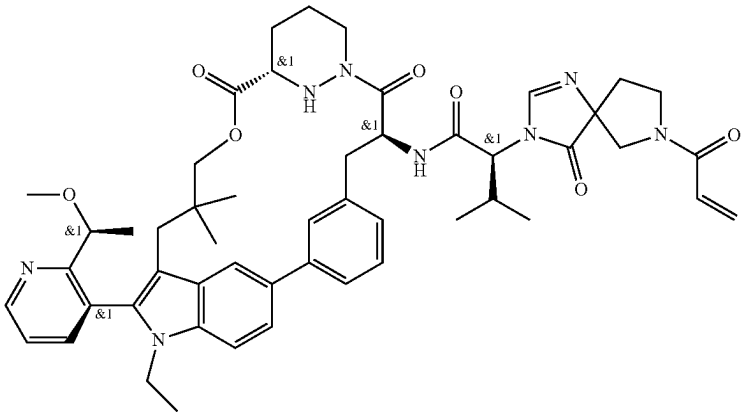
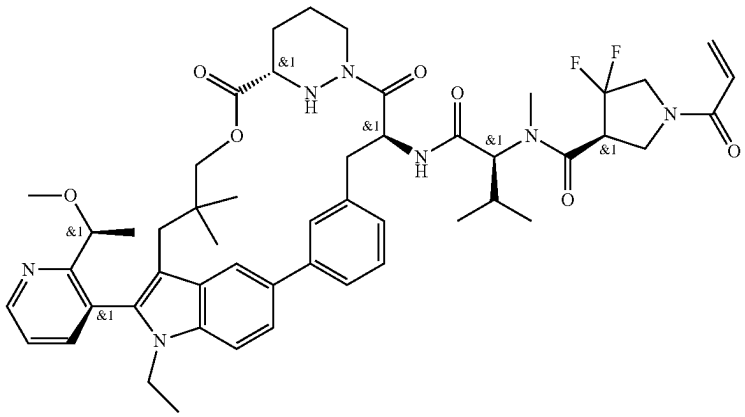
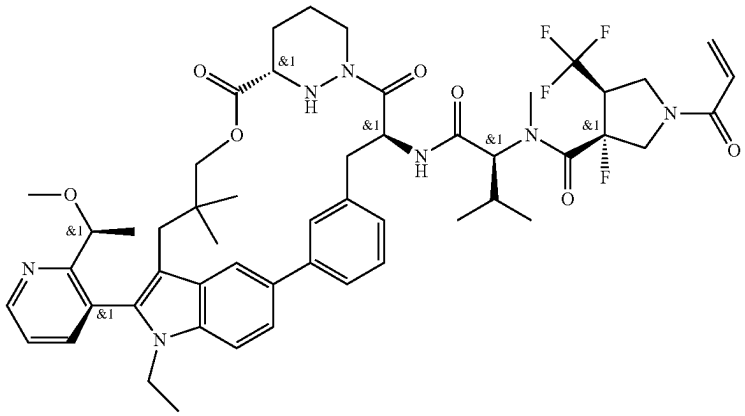
B399	
B400	
B401	

TABLE 2-continued

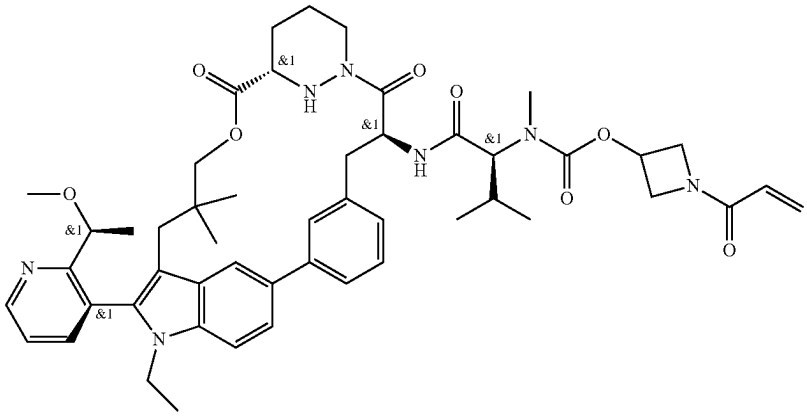
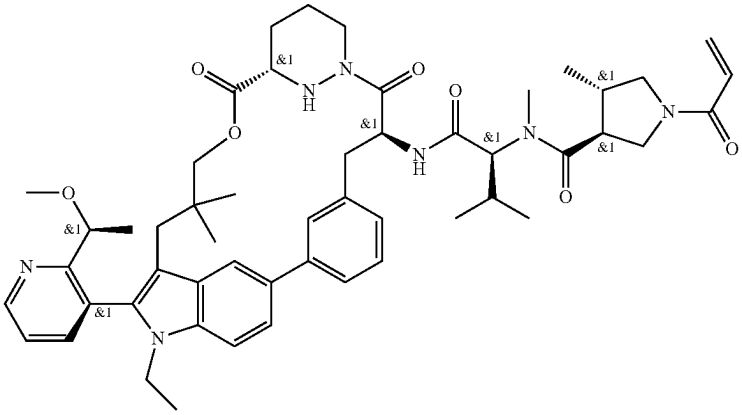
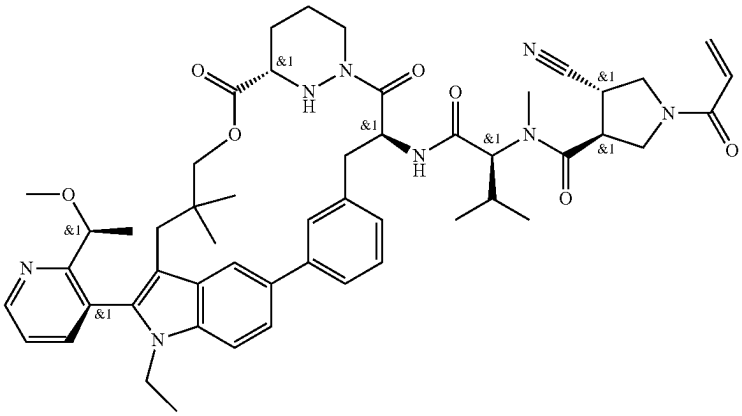
Certain Compounds of the Present Invention	
Ex #	Structure
B402	
B403	
B404	

TABLE 2-continued

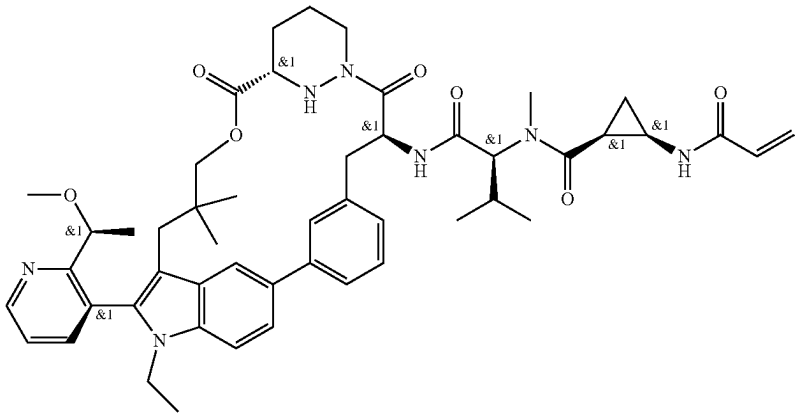
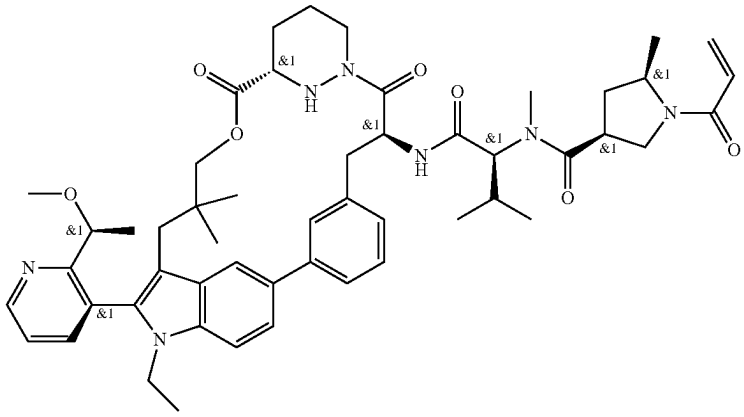
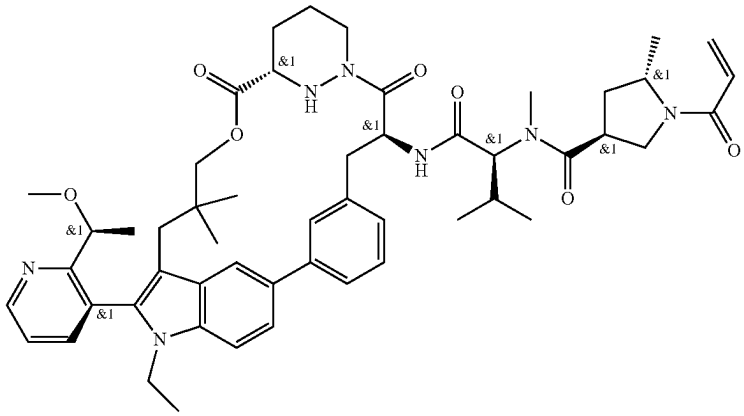
Certain Compounds of the Present Invention	
Ex #	Structure
B405	
B406	
B407	

TABLE 2-continued

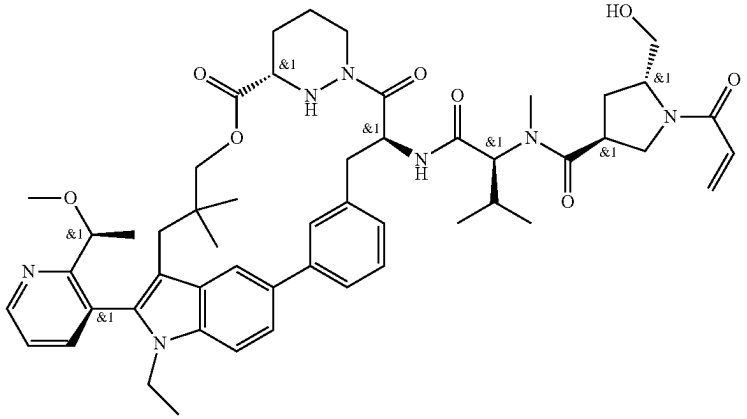
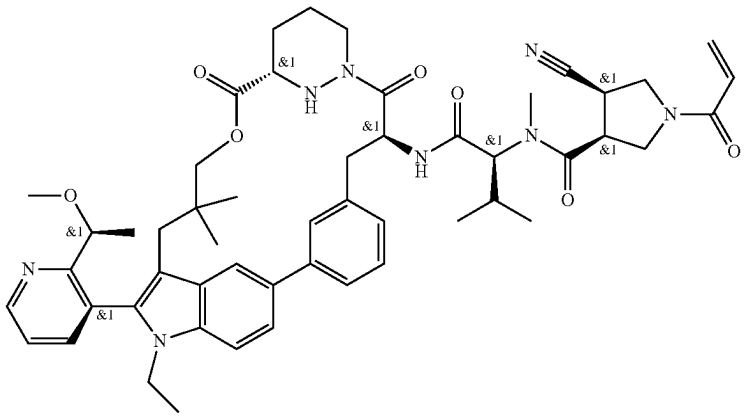
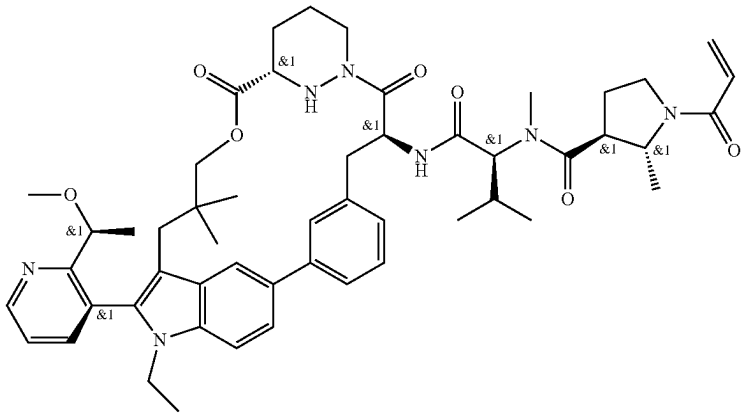
Certain Compounds of the Present Invention	
Ex #	Structure
B408	
B409	
B410	

TABLE 2-continued

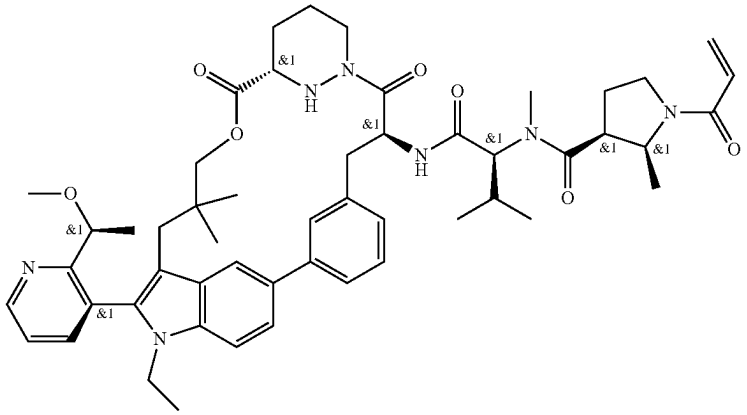
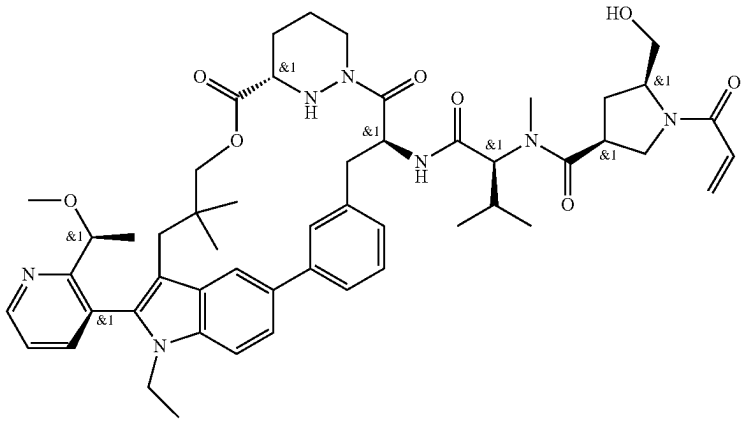
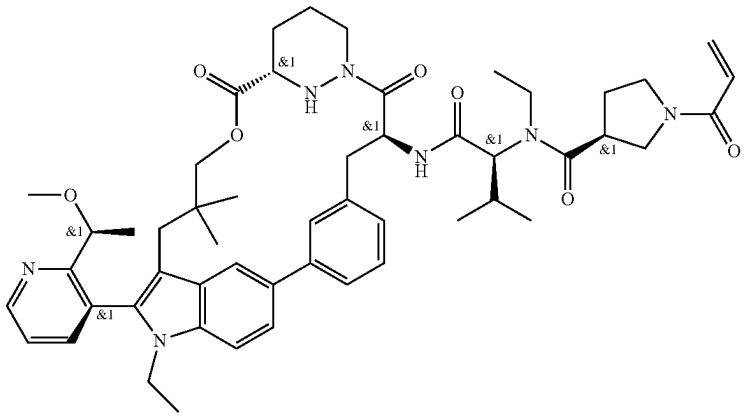
Certain Compounds of the Present Invention	
Ex #	Structure
B411	
B412	
B413	

TABLE 2-continued

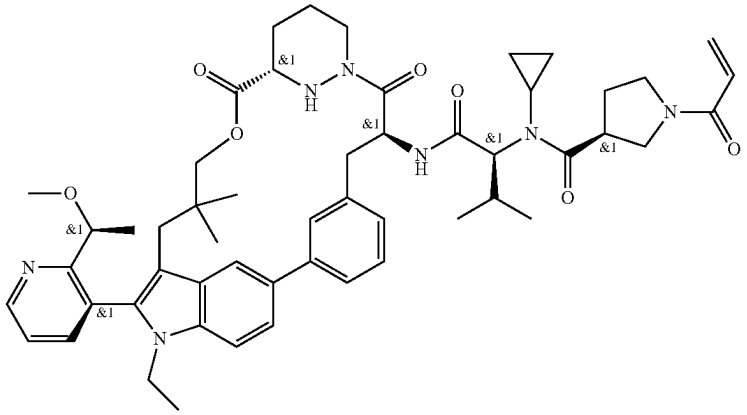
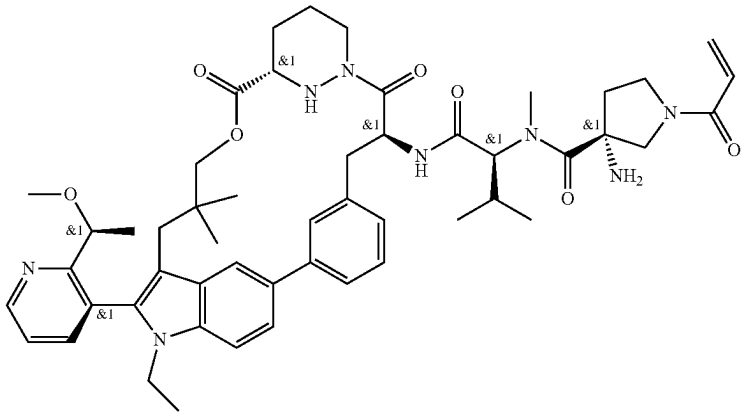
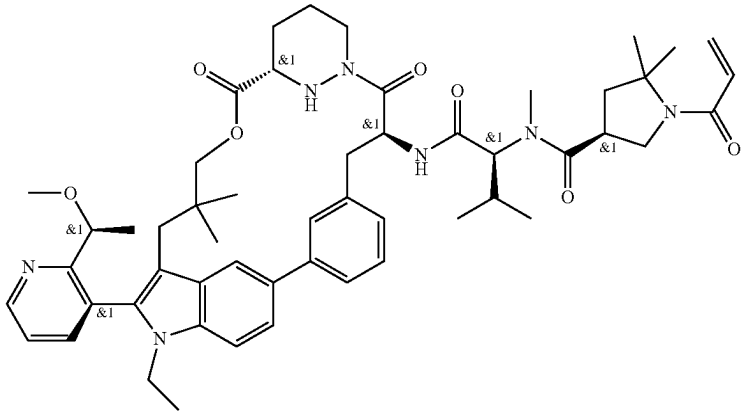
Certain Compounds of the Present Invention	
Ex #	Structure
B414	
B415	
B416	

TABLE 2-continued

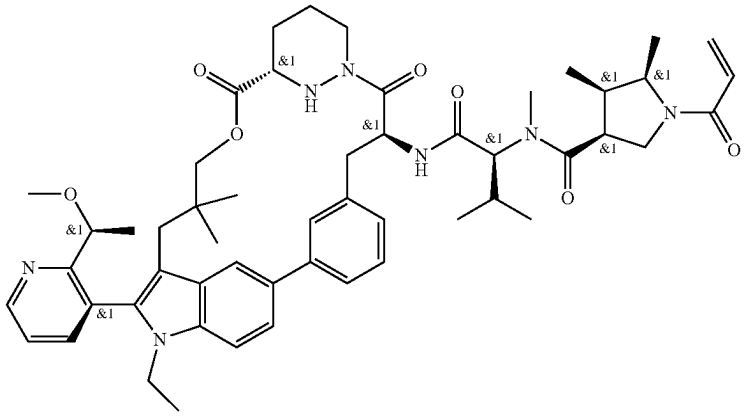
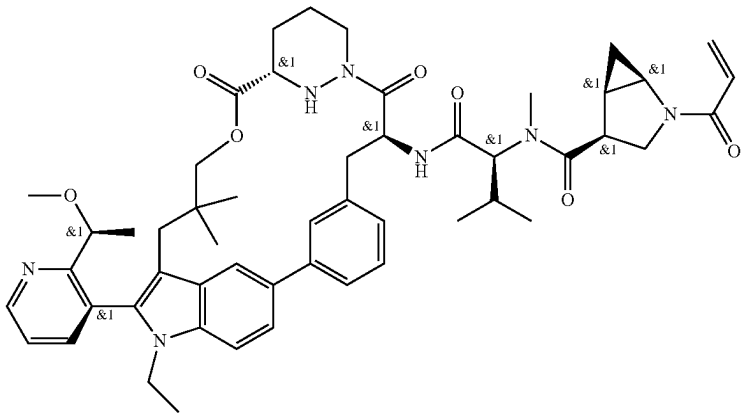
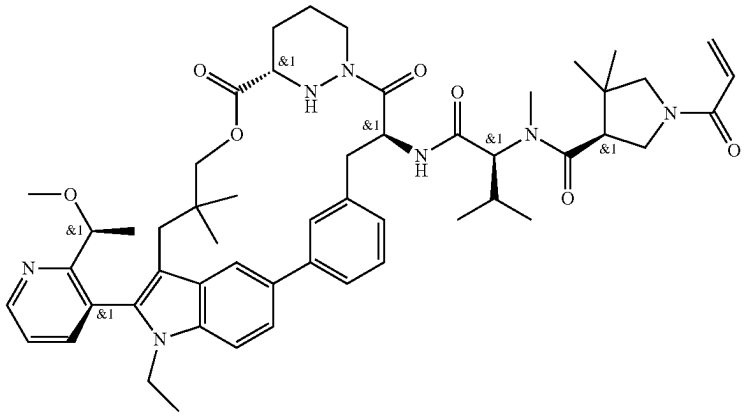
Certain Compounds of the Present Invention	
Ex #	Structure
B417	
B418	
B419	

TABLE 2-continued

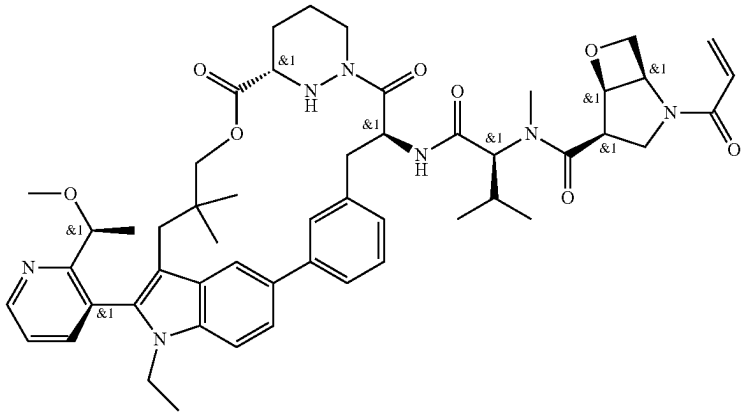
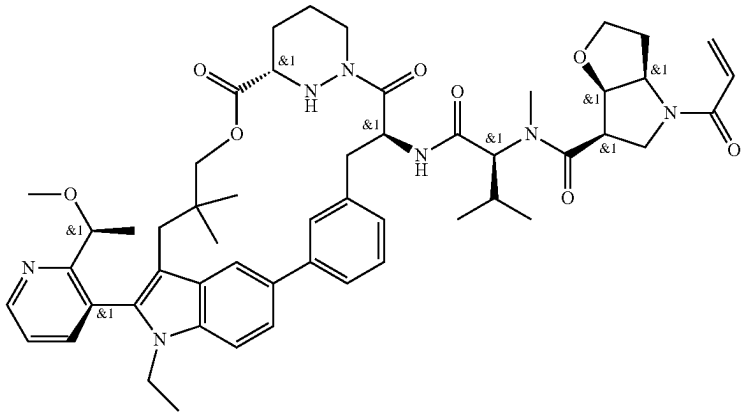
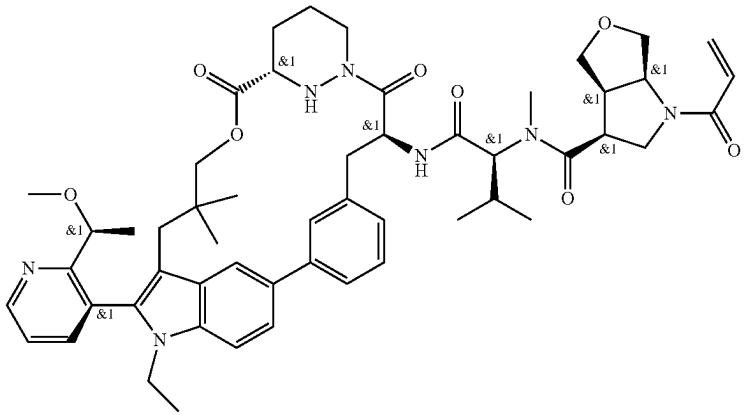
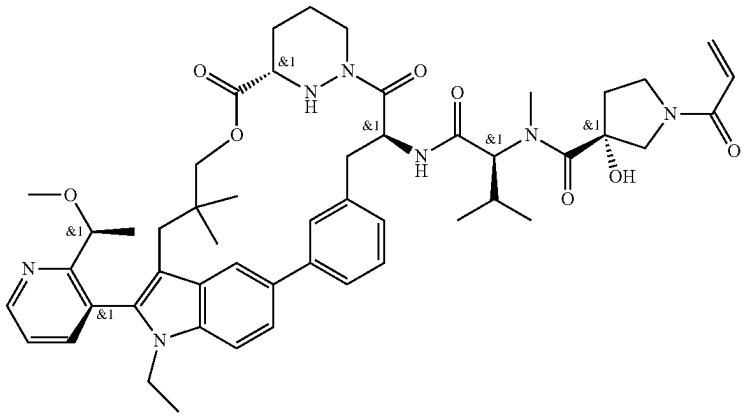
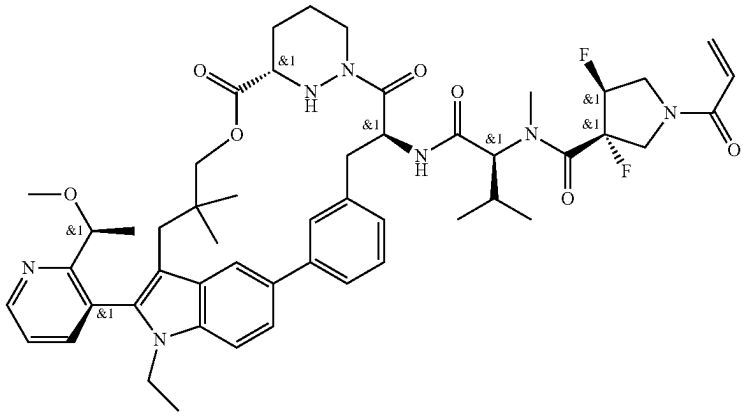
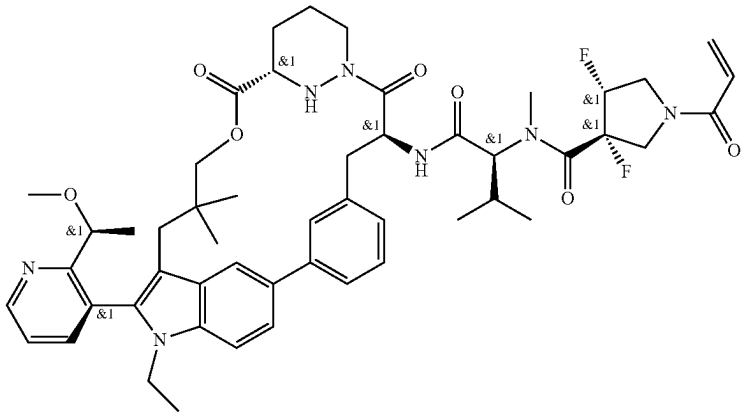
Certain Compounds of the Present Invention	
Ex #	Structure
B420	
B421	
B422	

TABLE 2-continued

Certain Compounds of the Present Invention	
Ex #	Structure
B423	
B424	
B425	

Note

that some compounds are shown with bonds as flat or wedged. In some instances, the relative stereochemistry of stereoisomers has been determined; in some instances, the absolute stereochemistry has been determined. All stereoisomers of the compounds of the foregoing table are contemplated by the present invention. In particular embodiments, an atropisomer of a compound of the foregoing table is contemplated.

In some embodiments, a compound of the present invention is or acts as a prodrug, such as with respect to administration to a cell or to a subject in need thereof.

Also provided are pharmaceutical compositions comprising a compound of the present invention, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient.

Further provided is a conjugate, or salt thereof, comprising the structure of Formula IV:

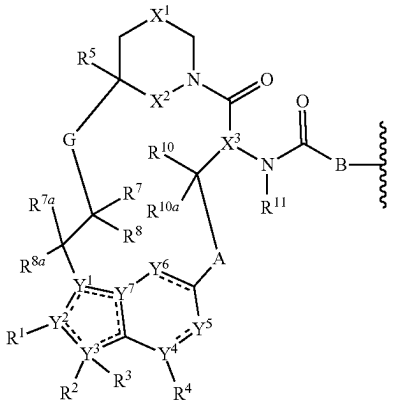
M-L-P Formula IV

wherein L is a linker;

P is a monovalent organic moiety; and

M has the structure of Formula Va:

Formula Va



wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is —N(H or CH_3)C(O)—(CH_2)— where the amino nitrogen is bound to the carbon atom of —CH(R^{10})—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is absent, —CH(R^9)—, >C═C$R^9R^{9'}$, or >C$R^9R^{9'}$ where the carbon is bound to the carbonyl carbon of —N(R^{11})C(O)—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, —C(O)O—CH(R^6)— where C is bound to —C(R^7R^8)—, —C(O)NH—CH(R^6)— where C is bound to —C(R^7R^8)—, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or $S(O)_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 is CH, CH_2, or N;

Y^6 is C(O), CH, CH_2, or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl, or

R^1 and R^2 combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

R^2 is absent, hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form C═C$R^{7'}R^{8'}$; C═N(OH), C═N(O—C_1-C_3 alkyl), C═O, C═S, C═NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{7a} and R^{8a} are, independently, hydrogen, halo, optionally substituted C_1-C_3 alkyl, or combine with the carbon to which they are attached to form a carbonyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is H, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3

to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl, or

R^9 and L combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

$R^{9'}$ is hydrogen or optionally substituted C_1-C_6 alkyl; or

R^9 and $R^{9'}$, combined with the atoms to which they are attached, form a 3 to 6-membered cycloalkyl or a 3 to 6-membered heterocycloalkyl;

R^{10} is hydrogen, halo, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl;

R^{10a} is hydrogen or halo; and

R^{11} is hydrogen or C_1-C_3 alkyl.

In some embodiments the conjugate, or salt thereof, comprises the structure of Formula IV:

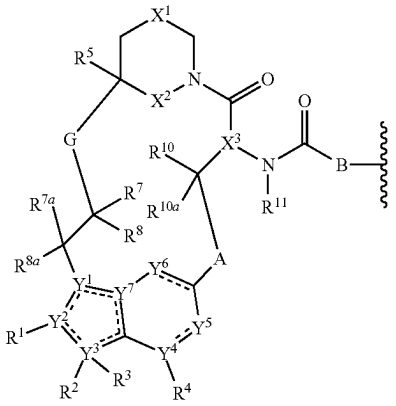
M-L-P Formula IV

wherein L is a linker;

P is a monovalent organic moiety; and

M has the structure of Formula Vb:

Formula Vb



wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is —N(H or CH_3)C(O)—(CH_2)— where the amino nitrogen is bound to the carbon atom of —CH(R^{10})—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is —CH(R^9)— or >C═C$R^9R^{9'}$ where the carbon is bound to the carbonyl carbon of —N(R^{11})C(O)—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, —C(O)O—CH(R^6)— where C is bound to —C(R^7R^8)—, —C(O)NH—CH(R^6)— where C is bound to —C(R^7R^8)—, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or $S(O)_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 is CH, CH_2, or N;

Y^6 is C(O), CH, CH_2, or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl, or

R^1 and R^2 combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

R^2 is absent, hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form C═C$R^{7'}R^{8'}$; C═N(OH), C═N(O—C_1-C_3 alkyl), C═O, C═S, C═NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{7a} and R^{8a} are, independently, hydrogen, halo, optionally substituted C_1-C_3 alkyl, or combine with the carbon to which they are attached to form a carbonyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl, or

R^9 and L combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

$R^{9'}$ is hydrogen or optionally substituted C_1-C_6 alkyl;

R^{10} is hydrogen, halo, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl;

R^{10a} a is hydrogen or halo; and

R^{11} is hydrogen or C_1-C_3 alkyl.

In some embodiments, the conjugate has the structure of Formula IV:

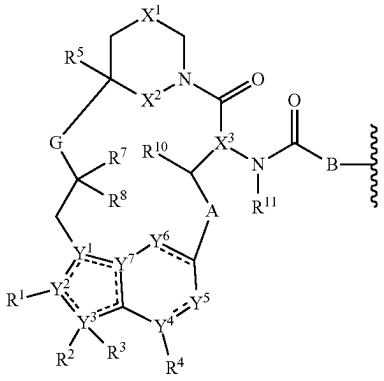
M-L-P Formula IV

wherein L is a linker;

P is a monovalent organic moiety; and

M has the structure of Formula Vc:

Formula Vc



wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is —N(H or CH_3)C(O)—(CH_2)— where the amino nitrogen is bound to the carbon atom of —CH(R^{10})—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is —CH(R^9)— where the carbon is bound to the carbonyl carbon of —N(R^{11})C(O)—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, —C(O)O—CH(R^6)— where C is bound to —C(R^7R^8)—, —C(O)NH—CH(R^6)— where C is bound to —C(R^7R^8)—, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or $S(O)_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 and Y^6 are, independently, CH or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

R^2 is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form C═C$R^{7'}R^{8'}$; C═N(OH), C═N(O—C_1-C_3 alkyl), C═O, C═S, C═NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{10} is hydrogen, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl; and

R^{11} is hydrogen or C_1-C_3 alkyl.

In some embodiments, a compound of the present invention has the structure of Formula IV:

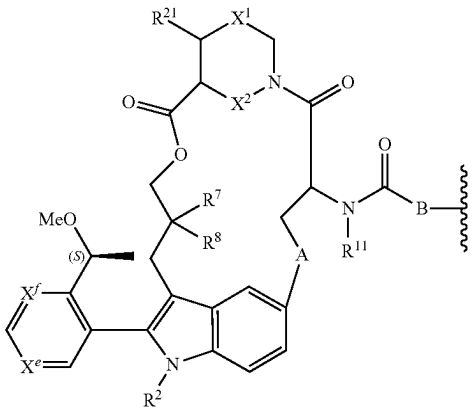
M-L-P Formula IV

wherein L is a linker;

P is a monovalent organic moiety; and

M has the structure of Formula Vd:

Formula Vd



wherein A optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene (e.g., phenyl or phenol), or optionally substituted 5 to 6-membered heteroarylene;

B is —CH(R^9)— where the carbon is bound to the carbonyl carbon of —NHC(O)—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or $S(O)_n$;

X^2 is O or NH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', $C(O)N(R')_2$, S(O)R', $S(O)_2R'$, or $S(O)_2N(R')_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

R^2 is C_1-C_6 alkyl, C_1-C_6 fluoroalkyl, or 3 to 6-membered cycloalkyl;

R^7 is C_1-C_3 alkyl;

R^8 is C_1-C_3 alkyl; and

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

X^e and X^f are, independently, N or CH;

R^{11} is hydrogen or C_1-C_3 alkyl; and

R^{21} is hydrogen or C_1-C_3 alkyl.

In some embodiments of a compound of the present invention, X^e is N and X^f is CH. In some embodiments, X^e is CH and X^f is N.

In some embodiments, a compound of the present invention has the structure of Formula IV:

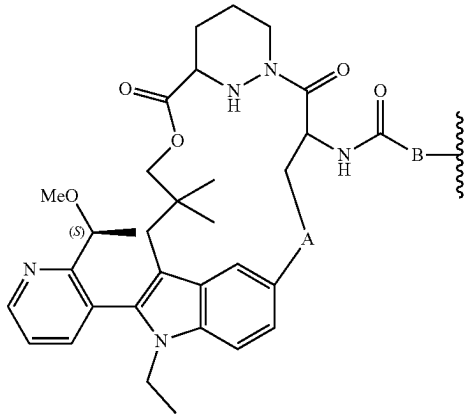
M-L-P Formula IV

wherein L is a linker;

P is a monovalent organic moiety; and

M has the structure of Formula Ve:

Formula Ve



wherein A is optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene (e.g., phenyl or phenol), or optionally substituted 5 to 6-membered heteroarylene;

B is —CH(R^9)— where the carbon is bound to the carbonyl carbon of —NHC(O)—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene; and

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl.

In some embodiments of a conjugate of the present invention, the linker has the structure of Formula II:

A^1-$(B^1)_f$—$(C^1)_g$—$(B^2)_h$-(D^1)-$(B^3)_i$—$(C^2)_j$—$(B^4)_k$-A^2 Formula II

where A^1 is a bond between the linker and B; A^2 is a bond between P and the linker; B^1, B^2, B^3, and B^4 each, independently, is selected from optionally substituted C_1-C_2 alkylene, optionally substituted C_1-C_3 heteroalkylene, O, S, and NR^N; R^N is hydrogen, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted C_1-C_7 heteroalkyl; C_1 and C_2 are each, independently, selected from carbonyl, thiocarbonyl, sulphonyl, or phosphoryl; f, g, h, i, j, and k are each, independently, 0 or 1; and D^1 is optionally substituted C_1-C_{10} alkylene, optionally substituted C_2-C_{10} alkenylene, optionally substituted C_2-C_{10} alkynylene, optionally substituted 3 to 14-membered heterocycloalkylene, optionally substituted 5 to 10-membered heteroarylene, optionally substituted 3 to 8-membered cycloalkylene, optionally substituted 6 to 10-membered arylene, optionally substituted C_2-C_{10} polyethylene glycolene, or optionally substituted C_1-C_{10} heteroalkylene, or a chemical bond linking A^1-$(B^1)_f$—$(C^1)_g$—$(B^2)_h$— to —$(B^3)_i$—$(C^2)_j$—$(B^4)_k$-A^2.

In some embodiments of a conjugate of the present invention, the monovalent organic moiety is a protein, such as a Ras protein. In some embodiments, the Ras protein is K-Ras G12C, K-Ras G13C, H-Ras G12C, H-Ras G13C, N-Ras G12C, or N-Ras G13C. Other Ras proteins are described herein. In some embodiments, the linker is bound to the monovalent organic moiety through a bond to a sulfhydryl group of an amino acid residue of the monovalent organic moiety. In some embodiments, the linker is bound to the monovalent organic moiety through a bond to a carboxyl group of an amino acid residue of the monovalent organic moiety.

Further provided is a method of treating cancer in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a compound of the present invention, or a pharmaceutically acceptable salt thereof. The cancer may, for example, be pancreatic cancer, colorectal cancer, non-small cell lung cancer, acute myeloid leukemia, multiple myeloma, thyroid gland adenocarcinoma, a myelodysplastic syndrome, or squamous cell lung carcinoma. In some embodiments, the cancer comprises a Ras mutation, such as K-Ras G12C, K-Ras G13C, H-Ras G12C, H-Ras G13C, N-Ras G12C, or N-Ras G13C. Other Ras mutations are described herein.

Further provided is a method of treating a Ras protein-related disorder in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a compound of the present invention, or a pharmaceutically acceptable salt thereof.

Further provided is a method of inhibiting a Ras protein in a cell, the method comprising contacting the cell with an effective amount of a compound of the present invention, or a pharmaceutically acceptable salt thereof. For example, the Ras protein is K-Ras G12C, K-Ras G13C, H-Ras G12C, H-Ras G13C, N-Ras G12C, or N-Ras G13C. Other Ras proteins are described herein. The cell may be a cancer cell, such as a pancreatic cancer cell, a colorectal cancer cell, a non-small cell lung cancer cell, an acute myeloid leukemia cell, a multiple myeloma cell, a thyroid gland adenocarcinoma cell, a myelodysplastic syndrome cell, or a squamous cell lung carcinoma cell. Other cancer types are described herein. The cell may be in vivo or in vitro.

With respect to compounds of the present invention, one stereoisomer may exhibit better inhibition than another stereoisomer. For example, one atropisomer may exhibit inhibition, whereas the other atropisomer may exhibit little or no inhibition.

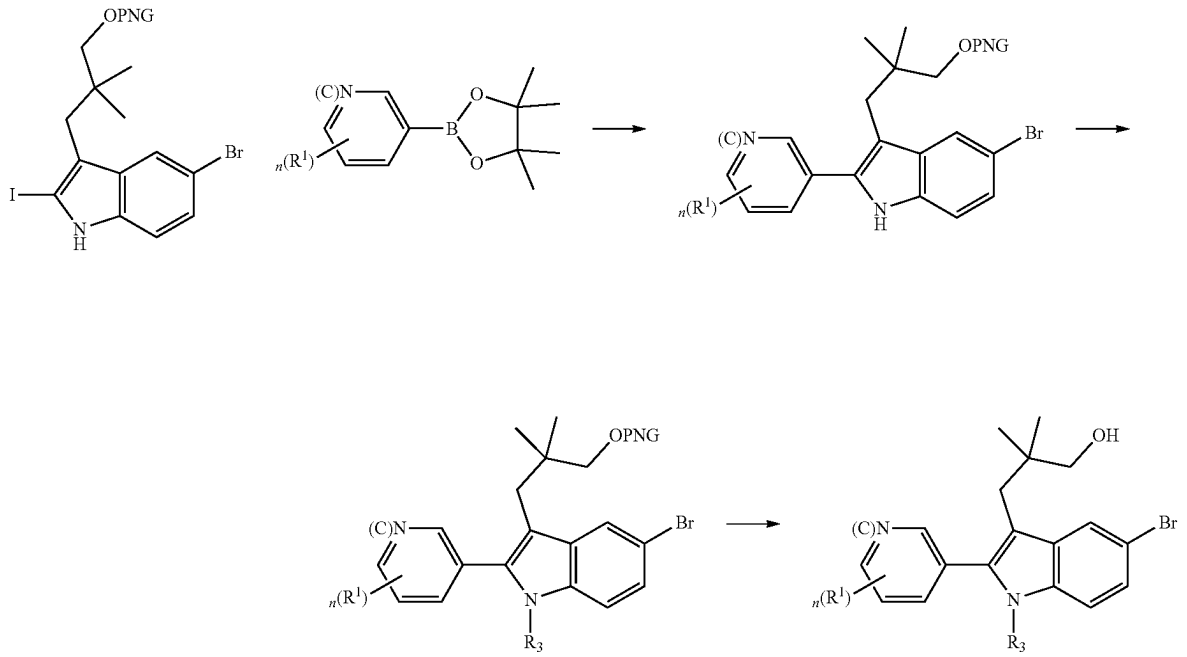
In some embodiments, a method or use described herein further comprises administering an additional anti-cancer therapy. In some embodiments, the additional anti-cancer therapy is a HER2 inhibitor, an EGFR inhibitor, a second Ras inhibitor, a SHP2 inhibitor, an SOS1 inhibitor, a Raf inhibitor, a MEK inhibitor, an ERK inhibitor, a PI3K inhibitor, a PTEN inhibitor, an AKT inhibitor, an mTORC1 inhibitor, a BRAF inhibitor, a PD-L1 inhibitor, a PD-1 inhibitor, a CDK4/6 inhibitor, or a combination thereof. In some embodiments, the additional anticancer therapy is a SHP2 inhibitor. Other additional anti-cancer therapies are described herein.

Methods of Synthesis

The compounds described herein may be made from commercially available starting materials or synthesized using known organic, inorganic, or enzymatic processes.

The compounds of the present invention can be prepared in a number of ways well known to those skilled in the art of organic synthesis. By way of example, compounds of the present invention can be synthesized using the methods described in the Schemes below, together with synthetic methods known in the art of synthetic organic chemistry, or variations thereon as appreciated by those skilled in the art. These methods include but are not limited to those methods described in the Schemes below.

Scheme 1. General synthesis of macrocyclic esters



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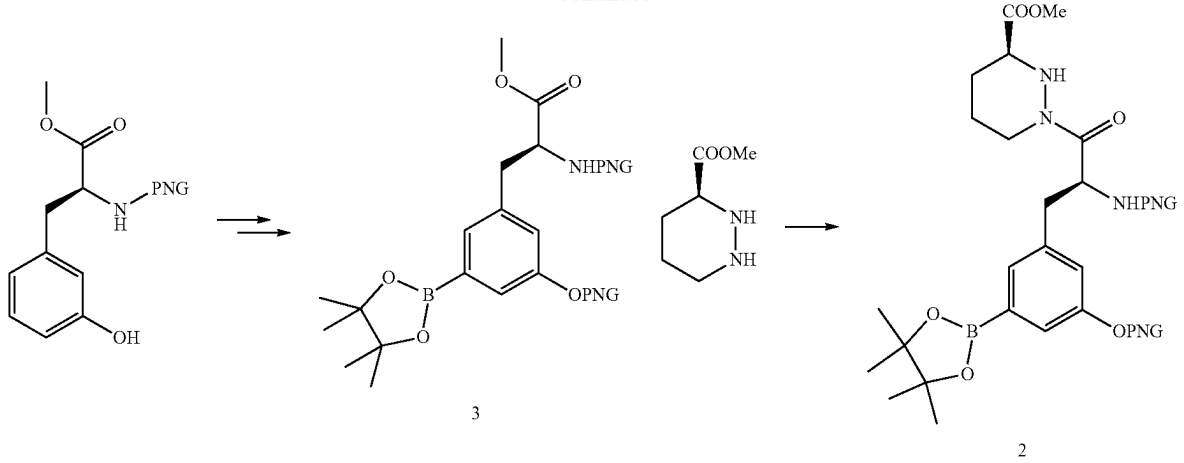
O
O
PNG
N
H
OH
O
O
NHPNG
O
B
O
OPNG
3
COOMe
NH
NH
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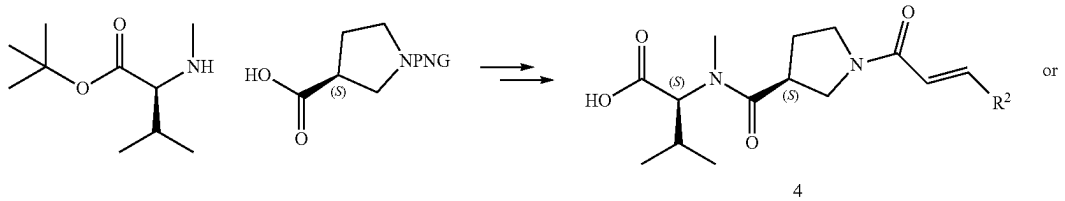
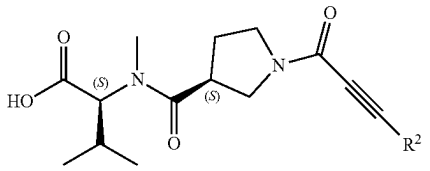
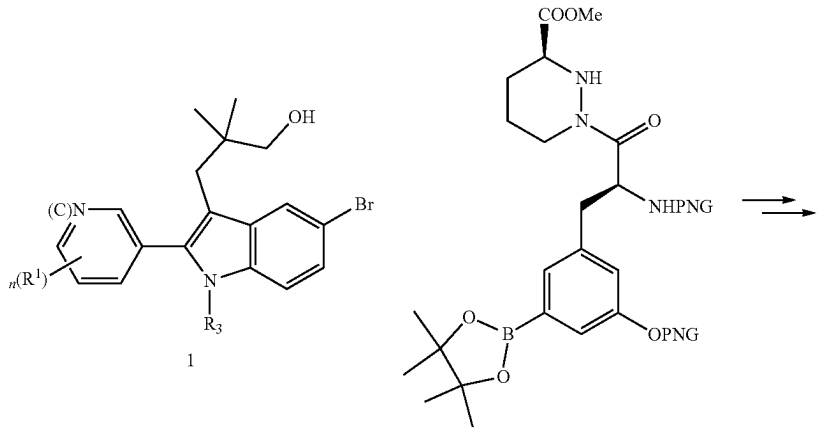
N
O
NHPNG
O
B
O
OPNG
2


O
O
NH
HO
O
NPNG
(S)
O
HO
(S)
N
(S)
N
O
R2
or
4


O
HO
(S)
N
(S)
N
O
O
R2
4


OH
(C)N
Br
n(R1)
N
R3
1
COOMe
NH
N
O
NHPNG
O
B
O
OPNG
2

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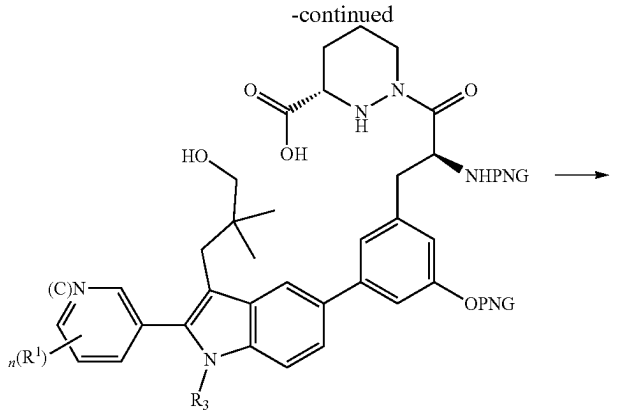

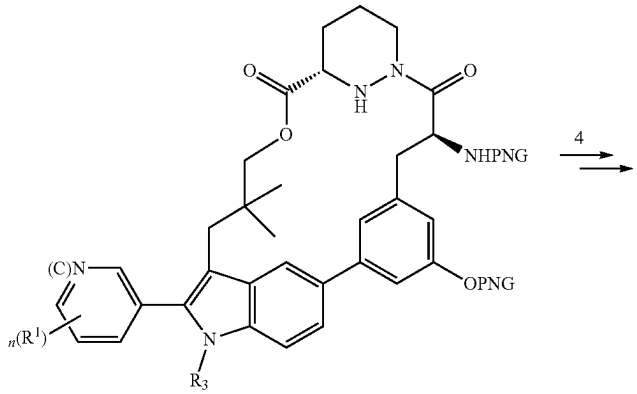

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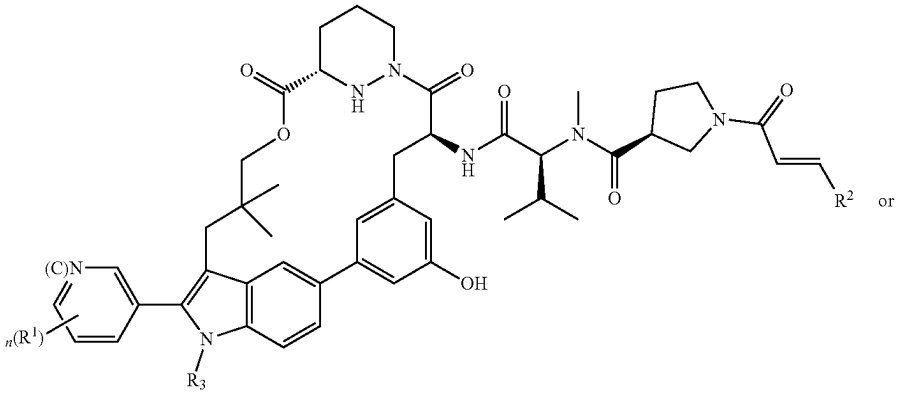
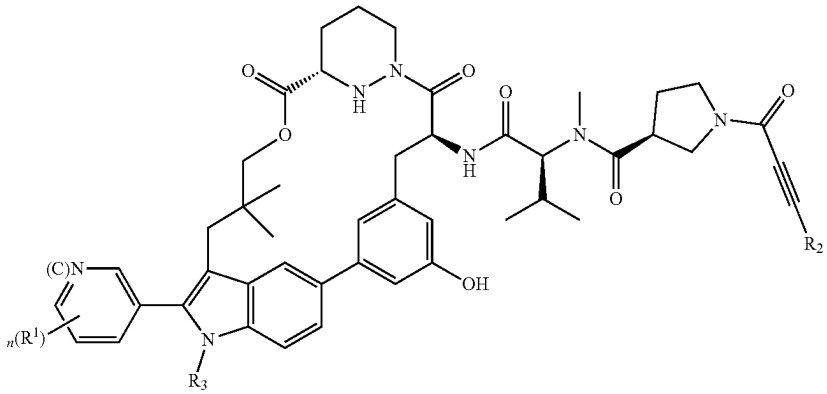




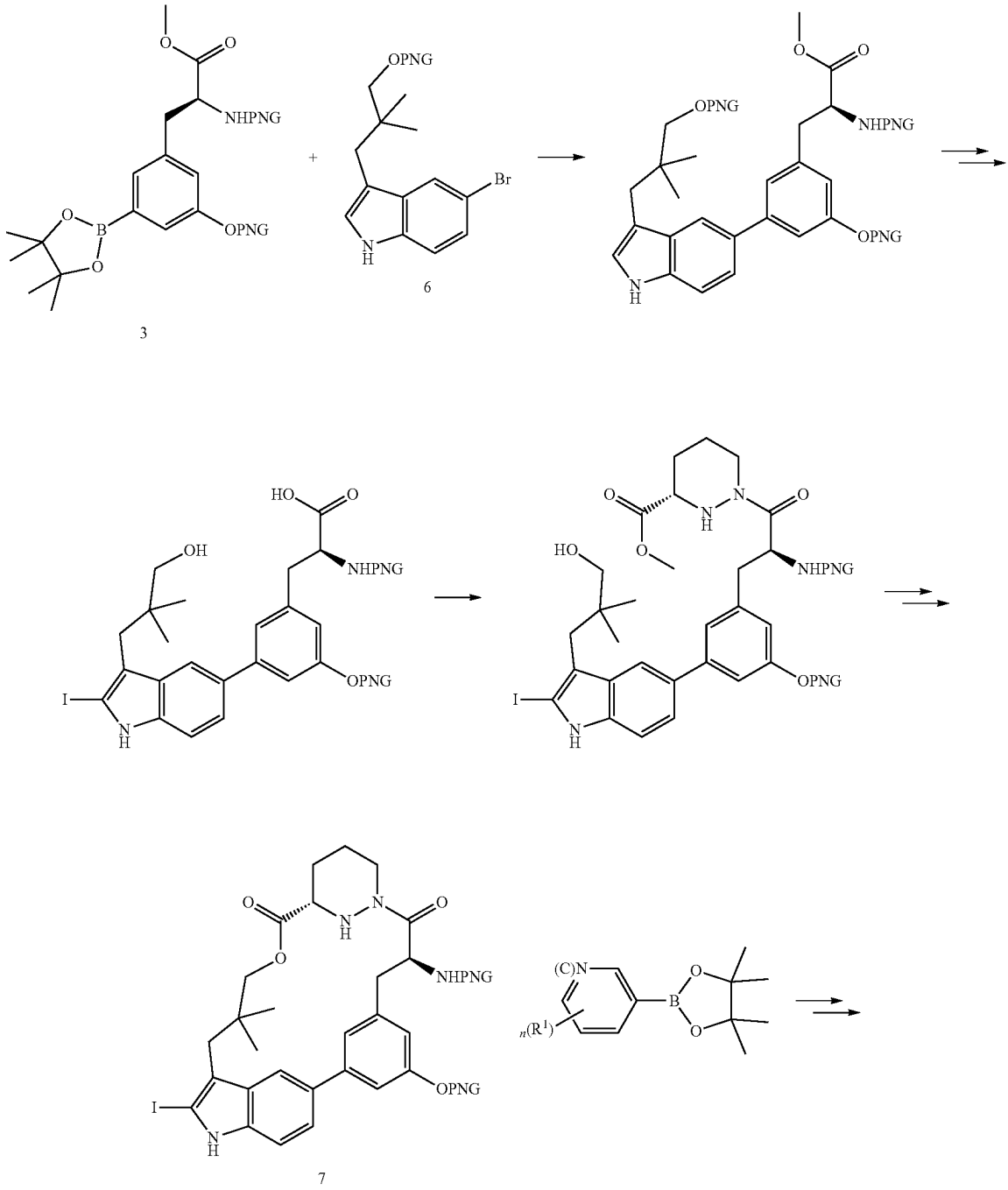
A general synthesis of macrocyclic esters is outlined in Scheme 1. An appropriately substituted aryl-3-(5-bromo-1-ethyl-1H-indol-3-yl)-2,2-dimethylpropan-1-ol (1) can be prepared in three steps starting from protected 3-(5-bromo-2-iodo-1H-indol-3-yl)-2,2-dimethylpropan-1-ol and appropriately substituted boronic acid, including palladium medi-

ated coupling, alkylation, and de-protection reactions. Methyl-amino-hexahydropyridazine-3-carboxylate-boronic ester (2) can be prepared in three steps, including protection, iridium catalyst mediated borylation, and coupling with methyl methyl (S)-hexahydropyridazine-3-carboxylate.

An appropriately substituted acetylpyrrolidine-3-carbonyl-N-methyl-L-valine (or an alternative aminoacid derivative (4) can be made by coupling of methyl-L-valinate and protected (S)-pyrrolidine-3-carboxylic acid, followed by deprotection, coupling with a carboxylic acid containing an appropriately substituted Michael acceptor, and a hydrolysis step.

The final macrocyclic esters can be made by coupling of methyl-amino-hexahydropyridazine-3-carboxylate-boronic ester (2) and aryl-3-(5-bromo-1-ethyl-1H-indol-3-yl)-2,2-dimethylpropan-1-ol (1) in the presence of a Pd catalyst followed by hydrolysis and macrolactonization steps to result in an appropriately protected macrocyclic intermediate (5). Deprotection and coupling with an appropriately substituted intermediate 4 results in a macrocyclic product. Additional deprotection and/or functionalization steps can be required to produce the final compound.

Scheme 2. Alternative general synthesis of macrocyclic esters



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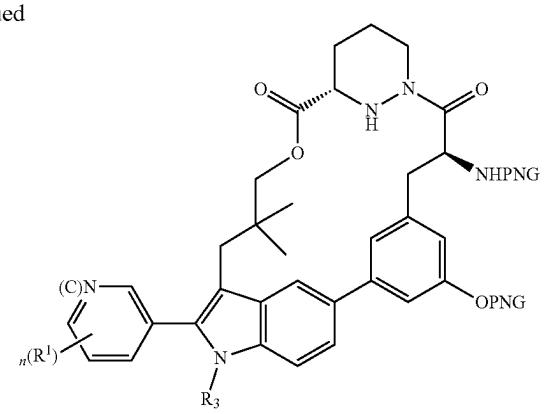

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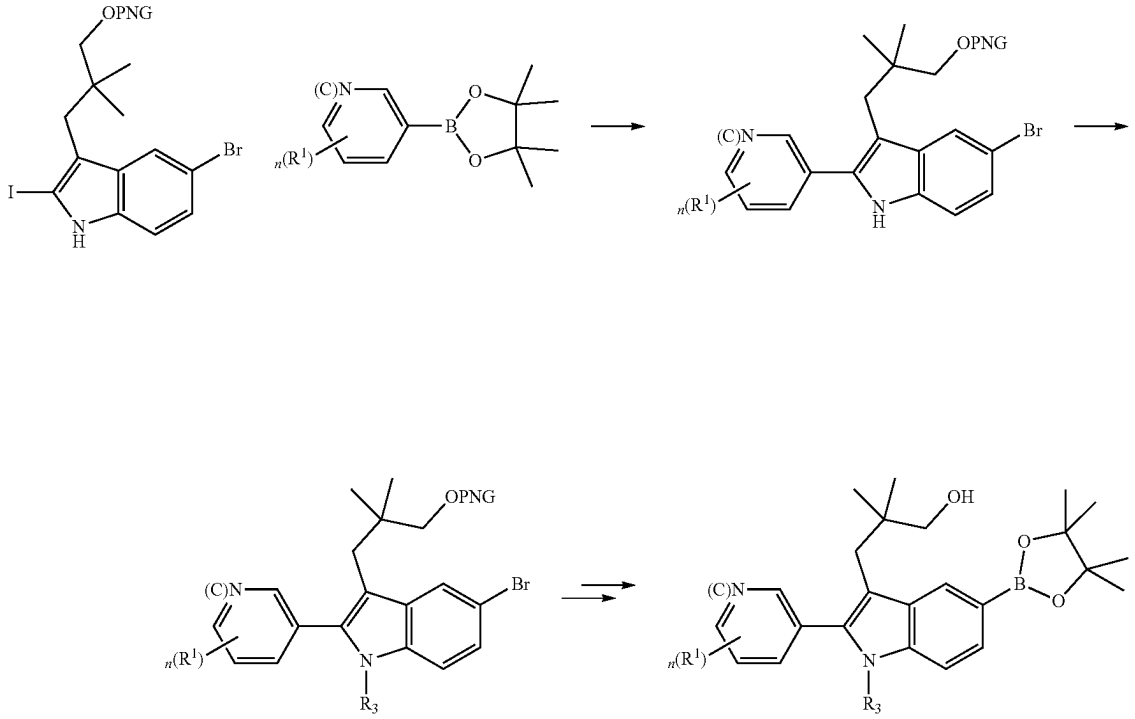
Alternatively, macrocyclic ester can be prepared as described in Scheme 2. An appropriately protected bromoindolyl (6) coupled in the presence of a Pd catalyst with boronic ester (3), followed by iodination, deprotection, and ester hydrolysis. Subsequent coupling with methyl (S)-hexahydropyridazine-3-carboxylate, followed by hydrolysis and macrolactonization can result in iodo intermediate (7). Coupling in the presence of a Pd catalyst with an appropriately substituted boronic ester and alkyllation can yield fully protected macrocycle (5). Additional deprotection or functionalization steps are required to produce the final compound.

In addition, compounds of the disclosure can be synthesized using the methods described in the Examples below, together with synthetic methods known in the art of synthetic organic chemistry, or variations thereon as appreciated by those skilled in the art. These methods include but are not limited to those methods described in the Examples below. For example, a person of skill in the art would be able to install into a macrocyclic ester a desired —B-L-W group of a compound of Formula (I), where B, L and W are defined herein, including by using methods exemplified in the Example section herein.

Compounds of Table 1 herein were prepared using methods disclosed herein or were prepared using methods disclosed herein combined with the knowledge of one of skill in the art. Compounds of Table 2 may be prepared using methods disclosed herein or may be prepared using methods disclosed herein combined with the knowledge of one of skill in the art.

Scheme 3. General synthesis of macrocyclic esters



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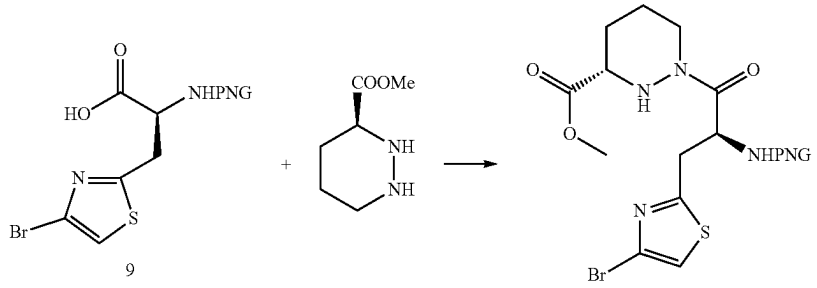
O
HO
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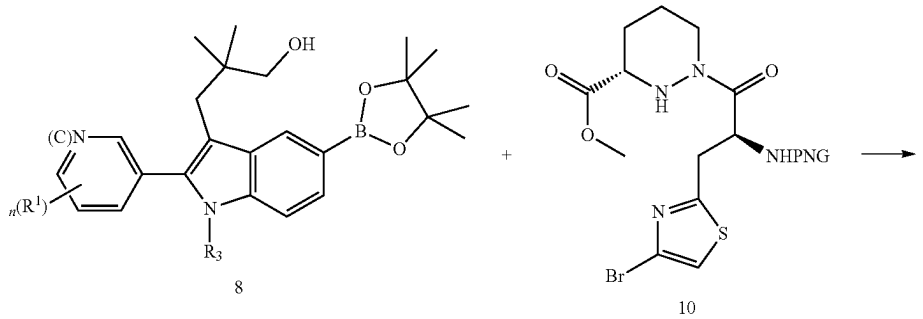
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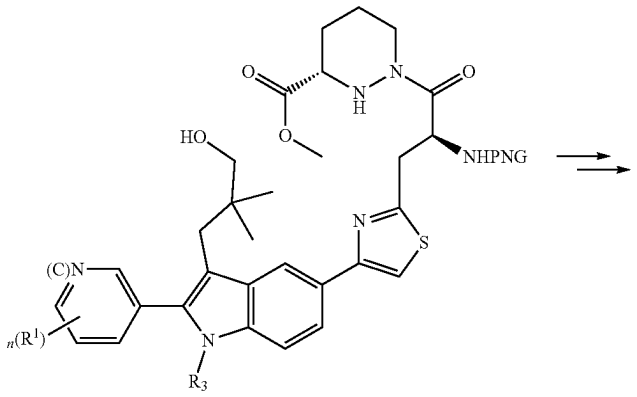
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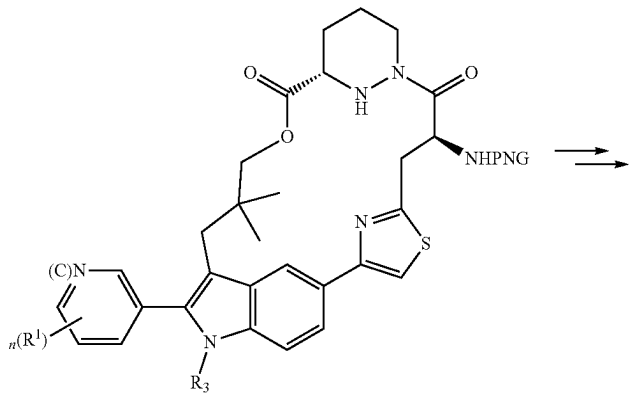
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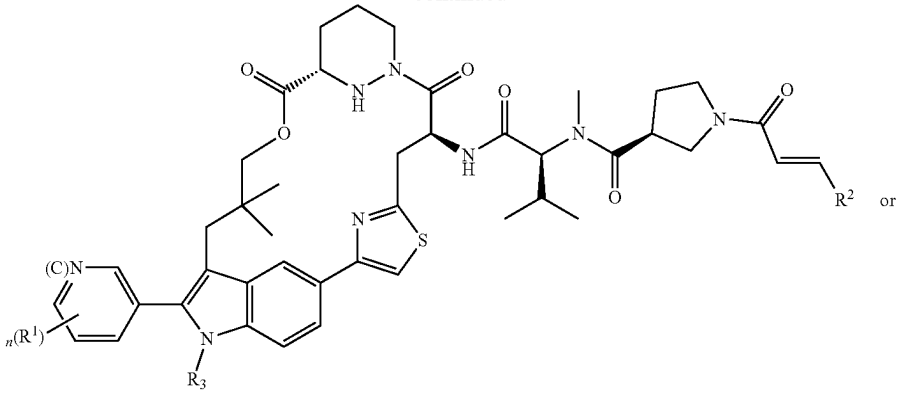

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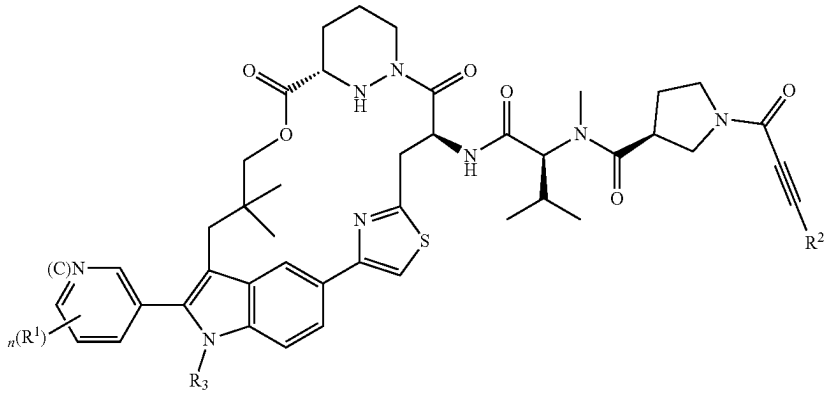


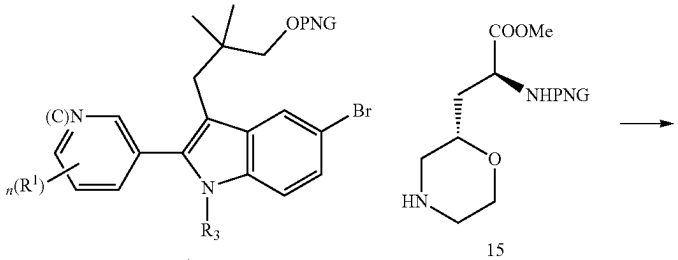
14

An alternative general synthesis of macrocyclic esters is outlined in Scheme 3. An appropriately substituted indolyl boronic ester (8) can be prepared in four steps starting from protected 3-(5-bromo-2-iodo-1H-indol-3-yl)-2,2-dimethyl-propan-1-ol and appropriately substituted boronic acid, including Palladium mediated coupling, alkylation, de-protection, and Palladium mediated borylation reactions.

Methyl-amino-3-(4-bromothiazol-2-yl)propanoyl)hexa-hydropyridazine-3-carboxylate (10) can be prepared via coupling of (S)-2-amino-3-(4-bromothiazol-2-yl)propanoic acid (9) with methyl (S)-hexahydropyridazine-3-carboxy-late.

The final macrocyclic esters can be made by coupling of Methyl-amino-3-(4-bromothiazol-2-yl)propanoyl)hexahy-dropyridazine-3-carboxylate (10) and an appropriately sub-stituted indolyl boronic ester (8) in the presence of Pd catalyst followed by hydrolysis and macrolactonization steps to result in an appropriately protected macrocyclic intermediate (11). Deprotection and coupling with an appro-priately substituted intermediate 4 can result in a macrocy-clic product. Additional deprotection or functionalization steps could be required to produce a final compound 13 or 14.

Scheme 4. General synthesis of macrocyclic esters



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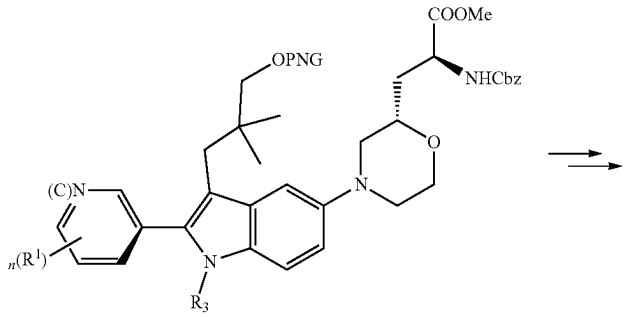

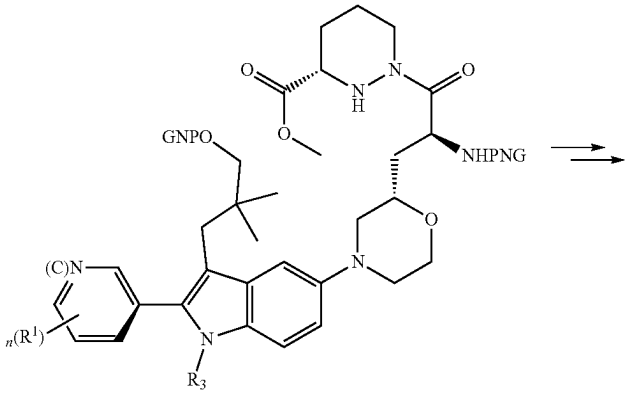

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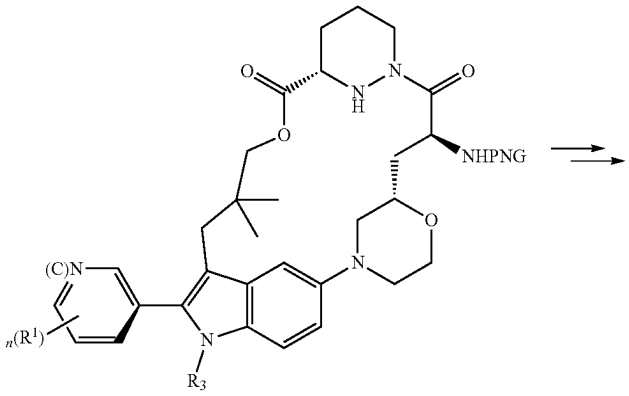

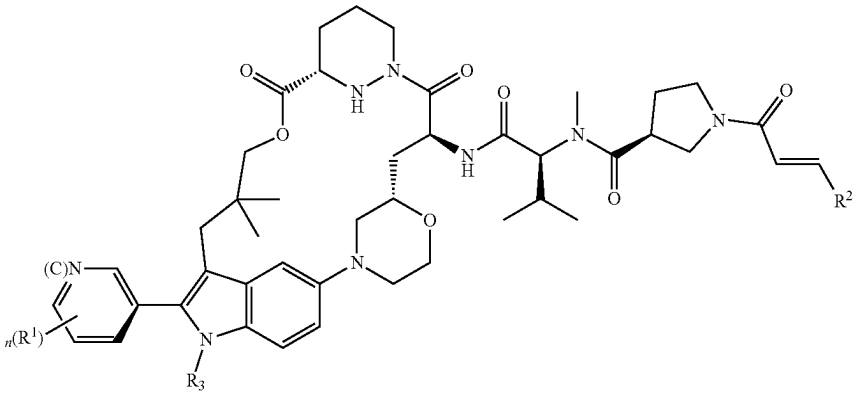

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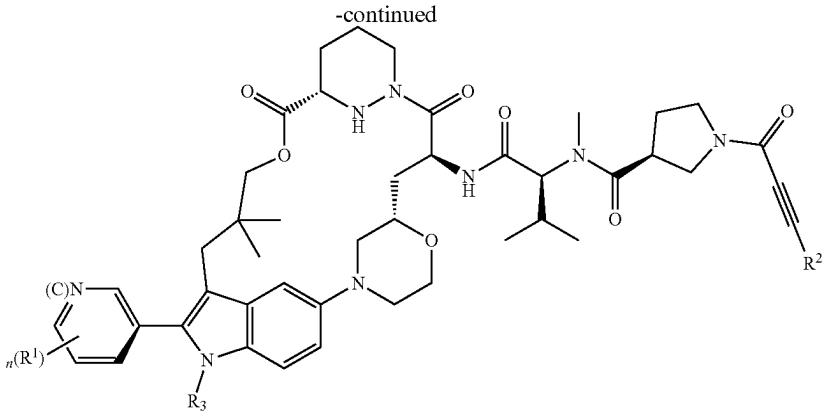
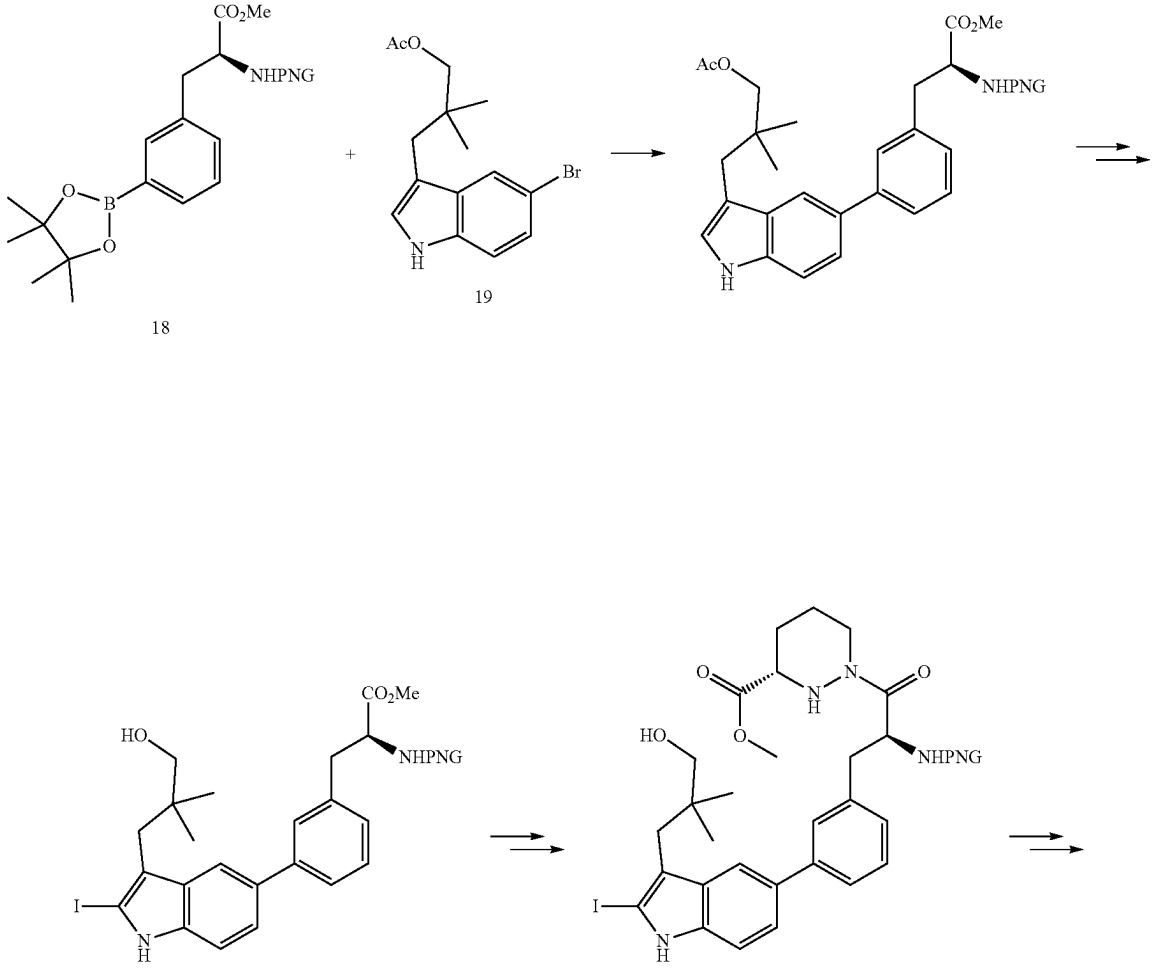


17

An alternative general synthesis of macrocyclic esters is outlined in Scheme 4. An appropriately substituted morpholine or an alternative herecyclic intermediate (15) can be coupled with appropriately protected Intermediate 1 via Palladium mediated coupling. Subsequent ester hydrolysis, and coupling with piperazoic ester results in intermediate 16.

The macrocyclic esters can be made by hydrolysis, deprotection and macrocyclization sequence. Subsequent deprotection and coupling with Intermediate 4 (or analogs) result in an appropriately substituted final macrocyclic products. Additional deprotection or functionalization steps could be required to produce a final compound 17.

Scheme 5. General synthesis of macrocyclic esters



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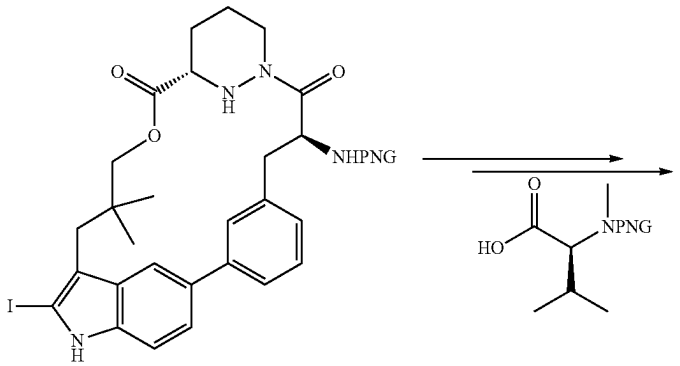

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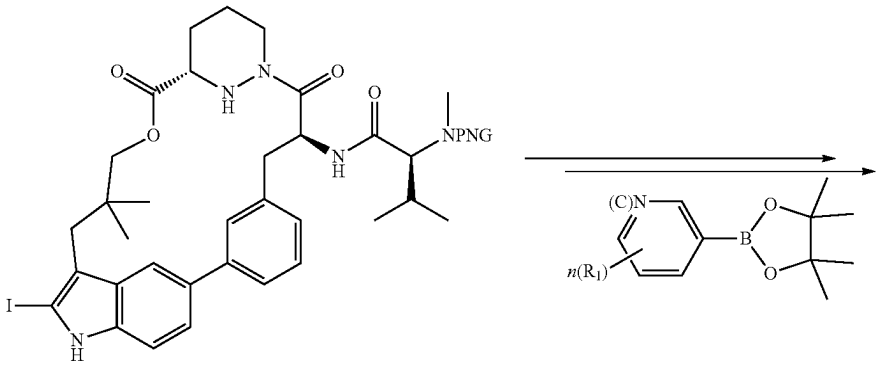

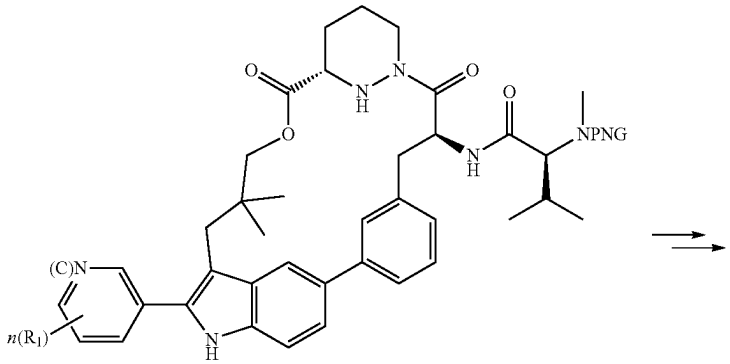

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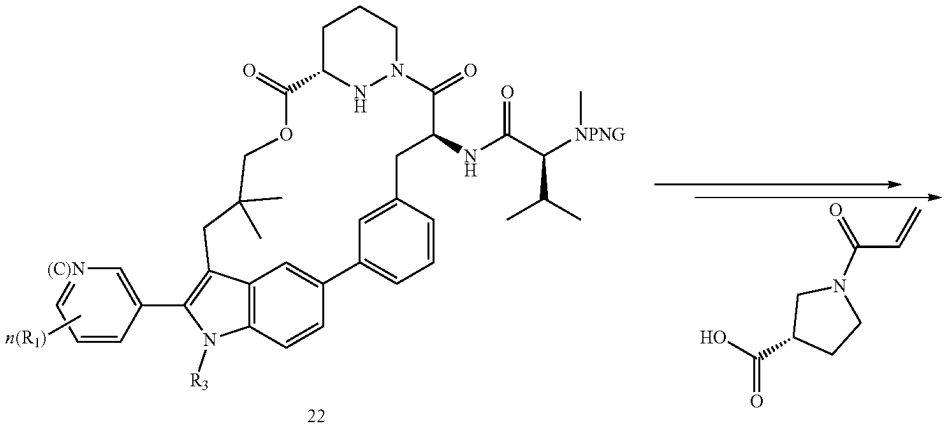

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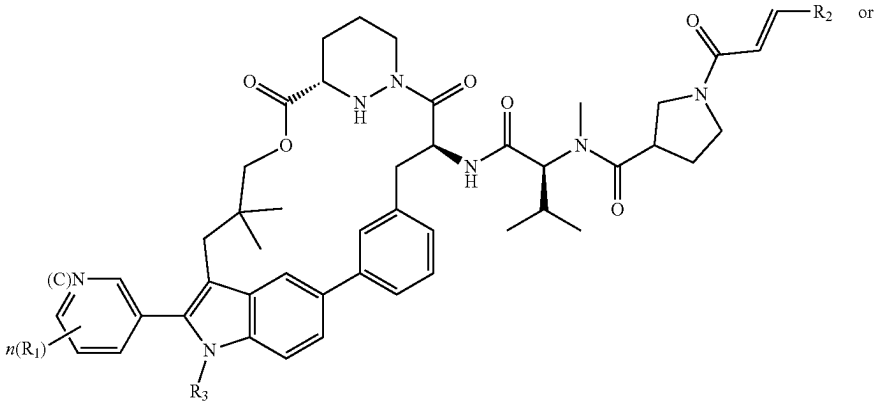

or

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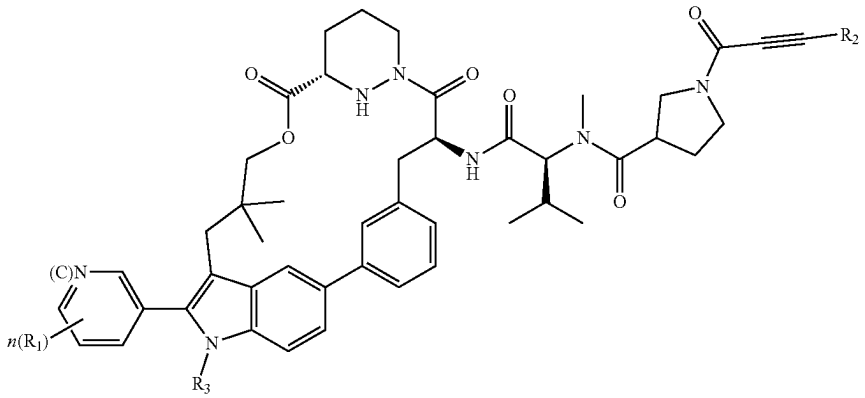


24

An alternative general synthesis of macrocyclic esters is outlined in Scheme 5. An appropriately substituted macrocycle (20) can be prepared starting from an appropriately protected boronic ester 18 and bromo indolyl intermediate (19), including Palladium mediated coupling, hydrolysis, coupling with piperazoic ester, hydrolysis, de-protection, and macrocyclizarion steps. Subsequent coupling with an appropriately substituted protected aminoacid followed by palladium mediated coupling yiels intermediate 21. Additional deprotection and derivatization steps, including alkyllation may be required at this point.

The final macrocyclic esters can be made by coupling of intermediate (22) and an appropriately substituted carboxylic acid intermediate (23). Additional deprotection or functionalization steps could be required to produce a final compound (24).

In addition, compounds of the disclosure can be synthesized using the methods described in the Examples below, together with synthetic methods known in the art of synthetic organic chemistry, or variations thereon as appreciated by those skilled in the art. These methods include but are not limited to those methods described in the Examples below. For example, a person of skill in the art would be able to install into a macrocyclic ester a desired —B-L-W group of a compound of Formula (I), where B, L and W are defined herein, including by using methods exemplified in the Example section herein.

Pharmaceutical Compositions and Methods of Use

Pharmaceutical Compositions and Methods of Administration

The compounds with which the invention is concerned are Ras inhibitors, and are useful in the treatment of cancer. Accordingly, one embodiment of the present invention provides pharmaceutical compositions containing a compound of the invention or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient, as well as methods of using the compounds of the invention to prepare such compositions.

As used herein, the term "pharmaceutical composition" refers to a compound, such as a compound of the present invention, or a pharmaceutically acceptable salt thereof, formulated together with a pharmaceutically acceptable excipient.

In some embodiments, a compound is present in a pharmaceutical composition in unit dose amount appropriate for administration in a therapeutic regimen that shows a statistically significant probability of achieving a predetermined therapeutic effect when administered to a relevant population. In some embodiments, pharmaceutical compositions may be specially formulated for administration in solid or liquid form, including those adapted for the following: oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, e.g., those targeted for buccal, sublingual, and systemic absorption, boluses, powders, granules, pastes for application to the tongue; parenteral administration, for example, by subcutaneous, intramuscular, intravenous or epidural injection as,

for example, a sterile solution or suspension, or sustained-release formulation; topical application, for example, as a cream, ointment, or a controlled-release patch or spray applied to the skin, lungs, or oral cavity; intravaginally or intrarectally, for example, as a pessary, cream, or foam; sublingually; ocularly; transdermally; or nasally, pulmonary, and to other mucosal surfaces.

A "pharmaceutically acceptable excipient," as used herein, refers any inactive ingredient (for example, a vehicle capable of suspending or dissolving the active compound) having the properties of being nontoxic and non-inflammatory in a subject. Typical excipients include, for example: antiadherents, antioxidants, binders, coatings, compression aids, disintegrants, dyes (colors), emollients, emulsifiers, fillers (diluents), film formers or coatings, flavors, fragrances, glidants (flow enhancers), lubricants, preservatives, printing inks, sorbents, suspensing or dispersing agents, sweeteners, or waters of hydration. Excipients include, but are not limited to: butylated optionally substituted hydroxyltoluene (BHT), calcium carbonate, calcium phosphate (dibasic), calcium stearate, croscarmellose, crosslinked polyvinyl pyrrolidone, citric acid, crospovidone, cysteine, ethylcellulose, gelatin, optionally substituted hydroxylpropyl cellulose, optionally substituted hydroxylpropyl methylcellulose, lactose, magnesium stearate, maltitol, mannitol, methionine, methylcellulose, methyl paraben, microcrystalline cellulose, polyethylene glycol, polyvinyl pyrrolidone, povidone, pregelatinized starch, propyl paraben, retinyl palmitate, shellac, silicon dioxide, sodium carboxymethyl cellulose, sodium citrate, sodium starch glycolate, sorbitol, starch (corn), stearic acid, stearic acid, sucrose, talc, titanium dioxide, vitamin A, vitamin E, vitamin C, and xylitol. Those of ordinary skill in the art are familiar with a variety of agents and materials useful as excipients. See, e.g., e.g., Ansel, et al., Ansel's Pharmaceutical Dosage Forms and Drug Delivery Systems. Philadelphia: Lippincott, Williams & Wilkins, 2004; Gennaro, et al., Remington: The Science and Practice of Pharmacy. Philadelphia: Lippincott, Williams & Wilkins, 2000; and Rowe, Handbook of Pharmaceutical Excipients. Chicago, Pharmaceutical Press, 2005. In some embodiments, a composition includes at least two different pharmaceutically acceptable excipients.

Compounds described herein, whether expressly stated or not, may be provided or utilized in salt form, e.g., a pharmaceutically acceptable salt form, unless expressly stated to the contrary. The term "pharmaceutically acceptable salt," as use herein, refers to those salts of the compounds described herein that are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and other animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, pharmaceutically acceptable salts are described in: Berge et al., *J. Pharmaceutical Sciences* 66:1-19, 1977 and in *Pharmaceutical Salts: Properties, Selection, and Use*, (Eds. P. H. Stahl and C. G. Wermuth), Wiley-VCH, 2008. The salts can be prepared in situ during the final isolation and purification of the compounds described herein or separately by reacting the free base group with a suitable organic acid.

The compounds of the invention may have ionizable groups so as to be capable of preparation as pharmaceutically acceptable salts. These salts may be acid addition salts involving inorganic or organic acids or the salts may, in the case of acidic forms of the compounds of the invention, be prepared from inorganic or organic bases. In some embodiments, the compounds are prepared or used as pharmaceutically acceptable salts prepared as addition products of pharmaceutically acceptable acids or bases. Suitable pharmaceutically acceptable acids and bases are well-known in the art, such as hydrochloric, sulfuric, hydrobromic, acetic, lactic, citric, or tartaric acids for forming acid addition salts, and potassium hydroxide, sodium hydroxide, ammonium hydroxide, caffeine, various amines, and the like for forming basic salts. Methods for preparation of the appropriate salts are well-established in the art.

Representative acid addition salts include acetate, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptonate, glycerophosphate, hemisulfate, heptonate, hexanoate, hydrobromide, hydrochloride, hydroiodide, 2-optionally substituted hydroxyl-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, toluenesulfonate, undecanoate, valerate salts and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium and the like, as well as nontoxic ammonium, quaternary ammonium, and amine cations, including, but not limited to ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, ethylamine, and the like.

As used herein, the term "subject" refers to any member of the animal kingdom. In some embodiments, "subject" refers to humans, at any stage of development. In some embodiments, "subject" refers to a human patient. In some embodiments, "subject" refers to non-human animals. In some embodiments, the non-human animal is a mammal (e.g., a rodent, a mouse, a rat, a rabbit, a monkey, a dog, a cat, a sheep, cattle, a primate, or a pig). In some embodiments, subjects include, but are not limited to, mammals, birds, reptiles, amphibians, fish, or worms. In some embodiments, a subject may be a transgenic animal, genetically-engineered animal, or a clone.

As used herein, the term "dosage form" refers to a physically discrete unit of a compound (e.g., a compound of the present invention) for administration to a subject. Each unit contains a predetermined quantity of compound. In some embodiments, such quantity is a unit dosage amount (or a whole fraction thereof) appropriate for administration in accordance with a dosing regimen that has been determined to correlate with a desired or beneficial outcome when administered to a relevant population (i.e., with a therapeutic dosing regimen). Those of ordinary skill in the art appreciate that the total amount of a therapeutic composition or compound administered to a particular subject is determined by one or more attending physicians and may involve administration of multiple dosage forms.

As used herein, the term "dosing regimen" refers to a set of unit doses (typically more than one) that are administered individually to a subject, typically separated by periods of time. In some embodiments, a given therapeutic compound (e.g., a compound of the present invention) has a recommended dosing regimen, which may involve one or more doses. In some embodiments, a dosing regimen comprises a plurality of doses each of which are separated from one another by a time period of the same length; in some embodiments, a dosing regimen comprises a plurality of doses and at least two different time periods separating individual doses. In some embodiments, all doses within a

dosing regimen are of the same unit dose amount. In some embodiments, different doses within a dosing regimen are of different amounts. In some embodiments, a dosing regimen comprises a first dose in a first dose amount, followed by one or more additional doses in a second dose amount different from the first dose amount. In some embodiments, a dosing regimen comprises a first dose in a first dose amount, followed by one or more additional doses in a second dose amount same as the first dose amount. In some embodiments, a dosing regimen is correlated with a desired or beneficial outcome when administered across a relevant population (i.e., is a therapeutic dosing regimen).

A "therapeutic regimen" refers to a dosing regimen whose administration across a relevant population is correlated with a desired or beneficial therapeutic outcome.

The term "treatment" (also "treat" or "treating"), in its broadest sense, refers to any administration of a substance (e.g., a compound of the present invention) that partially or completely alleviates, ameliorates, relieves, inhibits, delays onset of, reduces severity of, or reduces incidence of one or more symptoms, features, or causes of a particular disease, disorder, or condition. In some embodiments, such treatment may be administered to a subject who does not exhibit signs of the relevant disease, disorder or condition or of a subject who exhibits only early signs of the disease, disorder, or condition. Alternatively, or additionally, in some embodiments, treatment may be administered to a subject who exhibits one or more established signs of the relevant disease, disorder, or condition. In some embodiments, treatment may be of a subject who has been diagnosed as suffering from the relevant disease, disorder, or condition. In some embodiments, treatment may be of a subject known to have one or more susceptibility factors that are statistically correlated with increased risk of development of the relevant disease, disorder, or condition.

The term "therapeutically effective amount" means an amount that is sufficient, when administered to a population suffering from or susceptible to a disease, disorder, or condition in accordance with a therapeutic dosing regimen, to treat the disease, disorder, or condition. In some embodiments, a therapeutically effective amount is one that reduces the incidence or severity of, or delays onset of, one or more symptoms of the disease, disorder, or condition. Those of ordinary skill in the art will appreciate that the term "therapeutically effective amount" does not in fact require successful treatment be achieved in a particular individual. Rather, a therapeutically effective amount may be that amount that provides a particular desired pharmacological response in a significant number of subjects when administered to patients in need of such treatment. It is specifically understood that particular subjects may, in fact, be "refractory" to a "therapeutically effective amount." In some embodiments, reference to a therapeutically effective amount may be a reference to an amount as measured in one or more specific tissues (e.g., a tissue affected by the disease, disorder or condition) or fluids (e.g., blood, saliva, serum, sweat, tears, urine). Those of ordinary skill in the art will appreciate that, in some embodiments, a therapeutically effective amount may be formulated or administered in a single dose. In some embodiments, a therapeutically effective amount may be formulated or administered in a plurality of doses, for example, as part of a dosing regimen.

For use as treatment of subjects, the compounds of the invention, or a pharmaceutically acceptable salt thereof, can be formulated as pharmaceutical or veterinary compositions. Depending on the subject to be treated, the mode of administration, and the type of treatment desired, e.g., prevention, prophylaxis, or therapy, the compounds, or a pharmaceutically acceptable salt thereof, are formulated in ways consonant with these parameters. A summary of such techniques may be found in *Remington: The Science and Practice of Pharmacy,* 21st Edition, Lippincott Williams & Wilkins, (2005); and *Encyclopedia of Pharmaceutical Technology*, eds. J. Swarbrick and J. C. Boylan, 1988-1999, Marcel Dekker, New York, each of which is incorporated herein by reference.

Compositions can be prepared according to conventional mixing, granulating or coating methods, respectively, and the present pharmaceutical compositions can contain from about 0.1% to about 99%, from about 5% to about 90%, or from about 1% to about 20% of a compound of the present invention, or pharmaceutically acceptable salt thereof, by weight or volume. In some embodiments, compounds, or a pharmaceutically acceptable salt thereof, described herein may be present in amounts totaling 1-95% by weight of the total weight of a composition, such as a pharmaceutical composition.

The composition may be provided in a dosage form that is suitable for intraarticular, oral, parenteral (e.g., intravenous, intramuscular), rectal, cutaneous, subcutaneous, topical, transdermal, sublingual, nasal, vaginal, intravesicular, intraurethral, intrathecal, epidural, aural, or ocular administration, or by injection, inhalation, or direct contact with the nasal, genitourinary, reproductive or oral mucosa. Thus, the pharmaceutical composition may be in the form of, e.g., tablets, capsules, pills, powders, granulates, suspensions, emulsions, solutions, gels including hydrogels, pastes, ointments, creams, plasters, drenches, osmotic delivery devices, suppositories, enemas, injectables, implants, sprays, preparations suitable for iontophoretic delivery, or aerosols. The compositions may be formulated according to conventional pharmaceutical practice.

As used herein, the term "administration" refers to the administration of a composition (e.g., a compound, or a preparation that includes a compound as described herein) to a subject or system. Administration to an animal subject (e.g., to a human) may be by any appropriate route. For example, in some embodiments, administration may be bronchial (including by bronchial instillation), buccal, enteral, interdermal, intra-arterial, intradermal, intragastric, intramedullary, intramuscular, intranasal, intraperitoneal, intrathecal, intravenous, intraventricular, mucosal, nasal, oral, rectal, subcutaneous, sublingual, topical, tracheal (including by intratracheal instillation), transdermal, vaginal, or vitreal.

Formulations may be prepared in a manner suitable for systemic administration or topical or local administration. Systemic formulations include those designed for injection (e.g., intramuscular, intravenous or subcutaneous injection) or may be prepared for transdermal, transmucosal, or oral administration. A formulation will generally include a diluent as well as, in some cases, adjuvants, buffers, preservatives and the like. Compounds, or a pharmaceutically acceptable salt thereof, can be administered also in liposomal compositions or as microemulsions.

For injection, formulations can be prepared in conventional forms as liquid solutions or suspensions or as solid forms suitable for solution or suspension in liquid prior to injection or as emulsions. Suitable excipients include, for example, water, saline, dextrose, glycerol and the like. Such compositions may also contain amounts of nontoxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like, such as, for example, sodium acetate, sorbitan monolaurate, and so forth.

Various sustained release systems for drugs have also been devised. See, for example, U.S. Pat. No. 5,624,677.

Systemic administration may also include relatively non-invasive methods such as the use of suppositories, transdermal patches, transmucosal delivery and intranasal administration. Oral administration is also suitable for compounds of the invention, or a pharmaceutically acceptable salt thereof. Suitable forms include syrups, capsules, and tablets, as is understood in the art.

Each compound, or a pharmaceutically acceptable salt thereof, as described herein, may be formulated in a variety of ways that are known in the art. For example, the first and second agents of the combination therapy may be formulated together or separately. Other modalities of combination therapy are described herein.

The individually or separately formulated agents can be packaged together as a kit. Non-limiting examples include, but are not limited to, kits that contain, e.g., two pills, a pill and a powder, a suppository and a liquid in a vial, two topical creams, etc. The kit can include optional components that aid in the administration of the unit dose to subjects, such as vials for reconstituting powder forms, syringes for injection, customized IV delivery systems, inhalers, etc. Additionally, the unit dose kit can contain instructions for preparation and administration of the compositions. The kit may be manufactured as a single use unit dose for one subject, multiple uses for a particular subject (at a constant dose or in which the individual compounds, or a pharmaceutically acceptable salt thereof, may vary in potency as therapy progresses); or the kit may contain multiple doses suitable for administration to multiple subjects ("bulk packaging"). The kit components may be assembled in cartons, blister packs, bottles, tubes, and the like.

Formulations for oral use include tablets containing the active ingredient(s) in a mixture with non-toxic pharmaceutically acceptable excipients. These excipients may be, for example, inert diluents or fillers (e.g., sucrose, sorbitol, sugar, mannitol, microcrystalline cellulose, starches including potato starch, calcium carbonate, sodium chloride, lactose, calcium phosphate, calcium sulfate, or sodium phosphate); granulating and disintegrating agents (e.g., cellulose derivatives including microcrystalline cellulose, starches including potato starch, croscarmellose sodium, alginates, or alginic acid); binding agents (e.g., sucrose, glucose, sorbitol, acacia, alginic acid, sodium alginate, gelatin, starch, pregelatinized starch, microcrystalline cellulose, magnesium aluminum silicate, carboxymethylcellulose sodium, methylcellulose, optionally substituted hydroxylpropyl methylcellulose, ethylcellulose, polyvinylpyrrolidone, or polyethylene glycol); and lubricating agents, glidants, and antiadhesives (e.g., magnesium stearate, zinc stearate, stearic acid, silicas, hydrogenated vegetable oils, or talc). Other pharmaceutically acceptable excipients can be colorants, flavoring agents, plasticizers, humectants, buffering agents, and the like.

Two or more compounds may be mixed together in a tablet, capsule, or other vehicle, or may be partitioned. In one example, the first compound is contained on the inside of the tablet, and the second compound is on the outside, such that a substantial portion of the second compound is released prior to the release of the first compound.

Formulations for oral use may also be provided as chewable tablets, or as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent (e.g., potato starch, lactose, microcrystalline cellulose, calcium carbonate, calcium phosphate or kaolin), or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example, peanut oil, liquid paraffin, or olive oil. Powders, granulates, and pellets may be prepared using the ingredients mentioned above under tablets and capsules in a conventional manner using, e.g., a mixer, a fluid bed apparatus or a spray drying equipment.

Dissolution or diffusion-controlled release can be achieved by appropriate coating of a tablet, capsule, pellet, or granulate formulation of compounds, or by incorporating the compound, or a pharmaceutically acceptable salt thereof, into an appropriate matrix. A controlled release coating may include one or more of the coating substances mentioned above or, e.g., shellac, beeswax, glycowax, castor wax, carnauba wax, stearyl alcohol, glyceryl monostearate, glyceryl distearate, glycerol palmitostearate, ethylcellulose, acrylic resins, dl-polylactic acid, cellulose acetate butyrate, polyvinyl chloride, polyvinyl acetate, vinyl pyrrolidone, polyethylene, polymethacrylate, methylmethacrylate, 2-optionally substituted hydroxylmethacrylate, methacrylate hydrogels, 1,3 butylene glycol, ethylene glycol methacrylate, or polyethylene glycols. In a controlled release matrix formulation, the matrix material may also include, e.g., hydrated methylcellulose, carnauba wax and stearyl alcohol, carbopol 934, silicone, glyceryl tristearate, methyl acrylate-methyl methacrylate, polyvinyl chloride, polyethylene, or halogenated fluorocarbon.

The liquid forms in which the compounds, or a pharmaceutically acceptable salt thereof, and compositions of the present invention can be incorporated for administration orally include aqueous solutions, suitably flavored syrups, aqueous or oil suspensions, and flavored emulsions with edible oils such as cottonseed oil, sesame oil, coconut oil, or peanut oil, as well as elixirs and similar pharmaceutical vehicles.

Generally, when administered to a human, the oral dosage of any of the compounds of the invention, or a pharmaceutically acceptable salt thereof, will depend on the nature of the compound, and can readily be determined by one skilled in the art. A dosage may be, for example, about 0.001 mg to about 2000 mg per day, about 1 mg to about 1000 mg per day, about 5 mg to about 500 mg per day, about 100 mg to about 1500 mg per day, about 500 mg to about 1500 mg per day, about 500 mg to about 2000 mg per day, or any range derivable therein.

In some embodiments, the pharmaceutical composition may further comprise an additional compound having antiproliferative activity. Depending on the mode of administration, compounds, or a pharmaceutically acceptable salt thereof, will be formulated into suitable compositions to permit facile delivery. Each compound, or a pharmaceutically acceptable salt thereof, of a combination therapy may be formulated in a variety of ways that are known in the art. For example, the first and second agents of the combination therapy may be formulated together or separately. Desirably, the first and second agents are formulated together for the simultaneous or near simultaneous administration of the agents.

It will be appreciated that the compounds and pharmaceutical compositions of the present invention can be formulated and employed in combination therapies, that is, the compounds and pharmaceutical compositions can be formulated with or administered concurrently with, prior to, or subsequent to, one or more other desired therapeutics or medical procedures. The particular combination of therapies (therapeutics or procedures) to employ in a combination regimen will take into account compatibility of the desired therapeutics or procedures and the desired therapeutic effect to be achieved. It will also be appreciated that the therapies

employed may achieve a desired effect for the same disorder, or they may achieve different effects (e.g., control of any adverse effects).

Administration of each drug in a combination therapy, as described herein, can, independently, be one to four times daily for one day to one year, and may even be for the life of the subject. Chronic, long-term administration may be indicated.

Methods of Use

In some embodiments, the invention discloses a method of treating a disease or disorder that is characterized by aberrant Ras activity due to a Ras mutant. In some embodiments, the disease or disorder is a cancer.

Accordingly, also provided is a method of treating cancer in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a compound of the present invention, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition comprising such a compound or salt. In some embodiments, the cancer is colorectal cancer, non-small cell lung cancer, small-cell lung cancer, pancreatic cancer, appendiceal cancer, melanoma, acute myeloid leukemia, small bowel cancer, ampullary cancer, germ cell cancer, cervical cancer, cancer of unknown primary origin, endometrial cancer, esophagogastric cancer, GI neuroendocrine cancer, ovarian cancer, sex cord stromal tumor cancer, hepatobiliary cancer, or bladder cancer. In some embodiments, the cancer is appendiceal, endometrial or melanoma. Also provided is a method of treating a Ras protein-related disorder in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a compound of the present invention, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition comprising such a compound or salt.

In some embodiments, the compounds of the present invention or pharmaceutically acceptable salts thereof, pharmaceutical compositions comprising such compounds or salts, and methods provided herein may be used for the treatment of a wide variety of cancers including tumors such as lung, prostate, breast, brain, skin, cervical carcinomas, testicular carcinomas, etc. More particularly, cancers that may be treated by the compounds or salts thereof, pharmaceutical compositions comprising such compounds or salts, and methods of the invention include, but are not limited to tumor types such as astrocytic, breast, cervical, colorectal, endometrial, esophageal, gastric, head and neck, hepatocellular, laryngeal, lung, oral, ovarian, prostate and thyroid carcinomas and sarcomas. Other cancers include, for example:

Cardiac, for example: sarcoma (angiosarcoma, fibrosarcoma, rhabdomyosarcoma, liposarcoma), myxoma, rhabdomyoma, fibroma, lipoma, and teratoma;

Lung, for example: bronchogenic carcinoma (squamous cell, undifferentiated small cell, undifferentiated large cell, adenocarcinoma), alveolar (bronchiolar) carcinoma, bronchial adenoma, sarcoma, lymphoma, chondromatous hamartoma, mesothelioma;

Gastrointestinal, for example: esophagus (squamous cell carcinoma, adenocarcinoma, leiomyosarcoma, lymphoma), stomach (carcinoma, lymphoma, leiomyosarcoma), pancreas (ductal adenocarcinoma, insulinoma, glucagonoma, gastrinoma, carcinoid tumors, vipoma), small bowel (adenocarcinoma, lymphoma, carcinoid tumors, Kaposi's sarcoma, leiomyoma, hemangioma, lipoma, neurofibroma, fibroma), large bowel (adenocarcinoma, tubular adenoma, villous adenoma, hamartoma, leiomyoma);

Genitourinary tract, for example: kidney (adenocarcinoma, Wilm's tumor (nephroblastoma), lymphoma, leukemia), bladder and urethra (squamous cell carcinoma, transitional cell carcinoma, adenocarcinoma), prostate (adenocarcinoma, sarcoma), testis (seminoma, teratoma, embryonal carcinoma, teratocarcinoma, choriocarcinoma, sarcoma, interstitial cell carcinoma, fibroma, fibroadenoma, adenomatoid tumors, lipoma);

Liver, for example: hepatoma (hepatocellular carcinoma), cholangiocarcinoma, hepatoblastoma, angiosarcoma, hepatocellular adenoma, hemangioma;

Biliary tract, for example: gall bladder carcinoma, ampullary carcinoma, cholangiocarcinoma;

Bone, for example: osteogenic sarcoma (osteosarcoma), fibrosarcoma, malignant fibrous histiocytoma, chondrosarcoma, Ewing's sarcoma, malignant lymphoma (reticulum cell sarcoma), multiple myeloma, malignant giant cell tumor chordoma, osteochronfroma (osteocartilaginous exostoses), benign chondroma, chondroblastoma, chondromyxofibroma, osteoid osteoma, and giant cell tumors;

Nervous system, for example: skull (osteoma, hemangioma, granuloma, xanthoma, osteitis deformans), meninges (meningioma, meningiosarcoma, gliomatosis), brain (astrocytoma, medulloblastoma, glioma, ependymoma, germinoma (pinealoma), glioblastoma multiform, oligodendrogliorna, schwannoma, retinoblastoma, congenital tumors), spinal cord neurofibroma, neurofibromatosis type 1, meningioma, glioma, sarcoma);

Gynecological, for example: uterus (endometrial carcinoma, uterine carcinoma, uterine corpus endometrial carcinoma), cervix (cervical carcinoma, pre-tumor cervical dysplasia), ovaries (ovarian carcinoma (serous cystadenocarcinoma, mucinous cystadenocarcinoma, unclassified carcinoma), granulosa-thecal cell tumors, Sertoli-Leydig cell tumors, dysgerminoma, malignant teratoma), vulva (squamous cell carcinoma, intraepithelial carcinoma, adenocarcinoma, fibrosarcoma, melanoma), vagina (clear cell carcinoma, squamous cell carcinoma, botryoid sarcoma (embryonal rhabdomyosarcoma), fallopian tubes (carcinoma);

Hematologic, for example: blood (myeloid leukemia (acute and chronic), acute lymphoblastic leukemia, chronic lymphocytic leukemia, myeloproliferative diseases, multiple myeloma, myelodysplastic syndrome), Hodgkin's disease, non-Hodgkin's lymphoma (malignant lymphoma);

Skin, for example: malignant melanoma, basal cell carcinoma, squamous cell carcinoma, Kaposi's sarcoma, moles dysplastic nevi, lipoma, angioma, dermatofibroma, keloids, psoriasis; and

Adrenal glands, for example: neuroblastoma.

In some embodiments, the Ras protein is wild-type (Ras^{WT}). Accordingly, in some embodiments, a compound of the present invention is employed in a method of treating a patient having a cancer comprising a Ras^{WT} (e.g., K-Ras^{WT}, H-Ras^{WT} or N-Ras^{WT}). In some embodiments, the Ras protein is Ras amplification (e.g., K-Ras^{amp}). Accordingly, in some embodiments, a compound of the present invention is employed in a method of treating a patient having a cancer comprising a Ras^{amp} (K-Ras^{amp}, H-Ras^{amp} or N-Ras^{amp}). In some embodiments, the cancer comprises a Ras mutation, such as a Ras mutation described herein. In some embodiments, a mutation is selected from:

(a) the following K-Ras mutants: G12D, G12V, G12C, G13D, G12R, G12A, Q61H, G12S, A146T, G13C,

Q61L, Q61R, K117N, A146V, G12F, Q61K, L19F, Q22K, V14I, A59T, A146P, G13R, G12L, or G13V, and combinations thereof;

(b) the following H-Ras mutants: Q61R, G13R, Q61K, G12S, Q61L, G12D, G13V, G13D, G12C, K117N, A59T, G12V, G13C, Q61H, G13S, A18V, D119N, G13N, A146T, A66T, G12A, A146V, G12N, or G12R, and combinations thereof; and

(c) the following N-Ras mutants: Q61R, Q61K, G12D, Q61L, Q61H, G13R, G13D, G12S, G12C, G12V, G12A, G13V, G12R, P185S, G13C, A146T, G60E, Q61P, A59D, E132K, E49K, T50I, A146V, or A59T, and combinations thereof;

or a combination of any of the foregoing. In some embodiments, the cancer comprises a K-Ras mutation selected from the group consisting of G12C, G12D, G13C, G12V, G13D, G12R, G12S, Q61H, Q61K and Q61 L. In some embodiments, the cancer comprises an N-Ras mutation selected from the group consisting of G12C, Q61H, Q61K, Q61L, Q61P and Q61R. In some embodiments, the cancer comprises an H-Ras mutation selected from the group consisting of Q61H and Q61 L. In some embodiments, the cancer comprises a Ras mutation selected from the group consisting of G12C, G13C, G12A, G12D, G13D, G12S, G13S, G12V and G13V. In some embodiments, the cancer comprises at least two Ras mutations selected from the group consisting of G12C, G13C, G12A, G12D, G13D, G12S, G13S, G12V and G13V. In some embodiments, a compound of the present invention inhibits more than one Ras mutant. For example, a compound may inhibit both K-Ras G12C and K-Ras G13C. A compound may inhibit both N-Ras G12C and K-Ras G12C. In some embodiments, a compound may inhibit both K-Ras G12C and K-Ras G12D. In some embodiments, a compound may inhibit both K-Ras G12V and K-Ras G12C. In some embodiments, a compound may inhibit both K-Ras G12V and K-Ras G12S. In some embodiments, a compound of the present invention inhibits Ras^{WT} in addition to one or more additional Ras mutations (e.g., K-, H- or N-Ras^{WT} and K-Ras G12D, G12V, G12C, G13D, G12R, G12A, Q61H, G12S, A146T, G13C, Q61L, Q61R, K117N, A146V, G12F, Q61K, L19F, Q22K, V14I, A59T, A146P, G13R, G12L, or G13V; K, H or N-Ras^{WT} and H-Ras Q61R, G13R, Q61K, G12S, Q61L, G12D, G13V, G13D, G12C, K117N, A59T, G12V, G13C, Q61H, G13S, A18V, D119N, G13N, A146T, A66T, G12A, A146V, G12N, or G12R; or K, H or N-Ras^{WT} and N-Ras Q61R, Q61K, G12D, Q61L, Q61H, G13R, G13D, G12S, G12C, G12V, G12A, G13V, G12R, P185S, G13C, A146T, G60E, Q61P, A59D, E132K, E49K, T50I, A146V, or A59T). In some embodiments, a compound of the present invention inhibits Ras^{amp} in addition to one or more additional Ras mutations (e.g., K-, H- or N-Ras^{amp} and K-Ras G12D, G12V, G12C, G13D, G12R, G12A, Q61H, G12S, A146T, G13C, Q61L, Q61R, K117N, A146V, G12F, Q61K, L19F, Q22K, V14I, A59T, A146P, G13R, G12L, or G13V; K, H or N-Ras^{amp} and H-Ras Q61R, G13R, Q61K, G12S, Q61L, G12D, G13V, G13D, G12C, K117N, A59T, G12V, G13C, Q61H, G13S, A18V, D119N, G13N, A146T, A66T, G12A, A146V, G12N, or G12R; or K, H or N-Ras^{amp} and N-Ras Q61R, Q61K, G12D, Q61L, Q61H, G13R, G13D, G12S, G12C, G12V, G12A, G13V, G12R, P185S, G13C, A146T, G60E, Q61P, A59D, E132K, E49K, T50I, A146V, or A59T).

Methods of detecting Ras mutations are known in the art. Such means include, but are not limited to direct sequencing, and utilization of a high-sensitivity diagnostic assay (with CE-IVD mark), e.g., as described in Domagala, et al., Pol J Pathol 3: 145-164 (2012), incorporated herein by reference in its entirety, including TheraScreen PCR; AmoyDx; PNA-Clamp; RealQuality; EntroGen; LightMix; StripAssay; Hybcell plexA; Devyser; Surveyor; Cobas; and TheraScreen Pyro. See, also, e.g., WO 2020/106640.

In some embodiments, the cancer is non-small cell lung cancer and the Ras mutation comprises a K-Ras mutation, such as K-Ras G12C, K-Ras G12V or K-Ras G12D. In some embodiments, the cancer is colorectal cancer and the Ras mutation comprises a K-Ras mutation, such as K-Ras G12C, K-Ras G12V or K-Ras G12D. In some embodiments, the cancer is pancreatic cancer and the Ras mutation comprises an K-Ras mutation, such as K-Ras G12D or K-Ras G12V. In some embodiments, the cancer is pancreatic cancer and the Ras mutation comprises an N-Ras mutation, such as N-Ras G12D. In some embodiments, the cancer is melanoma and the Ras mutation comprises an N-Ras mutation, such as N-Ras Q61R or N-Ras Q61K. In some embodiments, the cancer is non-small cell lung cancer and the Ras protein is K-Ras^{am}P. In any of the foregoing if not already specified, a compound may inhibit Ras^{WT} (e.g., K-, H- or N-Ras^{WT}) or Ras^{amp} (e.g., K-, H- or N-Ras^{amp}) as well.

In some embodiments, a cancer comprises a Ras mutation and an $STK11^{LOF}$, a KEAP1, an EPHA5 or an NF1 mutation. In some embodiments, the cancer is non-small cell lung cancer and comprises a K-Ras G12C mutation. In some embodiments, the cancer is non-small cell lung cancer and comprises a K-Ras G12C mutation and an $STK11^{LOF}$ mutation. In some embodiments, the cancer is non-small cell lung cancer and comprises a K-Ras G12C mutation and an $STK11^{LOF}$ mutation. In some embodiments, a cancer comprises a K-Ras G13C Ras mutation and an $STK11^{LOF}$, a KEAP1, an EPHA5 or an NF1 mutation. In some embodiments, the cancer is non-small cell lung cancer and comprises a K-Ras G12D mutation. In some embodiments, the cancer is non-small cell lung cancer and comprises a K-Ras G12V mutation. In some embodiments, the cancer is colorectal cancer and comprises a K-Ras G12C mutation. In some embodiments, the cancer is pancreatic cancer and comprises a K-Ras G12C or K-Ras G12D mutation. In some embodiments, the cancer is pancreatic cancer and comprises a K-Ras G12V mutation. In some embodiments, the cancer is endometrial cancer, ovarian cancer, cholangiocarcinoma, or mucinous appendiceal cancer and comprises a K-Ras G12C mutation. In some embodiments, the cancer is gastric cancer and comprises a K-Ras G12C mutation. In any of the foregoing, a compound may inhibit Ras^{WT} (e.g., K-, H- or N-Ras^{WT}) or Ras^{amp} (e.g., K-, H- or N-Ras^{amp}) as well.

Also provided is a method of inhibiting a Ras protein in a cell, the method comprising contacting the cell with an effective amount of a compound of the present invention, or a pharmaceutically acceptable salt thereof. A method of inhibiting RAF-Ras binding, the method comprising contacting the cell with an effective amount of a compound of the present invention, or a pharmaceutically acceptable salt thereof, is also provided. The cell may be a cancer cell. The cancer cell may be of any type of cancer described herein. The cell may be in vivo or in vitro.

Combination Therapy

The methods of the invention may include a compound of the invention used alone or in combination with one or more additional therapies (e.g., non-drug treatments or therapeutic agents). The dosages of one or more of the additional therapies (e.g., non-drug treatments or therapeutic agents) may be reduced from standard dosages when administered alone. For example, doses may be determined empirically

from drug combinations and permutations or may be deduced by isobolographic analysis (e.g., Black et al., *Neurology* 65:S3-S6 (2005)).

A compound of the present invention may be administered before, after, or concurrently with one or more of such additional therapies. When combined, dosages of a compound of the invention and dosages of the one or more additional therapies (e.g., non-drug treatment or therapeutic agent) provide a therapeutic effect (e.g., synergistic or additive therapeutic effect). A compound of the present invention and an additional therapy, such as an anti-cancer agent, may be administered together, such as in a unitary pharmaceutical composition, or separately and, when administered separately, this may occur simultaneously or sequentially. Such sequential administration may be close or remote in time.

In some embodiments, the additional therapy is the administration of side-effect limiting agents (e.g., agents intended to lessen the occurrence or severity of side effects of treatment. For example, in some embodiments, the compounds of the present invention can also be used in combination with a therapeutic agent that treats nausea. Examples of agents that can be used to treat nausea include: dronabinol, granisetron, metoclopramide, ondansetron, and prochlorperazine, or pharmaceutically acceptable salts thereof.

In some embodiments, the one or more additional therapies includes a non-drug treatment (e.g., surgery or radiation therapy). In some embodiments, the one or more additional therapies includes a therapeutic agent (e.g., a compound or biologic that is an anti-angiogenic agent, signal transduction inhibitor, antiproliferative agent, glycolysis inhibitor, or autophagy inhibitor). In some embodiments, the one or more additional therapies includes a non-drug treatment (e.g., surgery or radiation therapy) and a therapeutic agent (e.g., a compound or biologic that is an anti-angiogenic agent, signal transduction inhibitor, antiproliferative agent, glycolysis inhibitor, or autophagy inhibitor). In other embodiments, the one or more additional therapies includes two therapeutic agents. In still other embodiments, the one or more additional therapies includes three therapeutic agents. In some embodiments, the one or more additional therapies includes four or more therapeutic agents.

In this Combination Therapy section, all references are incorporated by reference for the agents described, whether explicitly stated as such or not.

Non-Drug Therapies

Examples of non-drug treatments include, but are not limited to, radiation therapy, cryotherapy, hyperthermia, surgery (e.g., surgical excision of tumor tissue), and T cell adoptive transfer (ACT) therapy.

In some embodiments, the compounds of the invention may be used as an adjuvant therapy after surgery. In some embodiments, the compounds of the invention may be used as a neo-adjuvant therapy prior to surgery.

Radiation therapy may be used for inhibiting abnormal cell growth or treating a hyperproliferative disorder, such as cancer, in a subject (e.g., mammal (e.g., human)). Techniques for administering radiation therapy are known in the art Radiation therapy can be administered through one of several methods, or a combination of methods, including, without limitation, external-beam therapy, internal radiation therapy, implant radiation, stereotactic radiosurgery, systemic radiation therapy, radiotherapy, and permanent or temporary interstitial brachy therapy. The term "brachy therapy," as used herein, refers to radiation therapy delivered by a spatially confined radioactive material inserted into the body at or near a tumor or other proliferative tissue disease site. The term is intended, without limitation, to include exposure to radioactive isotopes (e.g., At-211, I-131, I-125, Y-90, Re-186, Re-188, Sm-153, Bi-212, P-32, and radioactive isotopes of Lu). Suitable radiation sources for use as a cell conditioner of the present invention include both solids and liquids. By way of non-limiting example, the radiation source can be a radionuclide, such as I-125, I-131, Yb-169, Ir-192 as a solid source, 1-125 as a solid source, or other radionuclides that emit photons, beta particles, gamma radiation, or other therapeutic rays. The radioactive material can also be a fluid made from any solution of radionuclide(s), e.g., a solution of 1-125 or 1-131, or a radioactive fluid can be produced using a slurry of a suitable fluid containing small particles of solid radionuclides, such as Au-198, or Y-90. Moreover, the radionuclide(s) can be embodied in a gel or radioactive micro spheres.

In some embodiments, the compounds of the present invention can render abnormal cells more sensitive to treatment with radiation for purposes of killing or inhibiting the growth of such cells. Accordingly, this invention further relates to a method for sensitizing abnormal cells in a mammal to treatment with radiation which comprises administering to the mammal an amount of a compound of the present invention, which amount is effective to sensitize abnormal cells to treatment with radiation. The amount of the compound in this method can be determined according to the means for ascertaining effective amounts of such compounds described herein. In some embodiments, the compounds of the present invention may be used as an adjuvant therapy after radiation therapy or as a neo-adjuvant therapy prior to radiation therapy.

In some embodiments, the non-drug treatment is a T cell adoptive transfer (ACT) therapy. In some embodiments, the T cell is an activated T cell. The T cell may be modified to express a chimeric antigen receptor (CAR). CAR modified T (CAR-T) cells can be generated by any method known in the art. For example, the CAR-T cells can be generated by introducing a suitable expression vector encoding the CAR to a T cell. Prior to expansion and genetic modification of the T cells, a source of T cells is obtained from a subject. T cells can be obtained from a number of sources, including peripheral blood mononuclear cells, bone marrow, lymph node tissue, cord blood, thymus tissue, tissue from a site of infection, ascites, pleural effusion, spleen tissue, and tumors. In certain embodiments of the present invention, any number of T cell lines available in the art may be used. In some embodiments, the T cell is an autologous T cell. Whether prior to or after genetic modification of the T cells to express a desirable protein (e.g., a CAR), the T cells can be activated and expanded generally using methods as described, for example, in U.S. Pat. Nos. 6,352,694; 6,534,055; 6,905,680; 6,692,964; 5,858,358; 6,887,466; 6,905,681; 7,144,575; 7,067,318; 7,172,869; 7,232,566; 7,175,843; 7,572,631; 5,883,223; 6,905,874; 6,797,514; and 6,867,041.

Therapeutic Agents

A therapeutic agent may be a compound used in the treatment of cancer or symptoms associated therewith.

For example, a therapeutic agent may be a steroid. Accordingly, in some embodiments, the one or more additional therapies includes a steroid. Suitable steroids may include, but are not limited to, 21-acetoxypregnenolone, alclometasone, algestone, amcinonide, beclomethasone, betamethasone, budesonide, chloroprednisone, clobetasol, clocortolone, cloprednol, corticosterone, cortisone, cortivazol, deflazacort, desonide, desoximetasone, dexamethasone, diflorasone, diflucortolone, difuprednate, enoxolone, fluazacort, fiucloronide, flumethasone, flunisolide, fluocinolone

acetonide, fluocinonide, fluocortin butyl, fluocortolone, fluorometholone, fluperolone acetate, fluprednidene acetate, fluprednisolone, flurandrenolide, fluticasone propionate, formocortal, halcinonide, halobetasol propionate, halometasone, hydrocortisone, loteprednol etabonate, mazipredone, medrysone, meprednisone, methylprednisolone, mometasone furoate, paramethasone, prednicarbate, prednisolone, prednisolone 25-diethylaminoacetate, prednisolone sodium phosphate, prednisone, prednival, prednylidene, rimexolone, tixocortol, triamcinolone, triamcinolone acetonide, triamcinolone benetonide, triamcinolone hexacetonide, and salts or derivatives thereof.

Further examples of therapeutic agents that may be used in combination therapy with a compound of the present invention include compounds described in the following patents: U.S. Pat. Nos. 6,258,812, 6,630,500, 6,515,004, 6,713,485, 5,521,184, 5,770,599, 5,747,498, 5,990,141, 6,235,764, and 8,623,885, and International Patent Applications WO01/37820, WO01/32651, WO02/68406, WO02/66470, WO02/55501, WO04/05279, WO04/07481, WO04/07458, WO04/09784, WO02/59110, WO99/45009, WO00/59509, WO99/61422, WO00/12089, and WO00/02871.

A therapeutic agent may be a biologic (e.g., cytokine (e.g., interferon or an interleukin such as IL-2)) used in treatment of cancer or symptoms associated therewith. In some embodiments, the biologic is an immunoglobulin-based biologic, e.g., a monoclonal antibody (e.g., a humanized antibody, a fully human antibody, an Fc fusion protein, or a functional fragment thereof) that agonizes a target to stimulate an anti-cancer response or antagonizes an antigen important for cancer. Also included are antibody-drug conjugates.

A therapeutic agent may be a T-cell checkpoint inhibitor. In one embodiment, the checkpoint inhibitor is an inhibitory antibody (e.g., a monospecific antibody such as a monoclonal antibody). The antibody may be, e.g., humanized or fully human. In some embodiments, the checkpoint inhibitor is a fusion protein, e.g., an Fc-receptor fusion protein. In some embodiments, the checkpoint inhibitor is an agent, such as an antibody, that interacts with a checkpoint protein. In some embodiments, the checkpoint inhibitor is an agent, such as an antibody, that interacts with the ligand of a checkpoint protein. In some embodiments, the checkpoint inhibitor is an inhibitor (e.g., an inhibitory antibody or small molecule inhibitor) of CTLA-4 (e.g., an anti-CTLA-4 antibody or fusion a protein). In some embodiments, the checkpoint inhibitor is an inhibitor or antagonist (e.g., an inhibitory antibody or small molecule inhibitor) of PD-1. In some embodiments, the checkpoint inhibitor is an inhibitor or antagonist (e.g., an inhibitory antibody or small molecule inhibitor) of PDL-1. In some embodiments, the checkpoint inhibitor is an inhibitor or antagonist (e.g., an inhibitory antibody or Fc fusion or small molecule inhibitor) of PDL-2 (e.g., a PDL-2/lg fusion protein). In some embodiments, the checkpoint inhibitor is an inhibitor or antagonist (e.g., an inhibitory antibody or small molecule inhibitor) of B7-H3, B7-H4, BTLA, HVEM, TIM3, GAL9, LAG3, VISTA, KIR, 2B4, CD160, CGEN-15049, CHK 1, CHK2, A2aR, B-7 family ligands, or a combination thereof. In some embodiments, the checkpoint inhibitor is pembrolizumab, nivolumab, PDR001 (NVS), $REGN_{2810}$ (Sanofi/Regeneron), a PD-L1 antibody such as, e.g., avelumab, durvalumab, atezolizumab, pidilizumab, JNJ-63723283 (JNJ), BGB-A317 (BeiGene & Celgene) or a checkpoint inhibitor disclosed in Preusser, M. et al. (2015) Nat. Rev. Neurol., including, without limitation, ipilimumab, tremelimumab, nivolumab, pembrolizumab, AMP224, AMP514/MEDI0680, BMS936559, MEDI4736, MPDL3280A, MSB0010718C, BMS986016, IMP321, lirilumab, IPH2101, 1-7F9, and KW-6002.

A therapeutic agent may be an anti-TIGIT antibody, such as MBSA43, BMS-986207, MK-7684, COM902, AB154, MTIG7192A or OMP-313M32 (etigilimab).

A therapeutic agent may be an agent that treats cancer or symptoms associated therewith (e.g., a cytotoxic agent, non-peptide small molecules, or other compound useful in the treatment of cancer or symptoms associated therewith, collectively, an "anti-cancer agent"). Anti-cancer agents can be, e.g., chemotherapeutics or targeted therapy agents.

Anti-cancer agents include mitotic inhibitors, intercalating antibiotics, growth factor inhibitors, cell cycle inhibitors, enzymes, topoisomerase inhibitors, biological response modifiers, alkylating agents, antimetabolites, folic acid analogs, pyrimidine analogs, purine analogs and related inhibitors, *vinca* alkaloids, epipodopyyllotoxins, antibiotics, L-Asparaginase, topoisomerase inhibitors, interferons, platinum coordination complexes, anthracenedione substituted urea, methyl hydrazine derivatives, adrenocortical suppressant, adrenocorticosteroides, progestins, estrogens, antiestrogen, androgens, antiandrogen, and gonadotropin-releasing hormone analog. Further anti-cancer agents include leucovorin (LV), irenotecan, oxaliplatin, capecitabine, paclitaxel, and doxetaxel. In some embodiments, the one or more additional therapies includes two or more anti-cancer agents. The two or more anti-cancer agents can be used in a cocktail to be administered in combination or administered separately. Suitable dosing regimens of combination anti-cancer agents are known in the art and described in, for example, Saltz et al., *Proc. Am. Soc. Clin. Oncol.* 18:233a (1999), and Douillard et al., Lancet 355(9209):1041-1047 (2000).

Other non-limiting examples of anti-cancer agents include Gleevec® (Imatinib Mesylate); Kyprolis® (carfilzomib); Velcade® (bortezomib); Casodex (bicalutamide); Iressa® (gefitinib); alkylating agents such as thiotepa and cyclosphosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethiylenethiophosphoramide and trimethylolomelamine; acetogenins (especially bullatacin and bullatacinone); a camptothecin (including the synthetic analogue topotecan); bryostatin; callystatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); cryptophycins (particularly cryptophycin 1 and cryptophycin 8); dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1); eleutherobin; pancratistatin; sarcodictyin A; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimustine; antibiotics such as the enediyne antibiotics (e.g., calicheamicin, such as calicheamicin gammall and calicheamicin omegall (see, e.g., Agnew, *Chem. Intl. Ed Engl.* 33:183-186 (1994)); dynemicin such as dynemicin A; bisphosphonates such as clodronate; an esperamicin; neocarzinostatin chromophore and related chromoprotein enediyne antiobiotic chromophores, aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, calicheamicin, carabicin, caminomycin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, adriamycin (doxo-

rubicin), morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin, deoxydoxorubicin, epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, zorubicin; anti-metabolites such as methotrexate and 5-fluorouracil (5-FU); folic acid analogues such as denopterin, pteropterin, trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, floxuridine; androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiostane, testolactone; anti-adrenals such as aminoglutethimide, mitotane, trilostane; folic acid replenishers such as frolinic acid; aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; bestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfomithine; elliptinium acetate; an epothilone such as epothilone B; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidamine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; losoxantrone; podophyllinic acid; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, OR); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes such as T-2 toxin, verracurin A, roridin A and anguidine; urethane; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiotepa; taxoids, e.g., Taxol® (paclitaxel), Abraxane® (cremophor-free, albumin-engineered nanoparticle formulation of paclitaxel), and Taxotere® (doxetaxel); chloranbucil; tamoxifen (Nolvadex™); raloxifene; aromatase inhibiting 4(5)-imidazoles; 4-hydroxytamoxifen; trioxifene; keoxifene; LY 117018; onapristone; toremifene (Fareston®); flutamide, nilutamide, bicalutamide, leuprolide, goserelin; chlorambucil; Gemzar® gemcitabine; 6-thioguanine; mercaptopurine; platinum coordination complexes such as cisplatin, oxaliplatin and carboplatin; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine; Navelbine® (vinorelbine); novantrone; teniposide; edatrexate; daunomycin; aminopterin; ibandronate; irinotecan (e.g., CPT-11); topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; esperamicins; capecitabine (e.g., Xeloda®); and pharmaceutically acceptable salts of any of the above.

Additional non-limiting examples of anti-cancer agents include trastuzumab (Herceptin®), bevacizumab (Avastin®), cetuximab (Erbitux®), rituximab (Rituxan®), Taxol®, Arimidex®, ABVD, avicine, abagovomab, acridine carboxamide, adecatumumab, 17-N-allylamino-17-demethoxygeldanamycin, alpharadin, alvocidib, 3-aminopyridine-2-carboxaldehyde thiosemicarbazone, amonafide, anthracenedione, anti-CD22 immunotoxins, antineoplastics (e.g., cell-cycle nonspecific antineoplastic agents, and other antineoplastics described herein), antitumorigenic herbs, apaziquone, atiprimod, azathioprine, belotecan, bendamustine, BIBW 2992, biricodar, brostallicin, bryostatin, buthionine sulfoximine, CBV (chemotherapy), calyculin, dichloroacetic acid, discodermolide, elsamitrucin, enocitabine, eribulin, exatecan, exisulind, ferruginol, forodesine, fosfestrol, ICE chemotherapy regimen, IT-101, imexon, imiquimod, indolocarbazole, irofulven, laniquidar, larotaxel, lenalidomide, lucanthone, lurtotecan, mafosfamide, mitozolomide, nafoxidine, nedaplatin, olaparib, ortataxel, PAC-1, pawpaw, pixantrone, proteasome inhibitors, rebeccamycin, resiquimod, rubitecan, SN-38, salinosporamide A, sapacitabine, Stanford V, swainsonine, talaporfin, tariquidar, tegafur-uracil, temodar, tesetaxel, triplatin tetranitrate, tris(2-chloroethyl)amine, troxacitabine, uramustine, vadimezan, vinflunine, ZD6126, and zosuquidar.

Further non-limiting examples of anti-cancer agents include natural products such as vinca alkaloids (e.g., vinblastine, vincristine, and vinorelbine), epidipodophyllotoxins (e.g., etoposide and teniposide), antibiotics (e.g., dactinomycin (actinomycin D), daunorubicin, and idarubicin), anthracyclines, mitoxantrone, bleomycins, plicamycin (mithramycin), mitomycin, enzymes (e.g., L-asparaginase which systemically metabolizes L-asparagine and deprives cells which do not have the capacity to synthesize their own asparagine), antiplatelet agents, antiproliferative/antimitotic alkylating agents such as nitrogen mustards (e.g., mechlorethamine, cyclophosphamide and analogs, melphalan, and chlorambucil), ethylenimines and methylmelamines (e.g., hexaamethylmelaamine and thiotepa), CDK inhibitors (e.g., a CDK4/6 inhibitor such as abemaciclib, ribociclib, palbociclib; seliciclib, UCN-01, P1446A-05, PD-0332991, dinaciclib, P27-00, AT-7519, RGB286638, and SCH727965), alkyl sulfonates (e.g., busulfan), nitrosoureas (e.g., carmustine (BCNU) and analogs, and streptozocin), trazenes-dacarbazinine (DTIC), antiproliferative/antimitotic antimetabolites such as folic acid analogs, pyrimidine analogs (e.g., fluorouracil, floxuridine, and cytarabine), purine analogs and related inhibitors (e.g., mercaptopurine, thioguanine, pentostatin, and 2-chlorodeoxyadenosine), aromatase inhibitors (e.g., anastrozole, exemestane, and letrozole), and platinum coordination complexes (e.g., cisplatin and carboplatin), procarbazine, hydroxyurea, mitotane, aminoglutethimide, histone deacetylase (HDAC) inhibitors (e.g., trichostatin, sodium butyrate, apicidan, suberoyl anilide hydroamic acid, vorinostat, LBH 589, romidepsin, ACY-1215, and panobinostat), mTOR inhibitors (e.g., vistusertib, temsirolimus, everolimus, ridaforolimus, and sirolimus), KSP(Eg5) inhibitors (e.g., Array 520), DNA binding agents (e.g., Zalypsis®), PI3K inhibitors such as PI3K delta inhibitor (e.g., GS-1101 and TGR-1202), PI3K delta and gamma inhibitor (e.g., CAL-130), copanlisib, alpelisib and idelalisib; multi-kinase inhibitor (e.g., TG02 and sorafenib), hormones (e.g., estrogen) and hormone agonists such as leutinizing hormone releasing hormone (LHRH) agonists (e.g., goserelin, leuprolide and triptorelin), BAFF-neutralizing antibody (e.g., LY2127399), IKK inhibitors, p38MAPK inhibitors, anti-IL-6 (e.g., CNT0328), telomerase inhibitors (e.g., GRN 163L), aurora kinase inhibitors (e.g., MLN8237), cell surface monoclonal antibodies (e.g., anti-CD38 (HUMAX-CD38), anti-CSI (e.g., elotuzumab), HSP90 inhibitors (e.g., 17 AAG and KOS 953), PI3K/Akt inhibitors (e.g., perifosine), Akt inhibitors (e.g., GSK-2141795), PKC inhibitors (e.g., enzastaurin), FTIs (e.g., Zarnestra™), anti-CD138 (e.g., BT062), Torcl/2 specific kinase inhibitors (e.g., INK128), ER/UPR targeting agents (e.g., MKC-3946), cFMS inhibitors (e.g., ARRY-382), JAK1/2 inhibitors (e.g., CYT387), PARP inhibitors (e.g., olaparib and veliparib (ABT-888)), and BCL-2 antagonists.

In some embodiments, an anti-cancer agent is selected from mechlorethamine, camptothecin, ifosfamide, tamoxifen, raloxifene, gemcitabine, Navelbine®, sorafenib, or any analog or derivative variant of the foregoing.

In some embodiments, the anti-cancer agent is a HER2 inhibitor. Non-limiting examples of HER2 inhibitors include monoclonal antibodies such as trastuzumab (Herceptin®)

and pertuzumab (Perjeta®); small molecule tyrosine kinase inhibitors such as gefitinib (Iressa®), erlotinib (Tarceva®), pilitinib, CP-654577, CP-724714, canertinib (CI 1033), HKI-272, lapatinib (GW-572016; Tykerb®), PKI-166, AEE788, BMS-599626, HKI-357, BIBW 2992, ARRY-334543, and JNJ-26483327.

In some embodiments, an anti-cancer agent is an ALK inhibitor. Non-limiting examples of ALK inhibitors include ceritinib, TAE-684 (NVP-TAE694), PF02341066 (crizotinib or 1066), alectinib; brigatinib; entrectinib; ensartinib (X-396); lorlatinib; ASP3026; CEP-37440; 4SC-203; TL-398; PLB1003; TSR-011; CT-707; TPX-0005, and AP26113. Additional examples of ALK kinase inhibitors are described in examples 3-39 of WO05016894.

In some embodiments, an anti-cancer agent is an inhibitor of a member downstream of a Receptor Tyrosine Kinase (RTK)/Growth Factor Receptor (e.g., a SHP2 inhibitor (e.g., SHP099, TNO155, RMC-4550, RMC-4630, JAB-3068, RLY-1971), a SOS1 inhibitor (e.g., BI-1701963, BI-3406), a Raf inhibitor, a MEK inhibitor, an ERK inhibitor, a PI3K inhibitor, a PTEN inhibitor, an AKT inhibitor, or an mTOR inhibitor (e.g., mTORC1 inhibitor or mTORC2 inhibitor). In some embodiments, the anti-cancer agent is JAB-3312. In some embodiments, an anti-cancer agent is an additional Ras inhibitor (e.g., AMG 510, MRTX1257, MRTX849, JNJ-74699157 (ARS-3248), LY3499446, ARS-853, or ARS-1620), or a Ras vaccine, or another therapeutic modality designed to directly or indirectly decrease the oncogenic activity of Ras. Other examples of Ras inhibitors that may be combined with a Ras inhibitor of the present invention are provided in the following, incorporated herein by reference in their entireties: WO 2020050890, WO 2020047192, WO 2020035031, WO 2020028706, WO 2019241157, WO 2019232419, WO 2019217691, WO 2019217307, WO 2019215203, WO 2019213526, WO 2019213516, WO 2019155399, WO 2019150305, WO 2019110751, WO 2019099524, WO 2019051291, WO 2018218070, WO 2018217651, WO 2018218071, WO 2018218069, WO 2018206539, WO 2018143315, WO 2018140600, WO 2018140599, WO 2018140598, WO 2018140514, WO 2018140513, WO 2018140512, WO 2018119183, WO 2018112420, WO 2018068017, WO 2018064510, WO 2017201161, WO 2017172979, WO 2017100546, WO 2017087528, WO 2017058807, WO 2017058805, WO 2017058728, WO 2017058902, WO 2017058792, WO 2017058768, WO 2017058915, WO 2017015562, WO 2016168540, WO 2016164675, WO 2016049568, WO 2016049524, WO 2015054572, WO 2014152588, WO 2014143659 and WO 2013155223.

In some embodiments, a therapeutic agent that may be combined with a compound of the present invention is an inhibitor of the MAP kinase (MAPK) pathway (or "MAPK inhibitor"). MAPK inhibitors include, but are not limited to, one or more MAPK inhibitor described in Cancers (Basel) 2015 September; 7(3): 1758-1784. For example, the MAPK inhibitor may be selected from one or more of trametinib, binimetinib, selumetinib, cobimetinib, LErafAON (NeoPharm), ISIS 5132; vemurafenib, pimasertib, TAK733, RO4987655 (CH4987655); CI-1040; PD-0325901; CH5126766; MAP855; AZD6244; refametinib (RDEA 119/BAY 86-9766); GDC-0973/XL581; AZD8330 (ARRY-424704/ARRY-704); R05126766 (Roche, described in PLoS One. 2014 Nov. 25; 9(11)); and GSK1120212 (or JTP-74057, described in Clin Cancer Res. 2011 Mar. 1; 17(5): 989-1000). The MAPK inhibitor may be PLX8394, LXH254, GDC-5573, or LY3009120.

In some embodiments, an anti-cancer agent is a disrupter or inhibitor of the RAS-RAF-ERK or PI3K-AKT-TOR or PI3K-AKT signaling pathways. The PI3K/AKT inhibitor may include, but is not limited to, one or more PI3K/AKT inhibitor described in Cancers (Basel) 2015 September; 7(3): 1758-1784. For example, the PI3K/AKT inhibitor may be selected from one or more of NVP-BEZ235; BGT226; XL765/SAR245409; SF1126; GDC-0980; PI-103; PF-04691502; PKI-587; GSK2126458.

In some embodiments, an anti-cancer agent is a PD-1 or PD-L1 antagonist.

In some embodiments, additional therapeutic agents include ALK inhibitors, HER2 inhibitors, EGFR inhibitors, IGF-1R inhibitors, MEK inhibitors, PI3K inhibitors, AKT inhibitors, TOR inhibitors, MCL-1 inhibitors, BCL-2 inhibitors, SHP2 inhibitors, proteasome inhibitors, and immune therapies. In some embodiments, a therapeutic agent may be a pan-RTK inhibitor, such as afatinib.

IGF-1R inhibitors include linsitinib, or a pharmaceutically acceptable salt thereof.

EGFR inhibitors include, but are not limited to, small molecule antagonists, antibody inhibitors, or specific antisense nucleotide or siRNA. Useful antibody inhibitors of EGFR include cetuximab (Erbitux®), panitumumab (Vectibix®), zalutumumab, nimotuzumab, and matuzumab. Further antibody-based EGFR inhibitors include any anti-EGFR antibody or antibody fragment that can partially or completely block EGFR activation by its natural ligand. Non-limiting examples of antibody-based EGFR inhibitors include those described in Modjtahedi et al., Br. J. Cancer 1993, 67:247-253; Teramoto et al., Cancer 1996, 77:639-645; Goldstein et al., Clin. Cancer Res. 1995, 1:1311-1318; Huang et al., 1999, Cancer Res. 15:59(8):1935-40; and Yang et al., Cancer Res. 1999, 59:1236-1243. The EGFR inhibitor can be monoclonal antibody Mab E7.6.3 (Yang, 1999 supra), or Mab C225 (ATCC Accession No. HB-8508), or an antibody or antibody fragment having the binding specificity thereof.

Small molecule antagonists of EGFR include gefitinib (Iressa®), erlotinib (Tarceva®), and lapatinib (TykerB®). See, e.g., Yan et al., Pharmacogenetics and Pharmacogenomics In Oncology Therapeutic Antibody Development, BioTechniques 2005, 39(4):565-8; and Paez et al., EGFR Mutations In Lung Cancer Correlation With Clinical Response To Gefitinib Therapy, Science 2004, 304(5676): 1497-500. In some embodiments, the EGFR inhibitor is osimertinib (Tagrisso®). Further non-limiting examples of small molecule EGFR inhibitors include any of the EGFR inhibitors described in the following patent publications, and all pharmaceutically acceptable salts of such EGFR inhibitors: EP 0520722; EP 0566226; WO96/33980; U.S. Pat. No. 5,747,498; WO96/30347; EP 0787772; WO97/30034; WO97/30044; WO97/38994; WO97/49688; EP 837063; WO98/02434; WO97/38983; WO95/19774; WO95/19970; WO97/13771; WO98/02437; WO98/02438; WO97/32881; DE 19629652; WO98/33798; WO97/32880; WO97/32880; EP 682027; WO97/02266; WO97/27199; WO98/07726; WO97/34895; WO96/31510; WO98/14449; WO98/14450; WO98/14451; WO95/09847; WO97/19065; WO98/17662; U.S. Pat. Nos. 5,789,427; 5,650,415; 5,656,643; WO99/35146; WO99/35132; WO99/07701; and WO92/20642. Additional non-limiting examples of small molecule EGFR inhibitors include any of the EGFR inhibitors described in Traxler et al., Exp. Opin. Ther. Patents 1998, 8(12):1599-1625. In some embodiments, an EGFR inhibitor is an ERBB

inhibitor. In humans, the ERBB family contains HER1 (EGFR, ERBB1), HER2 (NEU, ERBB2), HER3 (ERBB3), and HER (ERBB4).

MEK inhibitors include, but are not limited to, pimasertib, selumetinib, cobimetinib (Cotellic®), trametinib (Mekinist®), and binimetinib (Mektovi®). In some embodiments, a MEK inhibitor targets a MEK mutation that is a Class I MEK1 mutation selected from D67N; P124L; P124S; and L177V. In some embodiments, the MEK mutation is a Class II MEK1 mutation selected from ΔE51-Q58; ΔF53-Q58; E203K; L177M; C121S; F53L; K57E; Q56P; and K57N.

PI3K inhibitors include, but are not limited to, wortmannin; 17-hydroxywortmannin analogs described in WO06/044453; 4-[2-(1H-Indazol-4-yl)-6-[[4-(methylsulfonyl)piperazin-1-yl]methyl]thieno[3,2-d]pyrimidin-4-yl]morpholine (also known as pictilisib or GDC-0941 and described in WO09/036082 and WO09/055730); 2-methyl-2-[4-[3-methyl-2-oxo-8-(quinolin-3-yl)-2,3-dihydroimidazo[4,5-c]quinolin-1-yl]phenyl]propionitrile (also known as BEZ 235 or NVP-BEZ 235, and described in WO06/122806); (S)—I-(4-((2-(2-aminopyrimidin-5-yl)-7-methyl-4-morpholinothieno[3,2-d]pyrimidin-6-yl)methyl)piperazin-1-yl)-2-hydroxypropan-1-one (described in WO08/070740); LY294002 (2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (available from Axon Medchem); PI 103 hydrochloride (3-[4-(4-morpholinylpyrido-[3',2':4,5]furo[3,2-d]pyrimidin-2-yl]phenol hydrochloride (available from Axon Medchem); PIK 75 (2-methyl-5-nitro-2-[(6-bromoimidazo[1,2-a]pyridin-3-yl)methylene]-1-methylhydrazide-benzenesulfonic acid, monohydrochloride) (available from Axon Medchem); PIK 90 (N-(7,8-dimethoxy-2,3-dihydro-imidazo[1,2-c]quinazolin-5-yl)-nicotinamide (available from Axon Medchem); AS-252424 (5-[1-[5-(4-fluoro-2-hydroxy-phenyl)-furan-2-yl]-meth-(Z)-ylidene]-thiazolidine-2,4-dione (available from Axon Medchem); TGX-221 (7-methyl-2-(4-morpholinyl)-9-[1-(phenylamino)ethyl]-4H-pyrido-[1,2-a]pyrirnidin-4-one (available from Axon Medchem); XL-765; and XL-147. Other PI3K inhibitors include demethoxyviridin, perifosine, CAL101, PX-866, BEZ235, SF1126, INK1117, IPI-145, BKM120, XL147, XL765, Palomid 529, GSK1059615, ZSTK474, PWT33597, IC87114, TGI 00-115, CAL263, PI-103, GNE-477, CUDC-907, and AEZS-136.

AKT inhibitors include, but are not limited to, Akt-1-1 (inhibits Aktl) (Barnett et al., Biochem. J. 2005, 385(Pt. 2): 399-408); Akt-1-1,2 (inhibits Akl and 2) (Barnett et al., Biochem. J. 2005, 385(Pt. 2): 399-408); API-59CJ-Ome (e.g., Jin et al., Br. J. Cancer 2004, 91:1808-12); 1-H-imidazo[4,5-c]pyridinyl compounds (e.g., WO 05/011700); indole-3-carbinol and derivatives thereof (e.g., U.S. Pat. No. 6,656,963; Sarkar and Li J Nutr. 2004, 134(12 Suppl): 3493S-3498S); perifosine (e.g., interferes with Akt membrane localization; Dasmahapatra et al. Clin. Cancer Res. 2004, 10(15):5242-52); phosphatidylinositol ether lipid analogues (e.g., Gills and Dennis Expert. Opin. Investig. Drugs 2004, 13:787-97); and triciribine (TCN or API-2 or NCI identifier: NSC 154020; Yang et al., Cancer Res. 2004, 64:4394-9).

mTOR inhibitors include, but are not limited to, ATP-competitive mTORC1/mTORC2 inhibitors, e.g., PI-103, PP242, PP30; Torin 1; FKBP12 enhancers; 4H-1-benzopyran-4-one derivatives; and rapamycin (also known as sirolimus) and derivatives thereof, including: temsirolimus (Torisel®); everolimus (Afinitor®; WO94/09010); ridaforolimus (also known as deforolimus or AP23573); rapalogs, e.g., as disclosed in WO98/02441 and WO01/14387, e.g. AP23464 and AP23841; 40-(2-hydroxyethyl) rapamycin; 40-[3-hydroxy(hydroxymethyl)methylpropanoate]-rapamycin (also known as CC1779); 40-epi-(tetrazolyt)-rapamycin (also called ABT578); 32-deoxorapamycin; 16-pentynyloxy-32(S)-dihydrorapanycin; derivatives disclosed in WO05/005434; derivatives disclosed in U.S. Pat. Nos. 5,258,389, 5,118,677, 5,118,678, 5,100,883, 5,151,413, 5,120,842, and 5,256,790, and in WO94/090101, WO92/05179, WO93/111130, WO94/02136, WO94/02485, WO95/14023, WO94/02136, WO95/16691, WO96/41807, WO96/41807, and WO2018204416; and phosphorus-containing rapamycin derivatives (e.g., WO05/016252). In some embodiments, the mTOR inhibitor is a bisteric inhibitor (see, e.g., WO2018204416, WO2019212990 and WO2019212991), such as RMC-5552.

BRAF inhibitors that may be used in combination with compounds of the invention include, for example, vemurafenib, dabrafenib, and encorafenib. A BRAF may comprise a Class 3 BRAF mutation. In some embodiments, the Class 3 BRAF mutation is selected from one or more of the following amino acid substitutions in human BRAF: D287H; P367R; V459L; G466V; G466E; G466A; S467L; G469E; N581 S; N581 I; D594N; D594G; D594A; D594H; F595L; G596D; G596R and A762E.

MCL-1 inhibitors include, but are not limited to, AMG-176, MIK665, and S63845. The myeloid cell leukemia-1 (MCL-1) protein is one of the key anti-apoptotic members of the B-cell lymphoma-2 (BCL-2) protein family. Over-expression of MCL-1 has been closely related to tumor progression as well as to resistance, not only to traditional chemotherapies but also to targeted therapeutics including BCL-2 inhibitors such as ABT-263.

In some embodiments, the additional therapeutic agent is a SHP2 inhibitor. SHP2 is a non-receptor protein tyrosine phosphatase encoded by the PTPN11 gene that contributes to multiple cellular functions including proliferation, differentiation, cell cycle maintenance and migration. SHP2 has two N-terminal Src homology 2 domains (N—SH2 and C—SH2), a catalytic domain (PTP), and a C-terminal tail. The two SH2 domains control the subcellular localization and functional regulation of SHP2. The molecule exists in an inactive, self-inhibited conformation stabilized by a binding network involving residues from both the N—SH2 and PTP domains. Stimulation by, for example, cytokines or growth factors acting through receptor tyrosine kinases (RTKs) leads to exposure of the catalytic site resulting in enzymatic activation of SHP2.

SHP2 is involved in signaling through the RAS-mitogen-activated protein kinase (MAPK), the JAK-STAT or the phosphoinositol 3-kinase-AKT pathways. Mutations in the PTPN11 gene and subsequently in SHP2 have been identified in several human developmental diseases, such as Noonan Syndrome and Leopard Syndrome, as well as human cancers, such as juvenile myelomonocytic leukemia, neuroblastoma, melanoma, acute myeloid leukemia and cancers of the breast, lung and colon. Some of these mutations destabilize the auto-inhibited conformation of SHP2 and promote autoactivation or enhanced growth factor driven activation of SHP2. SHP2, therefore, represents a highly attractive target for the development of novel therapies for the treatment of various diseases including cancer. A SHP2 inhibitor (e.g., RMC-4550 or SHP099) in combination with a RAS pathway inhibitor (e.g., a MEK inhibitor) have been shown to inhibit the proliferation of multiple cancer cell lines in vitro (e.g., pancreas, lung, ovarian and breast cancer). Thus, combination therapy involving a SHP2

inhibitor with a RAS pathway inhibitor could be a general strategy for preventing tumor resistance in a wide range of malignancies.

Non-limiting examples of such SHP2 inhibitors that are known in the art, include: Chen et al. *Mol Pharmacol.* 2006, 70, 562; Sarver et al., *J. Med. Chem.* 2017, 62, 1793; Xie et al., *J. Med. Chem.* 2017, 60, 113734; and Igbe et al., *Oncotarget,* 2017, 8, 113734; and PCT applications: WO2015107493; WO2015107494; WO201507495; WO2016203404; WO2016203405; WO2016203406; WO2011022440; WO2017156397; WO2017079723; WO2017211303; WO2012041524; WO2017211303; WO2019051084; WO2017211303; US20160030594; US20110281942; WO2010011666; WO2014113584; WO2014176488; WO2017100279; WO2019051469; U.S. Pat. No. 8,637,684; WO2007117699; WO2015003094; WO2005094314; WO2008124815; WO2009049098; WO2009135000; WO2016191328; WO2016196591; WO2017078499; WO2017210134; WO2018013597; WO2018129402; WO2018130928; WO20181309928; WO2018136264; WO2018136265; WO2018160731; WO2018172984; and WO2010121212, each of which is incorporated herein by reference.

In some embodiments, a SHP2 inhibitor binds in the active site. In some embodiments, a SHP2 inhibitor is a mixed-type irreversible inhibitor. In some embodiments, a SHP2 inhibitor binds an allosteric site e.g., a non-covalent allosteric inhibitor. In some embodiments, a SHP2 inhibitor is a covalent SHP2 inhibitor, such as an inhibitor that targets the cysteine residue (C333) that lies outside the phosphatase's active site. In some embodiments a SHP2 inhibitor is a reversible inhibitor. In some embodiments, a SHP2 inhibitor is an irreversible inhibitor. In some embodiments, the SHP2 inhibitor is SHP099. In some embodiments, the SHP2 inhibitor is TNO155. In some embodiments, the SHP2 inhibitor is RMC-4550. In some embodiments, the SHP2 inhibitor is RMC-4630. In some embodiments, the SHP2 inhibitor is JAB-3068. In some embodiments, the SHP2 inhibitor is RLY-1971.

In some embodiments, the additional therapeutic agent is selected from the group consisting of a MEK inhibitor, a HER2 inhibitor, a SHP2 inhibitor, a CDK4/6 inhibitor, an mTOR inhibitor, a SOS1 inhibitor, and a PD-L1 inhibitor. In some embodiments, the additional therapeutic agent is selected from the group consisting of a MEK inhibitor, a SHP2 inhibitor, and a PD-L1 inhibitor. See, e.g., Hallin et al., Cancer Discovery, DOI: 10.1158/2159-8290 (Oct. 28, 2019) and Canon et al., Nature, 575:217 (2019). In some embodiments, a Ras inhibitor of the present invention is used in combination with a MEK inhibitor and a SOS1 inhibitor. In some embodiments, a Ras inhibitor of the present invention is used in combination with a PDL-1 inhibitor and a SOS1 inhibitor. In some embodiments, a Ras inhibitor of the present invention is used in combination with a PDL-1 inhibitor and a SHP2 inhibitor. In some embodiments, a Ras inhibitor of the present invention is used in combination with a MEK inhibitor and a SHP2 inhibitor. In some embodiments, the cancer is colorectal cancer and the treatment comprises administration of a Ras inhibitor of the present invention in combination with a second or third therapeutic agent.

Proteasome inhibitors include, but are not limited to, carfilzomib (Kyprolis®), bortezomib (Velcade®), and oprozomib.

Immune therapies include, but are not limited to, monoclonal antibodies, immunomodulatory imides (IMiDs), GITR agonists, genetically engineered T-cells (e.g., CAR-T cells), bispecific antibodies (e.g., BiTEs), and anti-PD-1, anti-PDL-1, anti-CTLA4, anti-LAGI, and anti-OX40 agents).

Immunomodulatory agents (IMiDs) are a class of immunomodulatory drugs (drugs that adjust immune responses) containing an imide group. The IMiD class includes thalidomide and its analogues (lenalidomide, pomalidomide, and apremilast).

Exemplary anti-PD-1 antibodies and methods for their use are described by Goldberg et al., Blood 2007, 110(1):186-192; Thompson et al., Clin. Cancer Res. 2007, 13(6):1757-1761; and WO06/121168 A1), as well as described elsewhere herein.

GITR agonists include, but are not limited to, GITR fusion proteins and anti-GITR antibodies (e.g., bivalent anti-GITR antibodies), such as, a GITR fusion protein described in U.S. Pat. Nos. 6,111,090, 8,586,023, WO2010/003118 and WO2011/090754; or an anti-GITR antibody described, e.g., in U.S. Pat. No. 7,025,962, EP 1947183, U.S. Pat. Nos. 7,812,135, 8,388,967, 8,591,886, 7,618,632, EP 1866339, and WO2011/028683, WO2013/039954, WO05/007190, WO07/133822, WO05/055808, WO99/40196, WO01/03720, WO99/20758, WO06/083289, WO05/115451, and WO2011/051726.

Another example of a therapeutic agent that may be used in combination with the compounds of the invention is an anti-angiogenic agent. Anti-angiogenic agents are inclusive of, but not limited to, in vitro synthetically prepared chemical compositions, antibodies, antigen binding regions, radionuclides, and combinations and conjugates thereof. An anti-angiogenic agent can be an agonist, antagonist, allosteric modulator, toxin or, more generally, may act to inhibit or stimulate its target (e.g., receptor or enzyme activation or inhibition), and thereby promote cell death or arrest cell growth. In some embodiments, the one or more additional therapies include an anti-angiogenic agent.

Anti-angiogenic agents can be MMP-2 (matrix-metalloproteinase 2) inhibitors, MMP-9 (matrix-metalloprotienase 9) inhibitors, and COX-II (cyclooxygenase 11) inhibitors. Non-limiting examples of anti-angiogenic agents include rapamycin, temsirolimus (CCI-779), everolimus (RAD001), sorafenib, sunitinib, and bevacizumab. Examples of useful COX-II inhibitors include alecoxib, valdecoxib, and rofecoxib. Examples of useful matrix metalloproteinase inhibitors are described in WO96/33172, WO96/27583, WO98/07697, WO98/03516, WO98/34918, WO98/34915, WO98/33768, WO98/30566, WO90/05719, WO99/52910, WO99/52889, WO99/29667, WO99007675, EP0606046, EP0780386, EP1786785, EP1181017, EP0818442, EP1004578, and US20090012085, and U.S. Pat. Nos. 5,863,949 and 5,861,510. Preferred MMP-2 and MMP-9 inhibitors are those that have little or no activity inhibiting MMP-1. More preferred, are those that selectively inhibit MMP-2 or AMP-9 relative to the other matrix-metalloproteinases (i.e., MAP-1, MMP-3, MMP-4, MMP-5, MMP-6, MMP-7, MMP-8, MMP-10, MMP-11, MMP-12, and MMP-13). Some specific examples of MMP inhibitors are AG-3340, RO 32-3555, and RS 13-0830.

Further exemplary anti-angiogenic agents include KDR (kinase domain receptor) inhibitory agents (e.g., antibodies and antigen binding regions that specifically bind to the kinase domain receptor), anti-VEGF agents (e.g., antibodies or antigen binding regions that specifically bind VEGF (e.g., bevacizumab), or soluble VEGF receptors or a ligand binding region thereof) such as VEGF-TRAP™, and anti-VEGF receptor agents (e.g., antibodies or antigen binding regions that specifically bind thereto), EGFR inhibitory agents (e.g.,

antibodies or antigen binding regions that specifically bind thereto) such as Vectibix® (panitumumab), erlotinib (Tarceva®), anti-Angl and anti-Ang2 agents (e.g., antibodies or antigen binding regions specifically binding thereto or to their receptors, e.g., Tie2/Tek), and anti-Tie2 kinase inhibitory agents (e.g., antibodies or antigen binding regions that specifically bind thereto). Other anti-angiogenic agents include Campath, IL-8, B-FGF, Tek antagonists (US2003/0162712; U.S. Pat. No. 6,413,932), anti-TWEAK agents (e.g., specifically binding antibodies or antigen binding regions, or soluble TWEAK receptor antagonists; see U.S. Pat. No. 6,727,225), ADAM distintegrin domain to antagonize the binding of integrin to its ligands (US 2002/0042368), specifically binding anti-eph receptor or anti-ephrin antibodies or antigen binding regions (U.S. Pat. Nos. 5,981,245; 5,728,813; 5,969,110; 6,596,852; 6,232,447; 6,057,124 and patent family members thereof), and anti-PDGF-BB antagonists (e.g., specifically binding antibodies or antigen binding regions) as well as antibodies or antigen binding regions specifically binding to PDGF-BB ligands, and PDGFR kinase inhibitory agents (e.g., antibodies or antigen binding regions that specifically bind thereto). Additional anti-angiogenic agents include: SD-7784 (Pfizer, USA); cilengitide (Merck KGaA, Germany, EPO 0770622); pegaptanib octasodium, (Gilead Sciences, USA); Alphastatin, (BioActa, UK); M-PGA, (Celgene, USA, U.S. Pat. No. 5,712,291); ilomastat, (Arriva, USA, U.S. Pat. No. 5,892,112); emaxanib, (Pfizer, USA, U.S. Pat. No. 5,792,783); vatalanib, (Novartis, Switzerland); 2-methoxyestradiol (EntreMed, USA); TLC ELL-12 (Elan, Ireland); anecortave acetate (Alcon, USA); alpha-D148 Mab (Amgen, USA); CEP-7055 (Cephalon, USA); anti-Vn Mab (Crucell, Netherlands), DACantiangiogenic (ConjuChem, Canada); Angiocidin (InKine Pharmaceutical, USA); KM-2550 (Kyowa Hakko, Japan); SU-0879 (Pfizer, USA); CGP-79787 (Novartis, Switzerland, EP 0970070); ARGENT technology (Ariad, USA); YIGSR-Stealth (Johnson & Johnson, USA); fibrinogen-E fragment (BioActa, UK); angiogenic inhibitor (Trigen, UK); TBC-1635 (Encysive Pharmaceuticals, USA); SC-236 (Pfizer, USA); ABT-567 (Abbott, USA); Metastatin (EntreMed, USA); maspin (Sosei, Japan); 2-methoxyestradiol (Oncology Sciences Corporation, USA); ER-68203-00 (IV AX, USA); BeneFin (Lane Labs, USA); Tz-93 (Tsumura, Japan); TAN-1120 (Takeda, Japan); FR-111142 (Fujisawa, Japan, JP 02233610); platelet factor 4 (RepliGen, USA, EP 407122); vascular endothelial growth factor antagonist (Borean, Denmark); bevacizumab (pINN) (Genentech, USA); angiogenic inhibitors (SUGEN, USA); XL 784 (Exelixis, USA); XL 647 (Exelixis, USA); MAb, alpha5beta3 integrin, second generation (Applied Molecular Evolution, USA and MedImmune, USA); enzastaurin hydrochloride (Lilly, USA); CEP 7055 (Cephalon, USA and Sanofi-Synthelabo, France); BC 1 (Genoa Institute of Cancer Research, Italy); rBPI 21 and BPI-derived antiangiogenic (XOMA, USA); PI 88 (Progen, Australia); cilengitide (Merck KGaA, German; Munich Technical University, Germany, Scripps Clinic and Research Foundation, USA); AVE 8062 (Ajinomoto, Japan); AS 1404 (Cancer Research Laboratory, New Zealand); SG 292, (Telios, USA); Endostatin (Boston Childrens Hospital, USA); ATN 161 (Attenuon, USA); 2-methoxyestradiol (Boston Childrens Hospital, USA); ZD 6474, (AstraZeneca, UK); ZD 6126, (Angiogene Pharmaceuticals, UK); PPI 2458, (Praecis, USA); AZD 9935, (AstraZeneca, UK); AZD 2171, (AstraZeneca, UK); vatalanib (pINN), (Novartis, Switzerland and Schering AG, Germany); tissue factor pathway inhibitors, (EntreMed, USA); pegaptanib (Pinn), (Gilead Sciences, USA); xanthorrhizol, (Yonsei University, South Korea); vaccine, gene-based, VEGF-2, (Scripps Clinic and Research Foundation, USA); SPV5.2, (Supratek, Canada); SDX 103, (University of California at San Diego, USA); PX 478, (ProIX, USA); METASTATIN, (EntreMed, USA); troponin I, (Harvard University, USA); SU 6668, (SUGEN, USA); OXI 4503, (OXiGENE, USA); o-guanidines, (Dimensional Pharmaceuticals, USA); motuporamine C, (British Columbia University, Canada); CDP 791, (Celltech Group, UK); atiprimod (pINN), (GlaxoSmithKline, UK); E 7820, (Eisai, Japan); CYC 381, (Harvard University, USA); AE 941, (Aeterna, Canada); vaccine, angiogenic, (EntreMed, USA); urokinase plasminogen activator inhibitor, (Dendreon, USA); oglufanide (pINN), (Melmotte, USA); HIF-lalfa inhibitors, (Xenova, UK); CEP 5214, (Cephalon, USA); BAY RES 2622, (Bayer, Germany); Angiocidin, (InKine, USA); A6, (Angstrom, USA); KR 31372, (Korea Research Institute of Chemical Technology, South Korea); GW 2286, (GlaxoSmithKline, UK); EHT 0101, (ExonHit, France); CP 868596, (Pfizer, USA); CP 564959, (OSI, USA); CP 547632, (Pfizer, USA); 786034, (GlaxoSmithKline, UK); KRN 633, (Kirin Brewery, Japan); drug delivery system, intraocular, 2-methoxyestradiol; anginex (Maastricht University, Netherlands, and Minnesota University, USA); ABT 510 (Abbott, USA); AAL 993 (Novartis, Switzerland); VEGI (ProteomTech, USA); tumor necrosis factor-alpha inhibitors; SU 11248 (Pfizer, USA and SUGEN USA); ABT 518, (Abbott, USA); YH16 (Yantai Rongchang, China); S-3APG (Boston Childrens Hospital, USA and EntreMed, USA); MAb, KDR (ImClone Systems, USA); MAb, alpha5 beta (Protein Design, USA); KDR kinase inhibitor (Celltech Group, UK, and Johnson & Johnson, USA); GFB 116 (South Florida University, USA and Yale University, USA); CS 706 (Sankyo, Japan); combretastatin A4 prodrug (Arizona State University, USA); chondroitinase AC (IBEX, Canada); BAY RES 2690 (Bayer, Germany); AGM 1470 (Harvard University, USA, Takeda, Japan, and TAP, USA); AG 13925 (Agouron, USA); Tetrathiomolybdate (University of Michigan, USA); GCS 100 (Wayne State University, USA) CV 247 (Ivy Medical, UK); CKD 732 (Chong Kun Dang, South Korea); irsogladine, (Nippon Shinyaku, Japan); RG 13577 (Aventis, France); WX 360 (Wilex, Germany); squalamine, (Genaera, USA); RPI 4610 (Sirna, USA); heparanase inhibitors (InSight, Israel); KL 3106 (Kolon, South Korea); Honokiol (Emory University, USA); ZK CDK (Schering AG, Germany); ZK Angio (Schering AG, Germany); ZK 229561 (Novartis, Switzerland, and Schering AG, Germany); XMP 300 (XOMA, USA); VGA 1102 (Taisho, Japan); VE-cadherin-2 antagonists (ImClone Systems, USA); Vasostatin (National Institutes of Health, USA); Flk-1 (ImClone Systems, USA); TZ 93 (Tsumura, Japan); TumStatin (Beth Israel Hospital, USA); truncated soluble FLT 1 (vascular endothelial growth factor receptor 1) (Merck & C_0, USA); Tie-2 ligands (Regeneron, USA); and thrombospondin 1 inhibitor (Allegheny Health, Education and Research Foundation, USA).

Further examples of therapeutic agents that may be used in combination with compounds of the invention include agents (e.g., antibodies, antigen binding regions, or soluble receptors) that specifically bind and inhibit the activity of growth factors, such as antagonists of hepatocyte growth factor (HGF, also known as Scatter Factor), and antibodies or antigen binding regions that specifically bind its receptor, c-Met.

Another example of a therapeutic agent that may be used in combination with compounds of the invention is an autophagy inhibitor. Autophagy inhibitors include, but are

not limited to chloroquine, 3-methyladenine, hydroxychloroquine (Plaquenil™), bafilomycin A1, 5-amino-4-imidazole carboxamide riboside (AICAR), okadaic acid, autophagy-suppressive algal toxins which inhibit protein phosphatases of type 2A or type 1, analogues of cAMP, and drugs which elevate cAMP levels such as adenosine, LY204002, N6-mercaptopurine riboside, and vinblastine. In addition, antisense or siRNA that inhibits expression of proteins including but not limited to ATG5 (which are implicated in autophagy), may also be used. In some embodiments, the one or more additional therapies include an autophagy inhibitor.

Another example of a therapeutic agent that may be used in combination with compounds of the invention is an anti-neoplastic agent. In some embodiments, the one or more additional therapies include an anti-neoplastic agent. Non-limiting examples of anti-neoplastic agents include acemannan, aclarubicin, aldesleukin, alemtuzumab, alitretinoin, altretamine, amifostine, aminolevulinic acid, amrubicin, amsacrine, anagrelide, anastrozole, ancer, ancestim, arglabin, arsenic trioxide, BAM-002 (Novelos), bexarotene, bicalutamide, broxuridine, capecitabine, celmoleukin, cetrorelix, cladribine, clotrimazole, cytarabine ocfosfate, DA 3030 (Dong-A), daclizumab, denileukin diftitox, deslorelin, dexrazoxane, dilazep, docetaxel, docosanol, doxercalciferol, doxifluridine, doxorubicin, bromocriptine, carmustine, cytarabine, fluorouracil, HIT diclofenac, interferon alfa, daunorubicin, doxorubicin, tretinoin, edelfosine, edrecolomab, eflornithine, emitefur, epirubicin, epoetin beta, etoposide phosphate, exemestane, exisulind, fadrozole, filgrastim, finasteride, fludarabine phosphate, formestane, fotemustine, gallium nitrate, gemcitabine, gemtuzumab zogamicin, gimeracil/oteracil/tegafur combination, glycopine, goserelin, heptaplatin, human chorionic gonadotropin, human fetal alpha fetoprotein, ibandronic acid, idarubicin, (imiquimod, interferon alfa, interferon alfa, natural, interferon alfa-2, interferon alfa-2a, interferon alfa-2b, interferon alfa-NI, interferon alfa-n3, interferon alfacon-1, interferon alpha, natural, interferon beta, interferon beta-la, interferon beta-Ib, interferon gamma, natural interferon gamma-Ia, interferon gamma-Ib, interleukin-1 beta, iobenguane, irinotecan, irsogladine, Ianreotide, LC 9018 (Yakult), leflunomide, lenograstim, lentinan sulfate, letrozole, leukocyte alpha interferon, leuprorelin, levamisole+fluorouracil, liarozole, lobaplatin, Ionidamine, lovastatin, masoprocol, melarsoprol, metoclopramide, mifepristone, miltefosine, mirimostim, mismatched double stranded RNA, mitoguazone, mitolactol, mitoxantrone, molgramostim, nafarelin, naloxone+pentazocine, nartograstim, nedaplatin, nilutamide, noscapine, novel erythropoiesis stimulating protein, NSC 631570 octreotide, oprelvekin, osaterone, oxaliplatin, paclitaxel, pamidronic acid, pegaspargase, peginterferon alfa-2b, pentosan polysulfate sodium, pentostatin, picibanil, pirarubicin, rabbit antithymocyte polyclonal antibody, polyethylene glycol interferon alfa-2a, porfimer sodium, raloxifene, raltitrexed, rasburiembodiment, rhenium Re 186 etidronate, RII retinamide, rituximab, romurtide, samarium (153 Sm) lexidronam, sargramostim, sizofiran, sobuzoxane, sonermin, strontium-89 chloride, suramin, tasonermin, tazarotene, tegafur, temoporfin, temozolomide, teniposide, tetrachlorodecaoxide, thalidomide, thymalfasin, thyrotropin alfa, topotecan, toremifene, tositumomab-iodine 131, trastuzumab, treosulfan, tretinoin, trilostane, trimetrexate, triptorelin, tumor necrosis factor alpha, natural, ubenimex, bladder cancer vaccine, Maruyama vaccine, melanoma lysate vaccine, valrubicin, verteporfin, vinorelbine, virulizin, zinostatin stimalamer, or zoledronic acid; abarelix; AE 941 (Aeterna), ambamustine, antisense oligonucleotide, bcl-2 (Genta), APC 8015 (Dendreon), decitabine, dexaminoglutethimide, diaziquone, EL 532 (Elan), EM 800 (Endorecherche), eniluracil, etanidazole, fenretinide, filgrastim SDO1 (Amgen), fulvestrant, galocitabine, gastrin 17 immunogen, HLA-B7 gene therapy (Vical), granulocyte macrophage colony stimulating factor, histamine dihydrochloride, ibritumomab tiuxetan, ilomastat, IM 862 (Cytran), interleukin-2, iproxifene, LDI 200 (Milkhaus), leridistim, lintuzumab, CA 125 MAb (Biomira), cancer MAb (Japan Pharmaceutical Development), HER-2 and Fc MAb (Medarex), idiotypic 105AD7 MAb (CRC Technology), idiotypic CEA MAb (Trilex), LYM-1-iodine 131 MAb (Techni clone), polymorphic epithelial mucin-yttrium 90 MAb (Antisoma), marimastat, menogaril, mitumomab, motexafin gadolinium, MX 6 (Galderma), nelarabine, nolatrexed, P 30 protein, pegvisomant, pemetrexed, porfiromycin, prinomastat, RL 0903 (Shire), rubitecan, satraplatin, sodium phenylacetate, sparfosic acid, SRL 172 (SR Pharma), SU 5416 (SUGEN), TA 077 (Tanabe), tetrathiomolybdate, thaliblastine, thrombopoietin, tin ethyl etiopurpurin, tirapazamine, cancer vaccine (Biomira), melanoma vaccine (New York University), melanoma vaccine (Sloan Kettering Institute), melanoma oncolysate vaccine (New York Medical College), viral melanoma cell lysates vaccine (Royal Newcastle Hospital), or valspodar.

Additional examples of therapeutic agents that may be used in combination with compounds of the invention include ipilimumab (Yervoy®); tremelimumab; galiximab; nivolumab, also known as BMS-936558 (Opdivo®); pembrolizumab (Keytruda®); avelumab (Bavencio®); AMP224; BMS-936559; MPDL3280A, also known as RG7446; MEDI-570; AMG557; MGA271; IMP321; BMS-663513; PF-05082566; CDX-1127; anti-OX40 (Providence Health Services); huMAbOX40L; atacicept; CP-870893; lucatumumab; dacetuzumab; muromonab-CD3; ipilumumab; MEDI4736 (Imfinzi®); MSB0010718C; AMP 224; adalimumab (Humira®); ado-trastuzumab emtansine (Kadcyla®); aflibercept (Eylea®); alemtuzumab (Campath®); basiliximab (Simulect®); belimumab (Benlysta®); basiliximab (Simulect®); belimumab (Benlysta®); brentuximab vedotin (Adcetris®); canakinumab (Ilaris®); certolizumab pegol (Cimzia®); daclizumab (Zenapax®); daratumumab (Darzalex®); denosumab (Prolia®); eculizumab (Soliris®); efalizumab (Raptiva®); gemtuzumab ozogamicin (Mylotarg®); golimumab (Simponi®); ibritumomab tiuxetan (Zevalin®); infliximab (Remicade®); motavizumab (Numax®); natalizumab (Tysabri®); obinutuzumab (Gazyva®); ofatumumab (Arzerra®); omalizumab (Xolair®); palivizumab (Synagis®); pertuzumab (Perjeta®); pertuzumab (Perjeta®); ranibizumab (Lucentis®); raxibacumab (Abthrax®); tocilizumab (Actemra®); tositumomab; tositumomab-i-131; tositumomab and tositumomab-i-131 (Bexxar®); ustekinumab (Stelara®); AMG 102; AMG 386; AMG 479; AMG 655; AMG 706; AMG 745; and AMG 951.

The compounds described herein can be used in combination with the agents disclosed herein or other suitable agents, depending on the condition being treated. Hence, in some embodiments the one or more compounds of the disclosure will be co-administered with other therapies as described herein. When used in combination therapy, the compounds described herein may be administered with the second agent simultaneously or separately. This administration in combination can include simultaneous administration of the two agents in the same dosage form, simultaneous administration in separate dosage forms, and separate administration. That is, a compound described herein and

any of the agents described herein can be formulated together in the same dosage form and administered simultaneously. Alternatively, a compound of the invention and any of the therapies described herein can be simultaneously administered, wherein both the agents are present in separate formulations. In another alternative, a compound of the present disclosure can be administered and followed by any of the therapies described herein, or vice versa. In some embodiments of the separate administration protocol, a compound of the invention and any of the therapies described herein are administered a few minutes apart, or a few hours apart, or a few days apart.

In some embodiments of any of the methods described herein, the first therapy (e.g., a compound of the invention) and one or more additional therapies are administered simultaneously or sequentially, in either order. The first therapeutic agent may be administered immediately, up to 1 hour, up to 2 hours, up to 3 hours, up to 4 hours, up to 5 hours, up to 6 hours, up to 7 hours, up to, 8 hours, up to 9 hours, up to 10 hours, up to 11 hours, up to 12 hours, up to 13 hours, 14 hours, up to hours 16, up to 17 hours, up 18 hours, up to 19 hours up to 20 hours, up to 21 hours, up to 22 hours, up to 23 hours, up to 24 hours, or up to 1-7, 1-14, 1-21 or 1-30 days before or after the one or more additional therapies.

The invention also features kits including (a) a pharmaceutical composition including an agent (e.g., a compound of the invention) described herein, and (b) a package insert with instructions to perform any of the methods described herein. In some embodiments, the kit includes (a) a pharmaceutical composition including an agent (e.g., a compound of the invention) described herein, (b) one or more additional therapies (e.g., non-drug treatment or therapeutic agent), and (c) a package insert with instructions to perform any of the methods described herein.

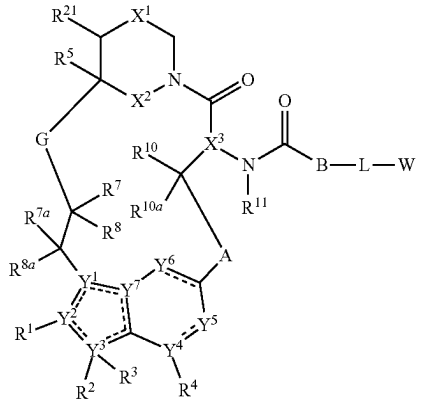
As one aspect of the present invention contemplates the treatment of the disease or symptoms associated therewith with a combination of pharmaceutically active compounds that may be administered separately, the invention further relates to combining separate pharmaceutical compositions in kit form.

The kit may comprise two separate pharmaceutical compositions: a compound of the present invention, and one or more additional therapies. The kit may comprise a container for containing the separate compositions such as a divided bottle or a divided foil packet. Additional examples of containers include syringes, boxes, and bags. In some embodiments, the kit may comprise directions for the use of the separate components. The kit form is particularly advantageous when the separate components are preferably administered in different dosage forms (e.g., oral and parenteral), are administered at different dosage intervals, or when titration of the individual components of the combination is desired by the prescribing health care professional.

NUMBERED EMBODIMENTS

[1] A compound, or pharmaceutically acceptable salt thereof, having the structure of Formula I:

Formula I



wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is —N(H or CH_3)C(O)—(CH_2)— where the amino nitrogen is bound to the carbon atom of —CH(R^{10})—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 10-membered heteroarylene;

B is absent, —CH(R^9)—, >C=$CR^9R^{9'}$, or >$CR^9R^{9'}$ where the carbon is bound to the carbonyl carbon of —N(R^{11})C(O)—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, —C(O)O—CH(R^6)— where C is bound to —C(R^7R^8)—, —C(O)NH—CH(R^6)— where C is bound to —C(R^7R^8)—, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, or an alkynyl sulfone;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or $S(O)_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 is CH, CH_2, or N;

Y^6 is C(O), CH, CH_2, or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl, or

R^1 and R^2 combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

R^2 is absent, hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C{=}CR^{7'}R^{8'}$; C=N(OH), C=N(O—C_1-C_3 alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{7a} and R^{8a} are, independently, hydrogen, halo, optionally substituted C_1-C_3 alkyl, or combine with the carbon to which they are attached to form a carbonyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is H, F, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl, or

R^9 and L combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

$R^{9'}$ is hydrogen or optionally substituted C_1-C_6 alkyl; or

R^9 and $R^{9'}$, combined with the atoms to which they are attached, form a 3 to 6-membered cycloalkyl or a 3 to 6-membered heterocycloalkyl;

R^{10} is hydrogen, halo, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl;

R^{10a} is hydrogen or halo;

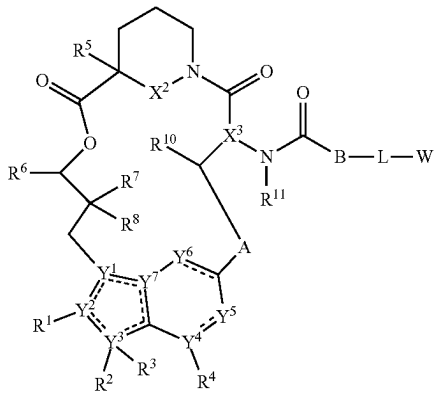
R^{11} is hydrogen or C_1-C_3 alkyl; and

R^{21} is H or C_1-C_3 alkyl.

[2] The compound, or pharmaceutically acceptable salt thereof, of paragraph [1], wherein G is optionally substituted C_1-C_4 heteroalkylene.

[3] The compound, or pharmaceutically acceptable salt thereof, of paragraph [1] or [2], wherein the compound has the structure of Formula Ic:

Formula Ic



wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is —N(H or CH_3)C(O)—(CH_2)— where the amino nitrogen is bound to the carbon atom of —CH(R^{10})—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is —CH(R^9)— where the carbon is bound to the carbonyl carbon of —N(R^{11})C(O)—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, or an alkynyl sulfone;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 and Y^6 are, independently, CH or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

R^2 is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substi-

tuted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C{=}CR^{7'}R^{8'}$; C═N(OH), C═N(O—C_1-C_3 alkyl), C═O, C═S, C═NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{10} is hydrogen, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl; and

R^{11} is hydrogen or C_1-C_3 alkyl.

[4] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [3], wherein X^2 is NH.

[5] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [4], wherein X^3 is CH.

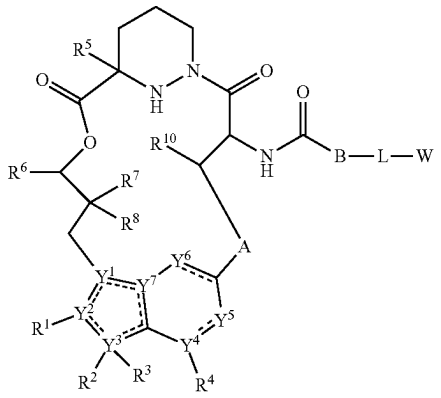
[6] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [5], wherein R^{11} is hydrogen.

[7] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [5], wherein R^{11} is C_1-C_3 alkyl.

[8] The compound, or pharmaceutically acceptable salt thereof, of paragraph [7], wherein R^{11} is methyl.

[9] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [6], wherein the compound has the structure of Formula Id:

Formula Id



wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is —N(H or CH_3)C(O)—(CH_2)— where the amino nitrogen is bound to the carbon atom of —CH(R^{10})—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is —CH(R^9)— where the carbon is bound to the carbonyl carbon of —NHC(O)—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, or an alkynyl sulfone;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 and Y^6 are, independently, CH or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

R^2 is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form C═$CR^{7'}R^{8'}$; C═N(OH), C═N(O—C_1-C_3 alkyl), C═O, C═S, C═NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl; and

R^{10} is hydrogen, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl.

[10] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [9] wherein X^1 is optionally substituted C_1-C_2 alkylene.

[11] The compound, or pharmaceutically acceptable salt thereof, of paragraph [10], wherein X^1 is methylene.

[12] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [11], wherein R^5 is hydrogen.

[13] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [11], wherein R^5 is C_1-C_4 alkyl optionally substituted with halogen.

[14] The compound, or pharmaceutically acceptable salt thereof, of paragraph [13], wherein R^5 is methyl.

[15] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [14], wherein Y^4 is C.

[16] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [15], wherein R^4 is hydrogen.

[17] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [16], wherein Y^5 is CH.

[18] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [17], wherein Y^6 is CH.

[19] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [18], wherein Y^1 is C.

[20] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [19] wherein Y^2 is C.

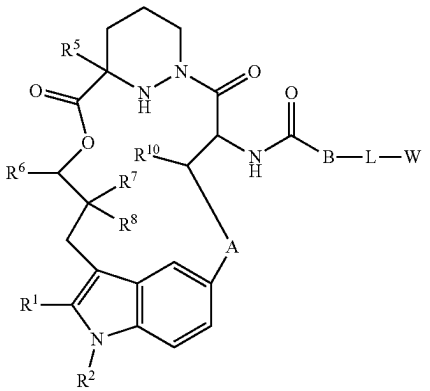
[21] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [20] wherein Y^3 is N.

[22] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [21], wherein R^3 is absent.

[23] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [22], wherein Y^7 is C.

[24] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [6] or [9] to [23], wherein the compound has the structure of Formula Ie:

Formula Ie



wherein A is —N(H or CH_3)C(O)—(CH_2)— where the amino nitrogen is bound to the carbon atom of —CH(R^{10})—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is —CH(R^9)— where the carbon is bound to the carbonyl carbon of —NHC(O)—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, or an alkynyl sulfone;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

R^2 is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form $C{=}CR^{7'}R^{8'}$; C═N(OH), C═N(O—C_1-C_3 alkyl), C═O, C═S, C═NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl; and

R^{10} is hydrogen, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl.

[25] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [3] to [24], wherein R^6 is hydrogen.

[26] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [25], wherein R^2 is hydrogen, cyano, optionally substituted C_1-C_6 alkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 6-membered heterocycloalkyl.

[27] The compound, or pharmaceutically acceptable salt thereof, of paragraph [26], wherein R^2 is optionally substituted C_1-C_6 alkyl.

[28] The compound, or pharmaceutically acceptable salt thereof, of paragraph [27], wherein R^2 is ethyl.

[29] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [28], wherein R^7 is optionally substituted C_1-C_3 alkyl.

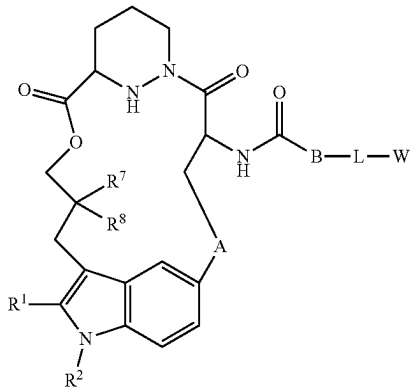
[30] The compound, or pharmaceutically acceptable salt thereof, of paragraph 29, wherein R^7 is C_1-C_3 alkyl.

[31] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to 30, wherein R^8 is optionally substituted C_1-C_3 alkyl.

[32] The compound, or pharmaceutically acceptable salt thereof, of paragraph [31], wherein R^8 is C_1-C_3 alkyl.

[33] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [32], wherein the compound has the structure of Formula If:

Formula If



wherein A is —N(H or CH_3)C(O)—(CH_2)— where the amino nitrogen is bound to the carbon atom of —CH(R^{10})—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is —CH(R^9)— where the carbon is bound to the carbonyl carbon of —NHC(O)—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, or an alkynyl sulfone;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl;

R^2 is C_1-C_6 alkyl or 3 to 6-membered cycloalkyl;

R^7 is C_1-C_3 alkyl;

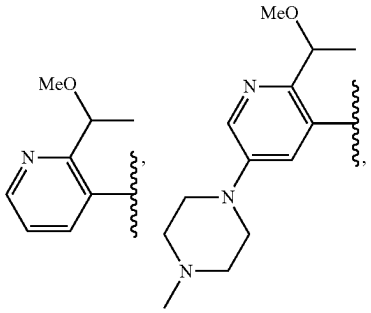
R^8 is C_1-C_3 alkyl; and

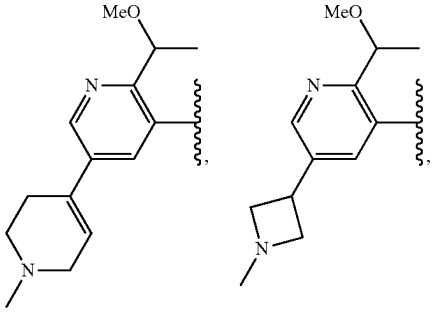
R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl.

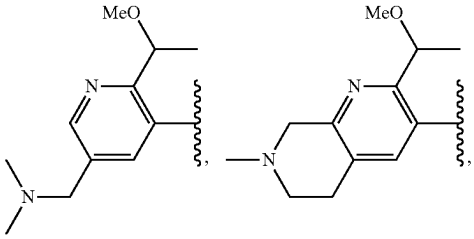
[34] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [33], wherein R^1 is optionally substituted 6 to 10-membered aryl, optionally substituted 3 to 6-membered cycloalkenyl, or optionally substituted 5 to 10-membered heteroaryl.

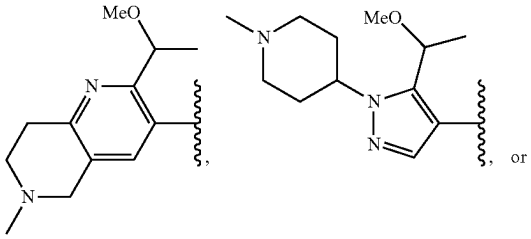
[35] The compound, or pharmaceutically acceptable salt thereof, of paragraph [34], wherein R^1 is optionally substituted 6-membered aryl, optionally substituted 6-membered cycloalkenyl, or optionally substituted 6-membered heteroaryl.

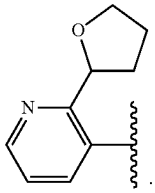
[36] The compound, or pharmaceutically acceptable salt thereof, of paragraph [35], wherein R^1 is



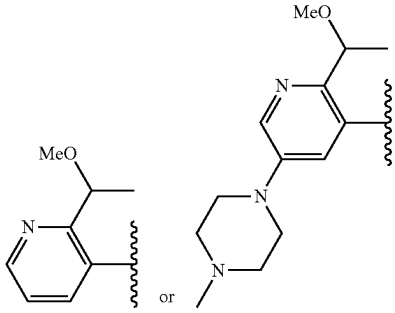






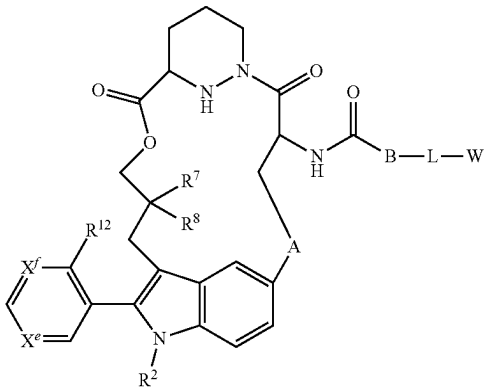


[37] The compound, or pharmaceutically acceptable salt thereof, of paragraph [36], wherein R^1 is



[38] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [37], wherein the compound has the structure of Formula Ig:

Formula Ig



wherein A is —N(H or CH_3)C(O)—(CH_2)— where the amino nitrogen is bound to the carbon atom of —CH(R^{10})—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is —CH(R^9)— where the carbon is bound to the carbonyl carbon of —NHC(O)—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, or an alkynyl sulfone;

R^2 is C_1-C_6 alkyl, C_1-C_6 fluoroalkyl, or 3 to 6-membered cycloalkyl;

R^7 is C_1-C_3 alkyl;

R^8 is C_1-C_3 alkyl; and

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl

X^e and X^f are, independently, N or CH; and

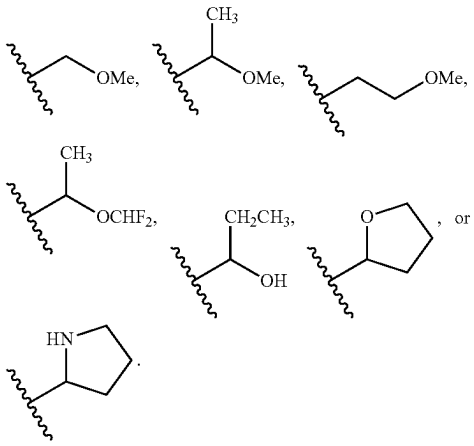
R^{12} is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl.

[39] The compound, or pharmaceutically acceptable salt thereof, of paragraph [38], wherein X^e is N and X^f is CH.

[40] The compound, or pharmaceutically acceptable salt thereof, of paragraph [38], wherein X^e is CH and X^f is N.

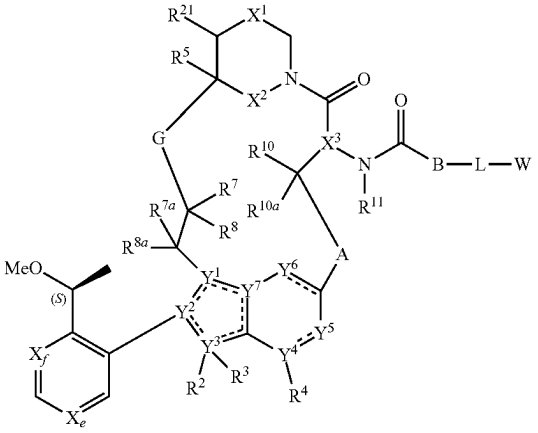
[41] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [38] to [40], wherein R^{12} is optionally substituted C_1-C_6 heteroalkyl.

[42] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [38] to [41], wherein R^{12} is



[43] The compound, or pharmaceutically acceptable salt thereof, of paragraph [1] or [2], wherein the compound has the structure of Formula VI:

Formula VI



wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is —N(H or CH_3)C(O)—(CH_2)— where the amino nitrogen is bound to the carbon atom of —CH(R^{10})—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 10-membered heteroarylene;

B is absent, —CH(R^9)—, >C═C$R^9R^{9'}$, or >C$R^9R^{9'}$ where the carbon is bound to the carbonyl carbon of —N(R^{11})C(O)—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, —C(O)O—CH(R^6)— where C is bound to —C(R^7R^8)—, —C(O)NH—CH(R^6)— where C is bound to —C(R^7R^8)—, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, a haloacetal, or an alkynyl sulfone;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or $S(O)_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 is CH, CH_2, or N;

Y^6 is C(O), CH, CH_2, or N;

R^2 is absent, hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form C═C$R^{7'}R^{8'}$; C═N(OH), C═N(O—C_1-C_3 alkyl), C═O, C═S, C═NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{7a} and R^{8a} are, independently, hydrogen, halo, optionally substituted C_1-C_3 alkyl, or combine with the carbon to which they are attached to form a carbonyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is H, F, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl; or

R^9 and L combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

$R^{9'}$ is hydrogen or optionally substituted C_1-C_6 alkyl; or

R^9 and $R^{9'}$, combined with the atoms to which they are attached, form a 3 to 6-membered cycloalkyl or a 3 to 6-membered heterocycloalkyl;

R^{10} is hydrogen, halo, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl;

R^{10a} is hydrogen or halo;

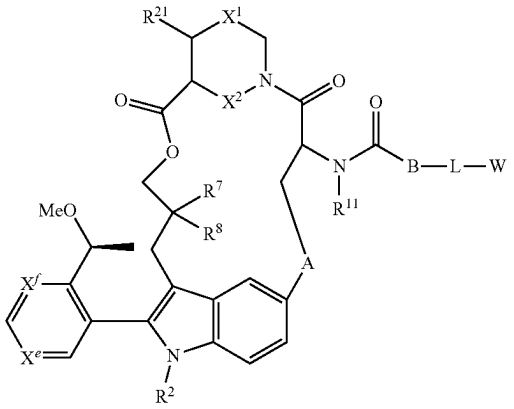
R^{11} is hydrogen or C_1-C_3 alkyl;

R^{21} is hydrogen or C_1-C_3 alkyl (e.g., methyl); and

X^e and X^f are, independently, N or CH.

[44] The compound, or pharmaceutically acceptable salt thereof, of paragraph [43], wherein the compound has the structure of Formula VIa:

Formula VIa



wherein A is optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is —CH(R^9)— where the carbon is bound to the carbonyl carbon of —NHC(O)—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

L is absent or a linker;

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, or an alkynyl sulfone;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or $S(O)_n$;

X^2 is O or NH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

R^2 is C_1-C_6 alkyl, C_1-C_6 fluoroalkyl, or 3 to 6-membered cycloalkyl;

R^7 is C_1-C_3 alkyl;

R^8 is C_1-C_3 alkyl; and

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

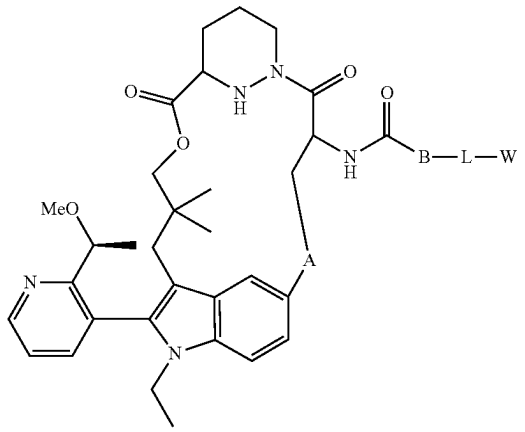
X^e and X^1 are, independently, N or CH;

R^{11} is hydrogen or C_1-C_3 alkyl; and

R^{21} is hydrogen or C_1-C_3 alkyl.

[45] The compound, or pharmaceutically acceptable salt thereof, of paragraph [43] or [44], wherein the compound has the structure of Formula VIb:

Formula VIb



wherein A is optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is —CH(R^9)— where the carbon is bound to the carbonyl carbon of —NHC(O)—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

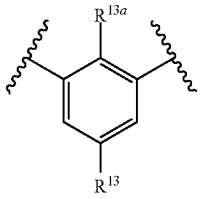
R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

L is absent or a linker; and

W is a cross-linking group comprising a vinyl ketone, a vinyl sulfone, an ynone, or an alkynyl sulfone.

[46] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [45], wherein A is optionally substituted 6-membered arylene.

[47] The compound, or pharmaceutically acceptable salt thereof, of paragraph [46], wherein A has the structure:



wherein R^{13} is hydrogen, halo, hydroxy, amino, optionally substituted C_1-C_6 alkyl, or optionally substituted C_1-C_6 heteroalkyl; and

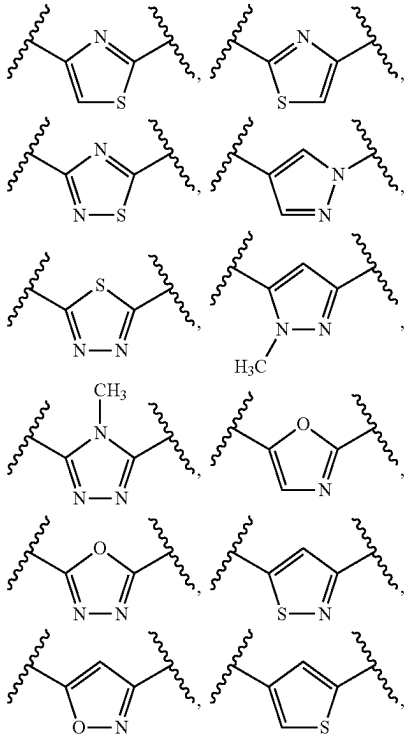
R^{13a} is hydrogen or halo.

[48] The compound, or pharmaceutically acceptable salt thereof, of paragraph [47], wherein R^{13} and R^{13a} are each hydrogen.

[49] The compound, or pharmaceutically acceptable salt thereof, of paragraph [47], wherein R^{13} is hydroxy, methyl, fluoro, or difluoromethyl.

[50] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [45], wherein A is optionally substituted 5 to 6-membered heteroarylene.

[51] The compound, or pharmaceutically acceptable salt thereof, of paragraph [50], wherein A is:



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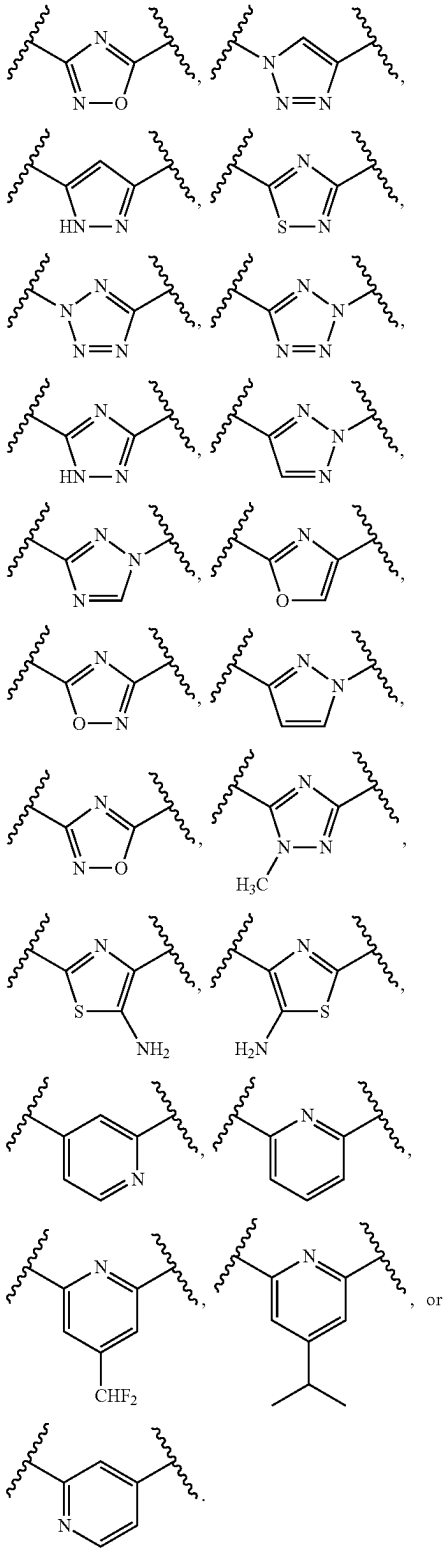
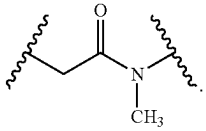


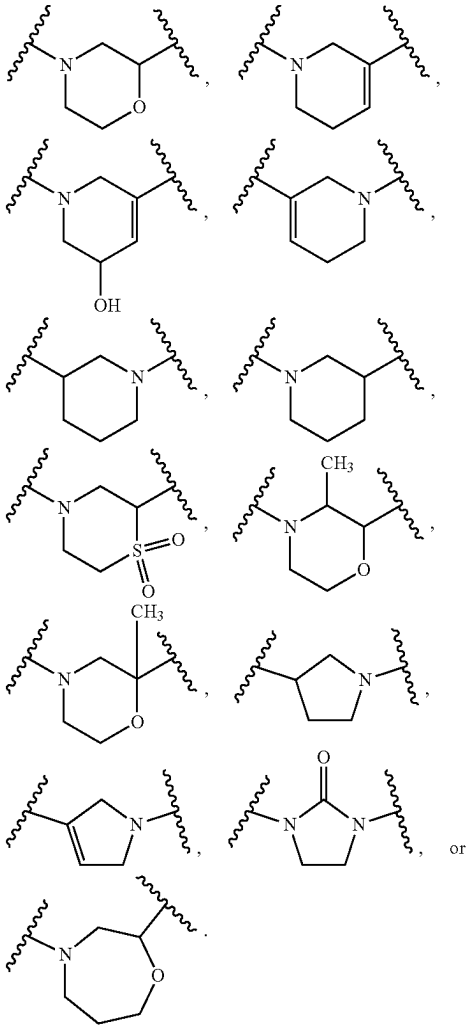
[52] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [45], wherein A is optionally substituted C_1-C_4 heteroalkylene.

[53] The compound, or pharmaceutically acceptable salt thereof, of paragraph [52], wherein A is:

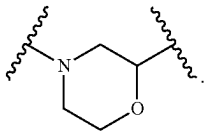


[54] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [45], wherein A is optionally substituted 3 to 6-membered heterocycloalkylene.

[55] The compound, or pharmaceutically acceptable salt thereof, of paragraph [54], wherein A is:



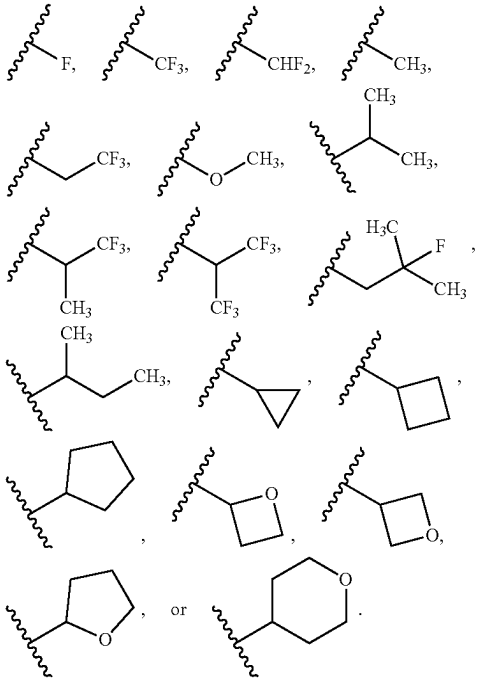
[56] The compound, or pharmaceutically acceptable salt thereof, of paragraph [55], wherein A is



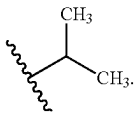
[57] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [56], wherein B is —CHR^9—.

[58] The compound, or pharmaceutically acceptable salt thereof, of paragraph [57], wherein R^9 is F, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl.

[59] The compound, or pharmaceutically acceptable salt thereof, of paragraph [58], wherein R^9 is:



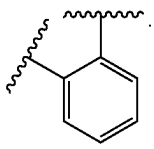
[60] The compound, or pharmaceutically acceptable salt thereof, of paragraph [59], wherein R^9 is:



[61] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [56], wherein B is optionally substituted 6-membered arylene.

[62] The compound, or pharmaceutically acceptable salt thereof, of paragraph [61], wherein B is 6-membered arylene.

[63] The compound, or pharmaceutically acceptable salt thereof, of paragraph [61], wherein B is:



[64] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [63], wherein R^7 is methyl.

[65] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [64], wherein R^8 is methyl.

[66] The compound, or pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [65], wherein the linker is the structure of Formula II:

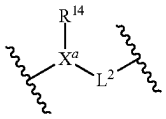
$$A^1\text{-}(B^1)_f\text{—}(C^1)_g\text{—}(B^2)_h\text{-}(D^1)\text{-}(B^3)_i\text{—}(C^2)_j\text{—}(B^4)_k\text{-}A^2 \qquad \text{Formula II}$$

where A^1 is a bond between the linker and B; A^2 is a bond between W and the linker; B^1, B^2, B^3, and B^4 each, independently, is selected from optionally substituted C_1-C_2 alkylene, optionally substituted C_1-C_3 heteroalkylene, O, S, and NR^N; R^N is hydrogen, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted C_1-C_7 heteroalkyl; C_1 and C^2 are each, independently, selected from carbonyl, thiocarbonyl, sulphonyl, or phosphoryl; f, g, h, i, j, and k are each, independently, 0 or 1; and D^1 is optionally substituted C_1-C_{10} alkylene, optionally substituted C_2-C_{10} alkenylene, optionally substituted C_2-C_{10} alkynylene, optionally substituted 3 to 14-membered heterocycloalkylene, optionally substituted 5 to 10-membered heteroarylene, optionally substituted 3 to 8-membered cycloalkylene, optionally substituted 6 to 10-membered arylene, optionally substituted C_2-C_{10} polyethylene glycolene, or optionally substituted C_1-C_{10} heteroalkylene, or a chemical bond linking A^1-$(B^1)_f$—$(C^1)_g$—$(B^2)_h$— to —$(B^3)_i$—$(C^2)_j$—$(B^4)_k$-A^2.

[67] The compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [66], wherein the linker is acyclic.

[68] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [67], wherein the linker has the structure of Formula IIa:

Formula IIa



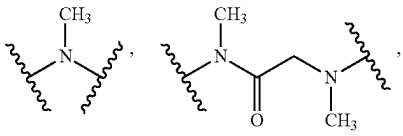
wherein X^a is absent or N;

R^{14} is absent, hydrogen or optionally substituted C_1-C_6 alkyl; and

L^2 is absent, —SO_2—, optionally substituted C_1-C_4 alkylene or optionally substituted C_1-C_4 heteroalkylene,

wherein at least one of X^a, R^{14}, or L^2 is present.

[69] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [68], wherein the linker has the structure:



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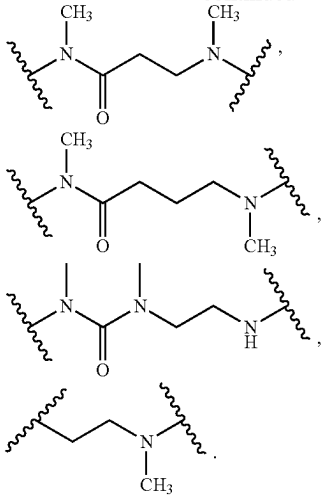
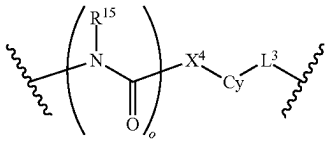


[70] The compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [66], wherein the linker is or comprises a cyclic moiety.

[71] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [70], wherein the linker has the structure of Formula IIb:

Formula IIb



wherein o is 0 or 1;

R^{15} is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted 3 to 8-membered cycloalkylene, or optionally substituted 3 to 8-membered heterocycloalkylene;

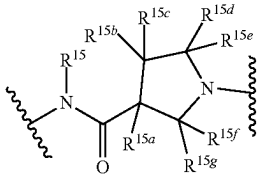
X^4 is absent, optionally substituted C_1-C_4 alkylene, 0, NCH_3, or optionally substituted C_1-C_4 heteroalkylene;

Cy is optionally substituted 3 to 8-membered cycloalkylene, optionally substituted 3 to 8-membered heterocycloalkylene, optionally substituted 6-10 membered arylene, or optionally substituted 5 to 10-membered heteroarylene; and

L^3 is absent, —SO_2—, optionally substituted C_1-C_4 alkylene or optionally substituted C_1-C_4 heteroalkylene.

[72] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [71], wherein the linker has the structure:

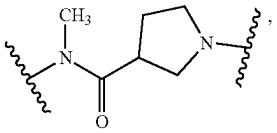
Formula IIc

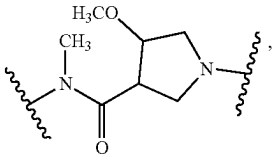


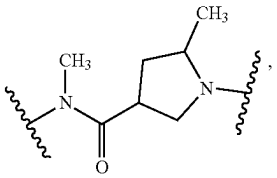
wherein R^{15} is hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted 3 to 8-membered cycloalkylene, or optionally substituted 3 to 8-membered heterocycloalkylene; and

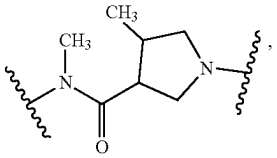
R^{15a}, R^{15b}, R^{15c}, R^{15d}, R^{15e}, R^{15f}, and R^{15g} are, independently, hydrogen, halo, hydroxy, cyano, amino, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 alkoxy, or, or R^{15b} and R^{15d} combine with the carbons to which they are attached to form an optionally substituted 3 to 8-membered cycloalkylene, or optionally substituted 3 to 8-membered heterocycloalkylene.

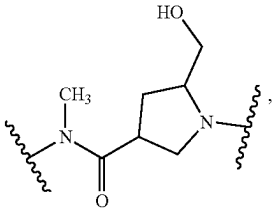
[73] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [72], wherein the linker has the structure:

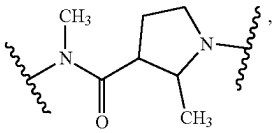


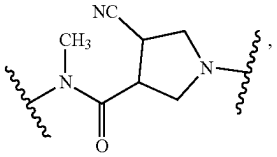


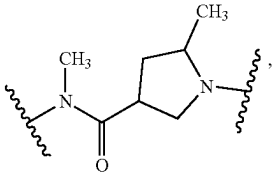












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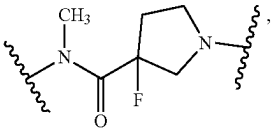

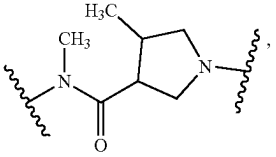

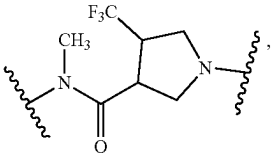

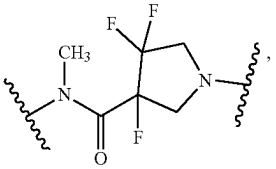

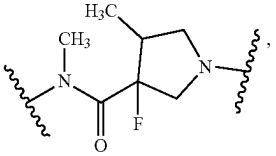

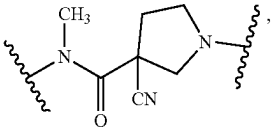

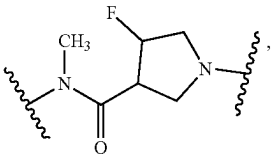

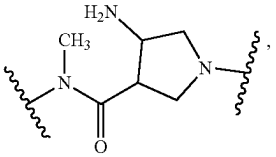

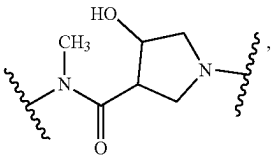

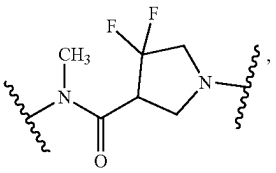

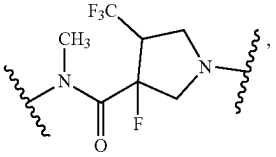

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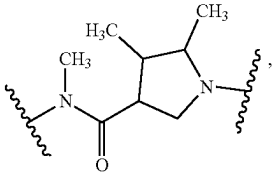

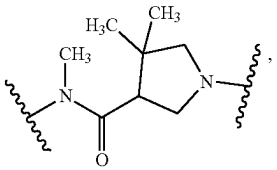

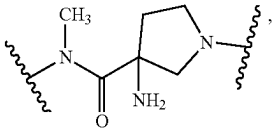

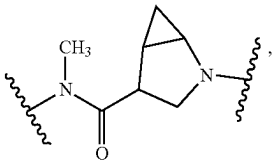

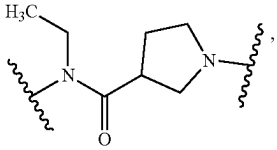

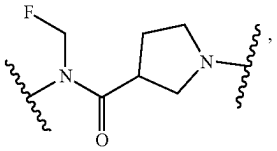

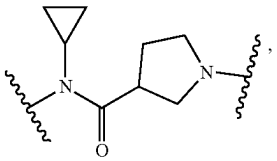

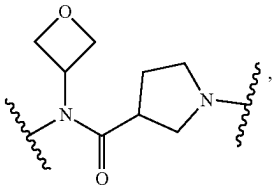

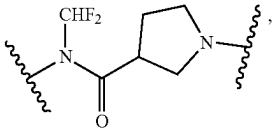

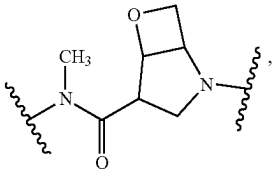

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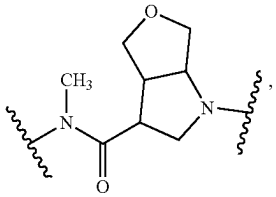

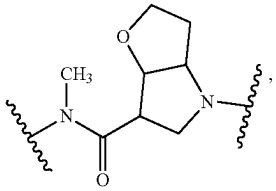

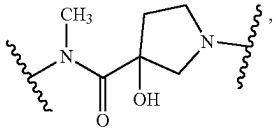

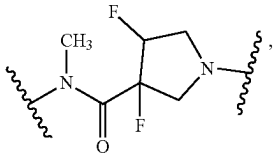

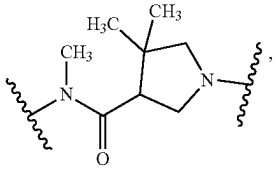

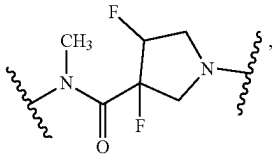

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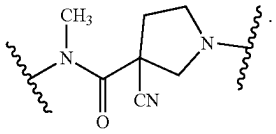
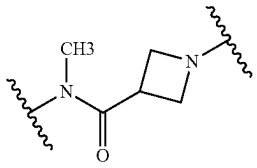
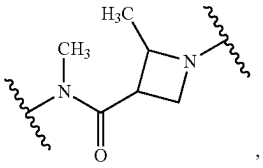
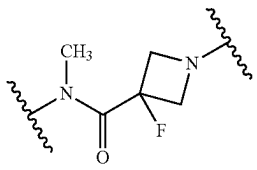


[74] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [71], wherein the linker has the structure:







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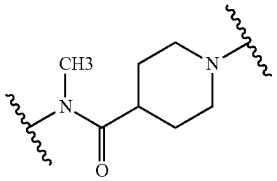

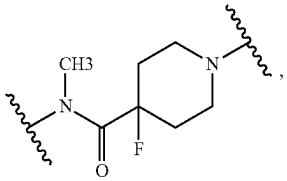

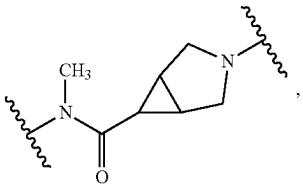

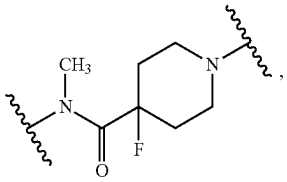

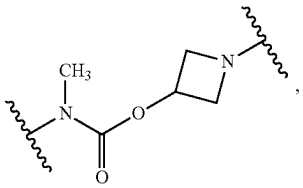

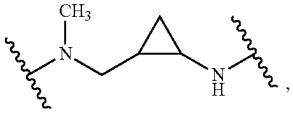

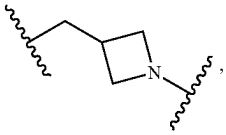

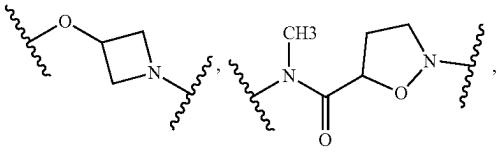

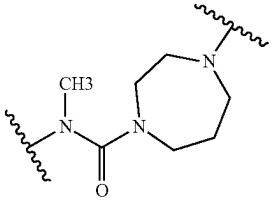

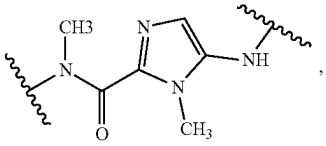

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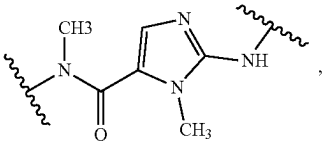

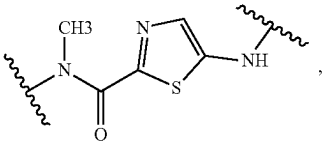

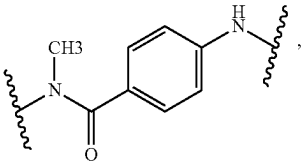

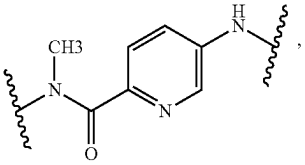

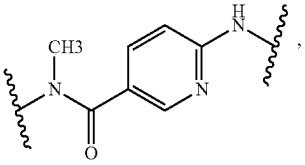

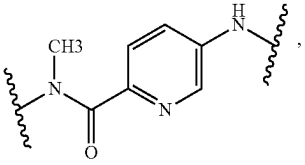

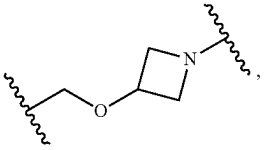

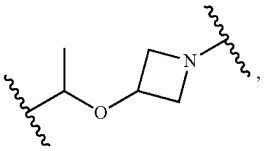

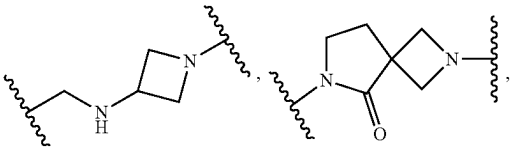

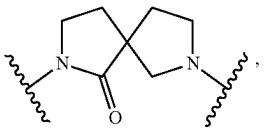

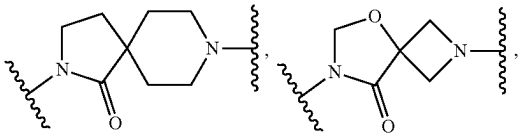

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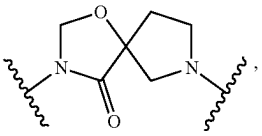

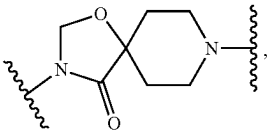

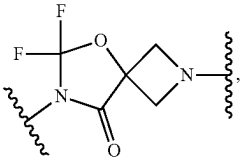

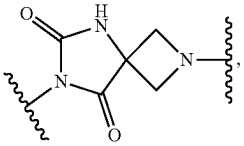

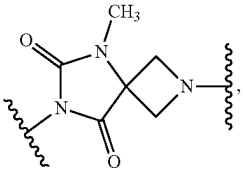

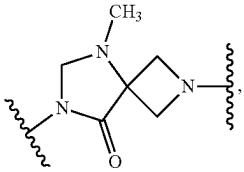

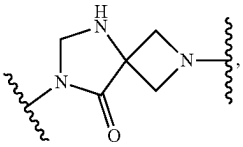

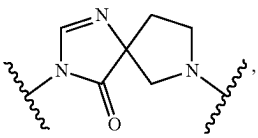

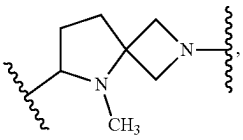

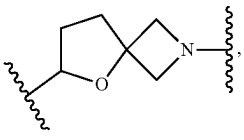

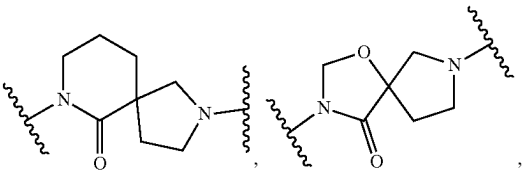

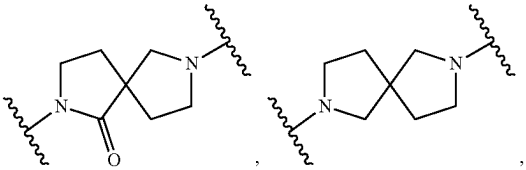

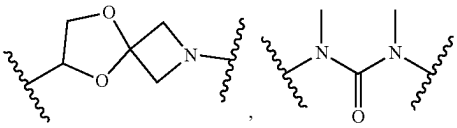

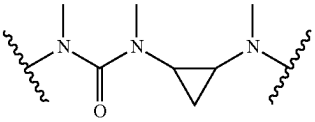

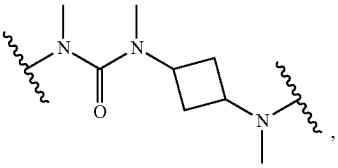

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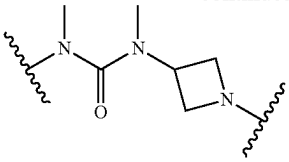

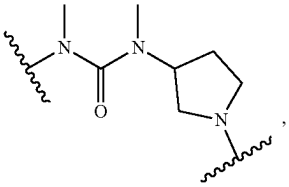

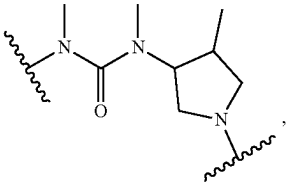

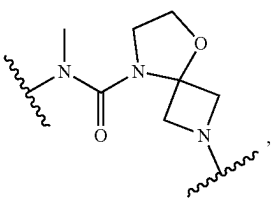

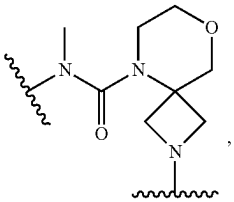

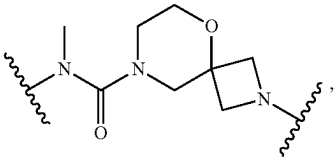

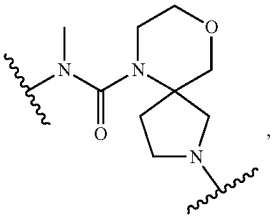

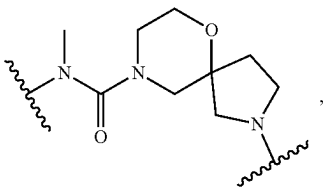

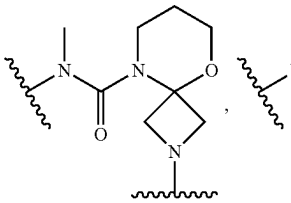

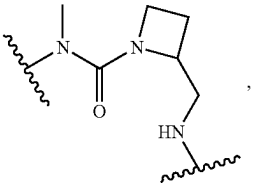

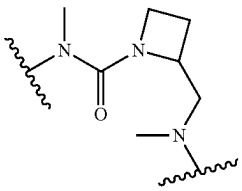

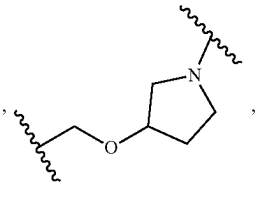

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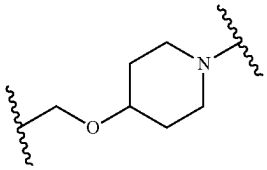

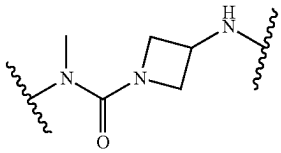

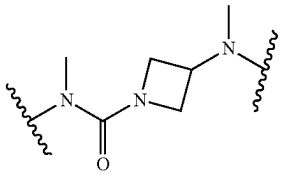

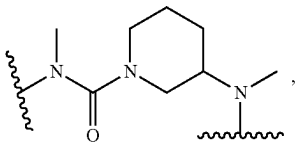

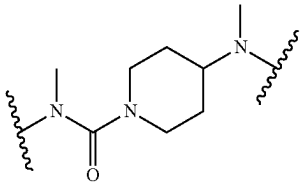

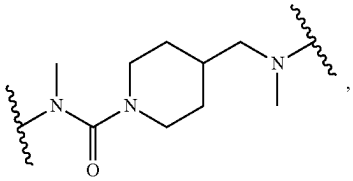

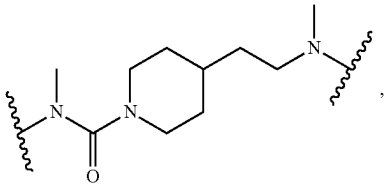

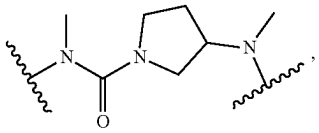

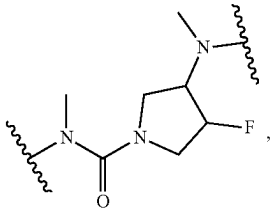

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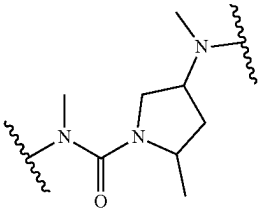

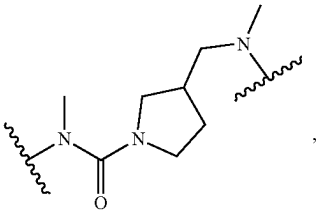

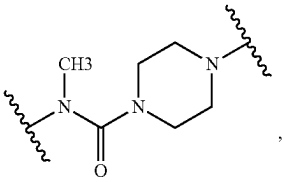

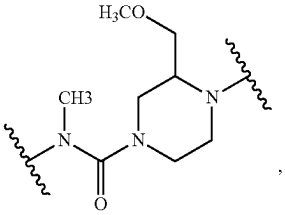

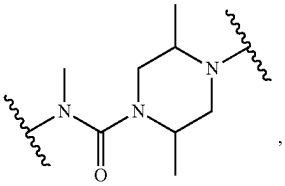

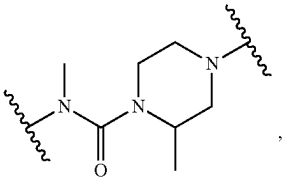

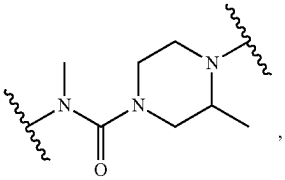

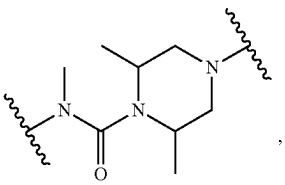

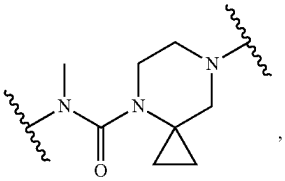

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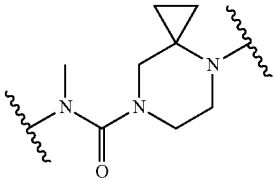

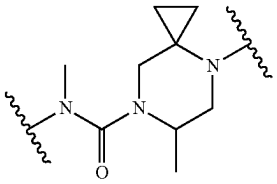

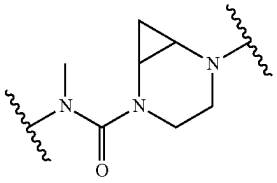

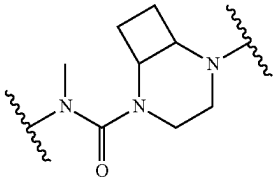

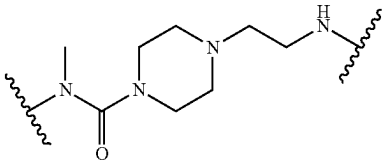

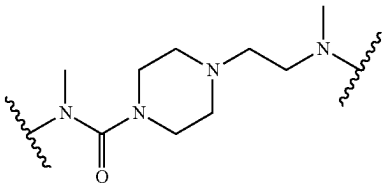

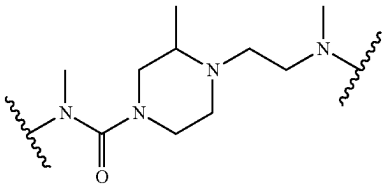

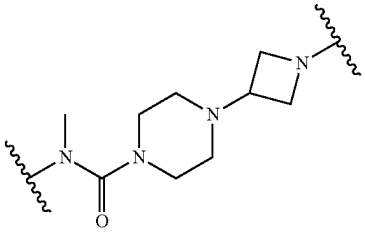

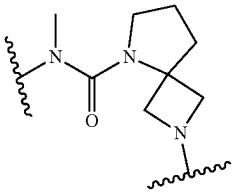

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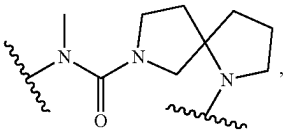

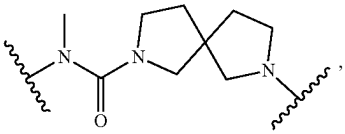

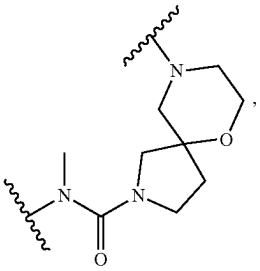

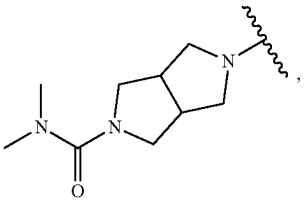

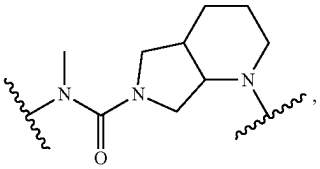

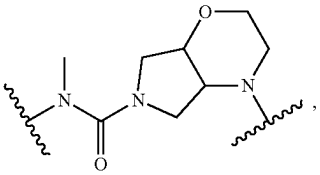

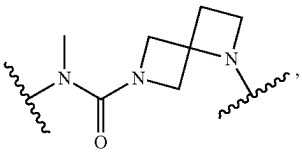

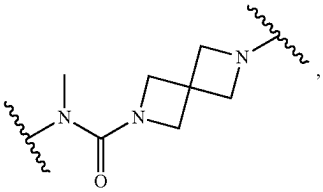

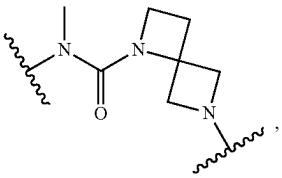

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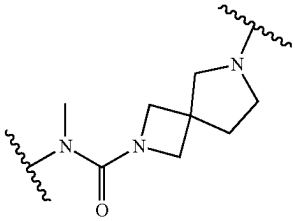

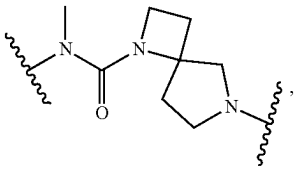

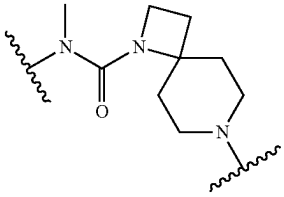

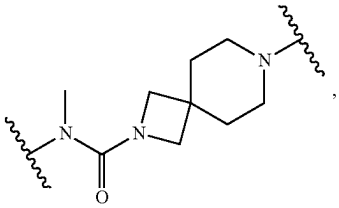

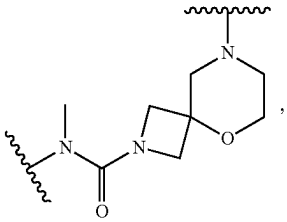

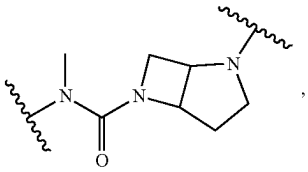

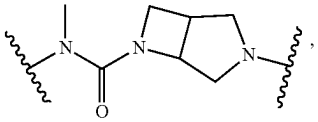

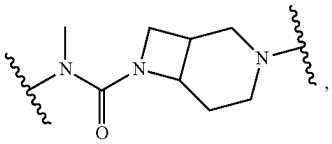

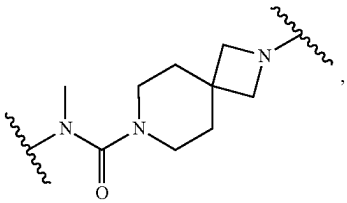

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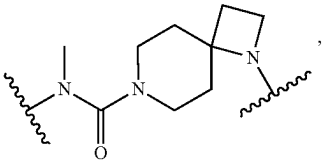

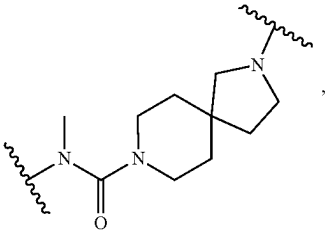

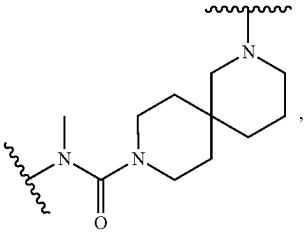

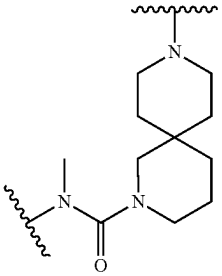

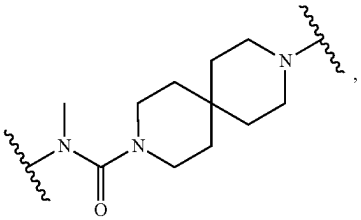

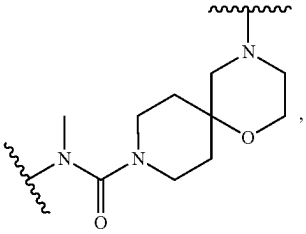

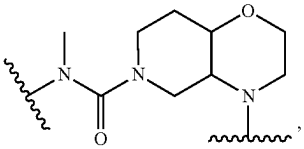

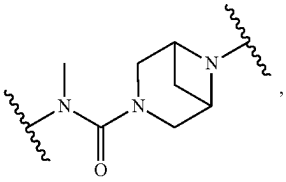

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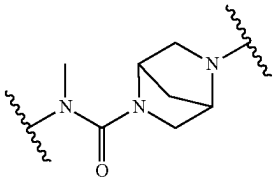
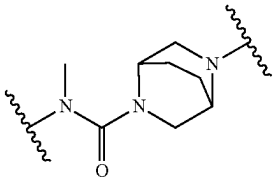
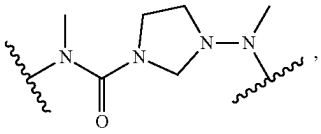
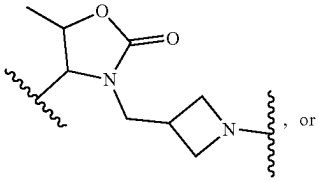
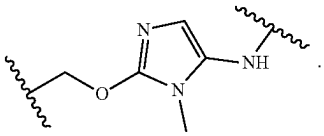






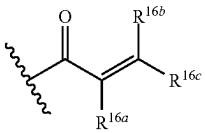




[75] The compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [74], wherein W is a cross-linking group comprising a vinyl ketone.

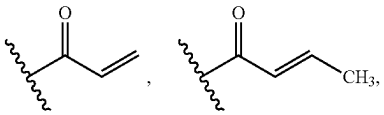
[76]. The compound, or a pharmaceutically acceptable salt thereof, of paragraph [75], wherein W has the structure of Formula IIIa:

Formula IIIa



wherein R^{16a}, R^{16b}, and R^{16c} are, independently, hydrogen, —CN, halogen, or —C_1-C_3 alkyl optionally substituted with one or more substituents independently selected from —OH, —O—C_1-C_3 alkyl, —NH_2, —NH (C_1-C_3 alkyl), —N(C_1-C_3 alkyl)$_2$, or a 4 to 7-membered saturated heterocycloalkyl.

[77] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [76], wherein W is:



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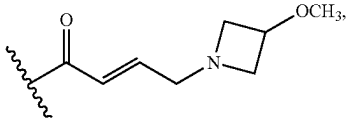
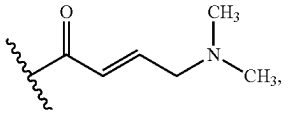
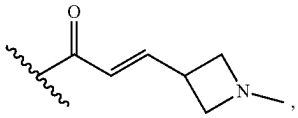
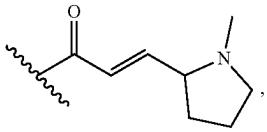
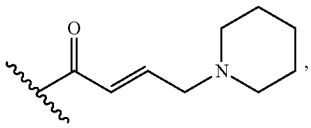
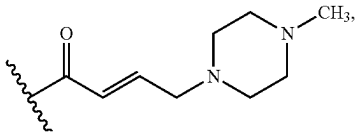


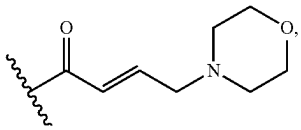


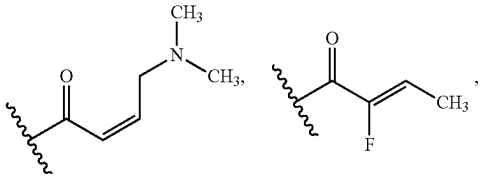


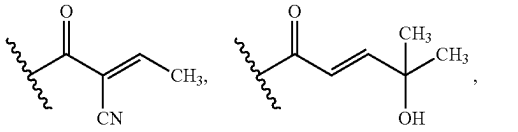


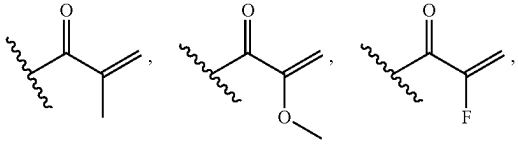


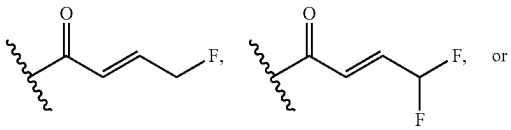


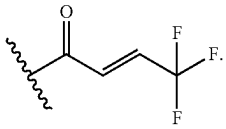








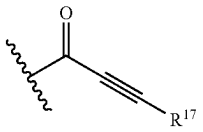




[78] The compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [74], wherein W is a cross-linking group comprising an ynone.

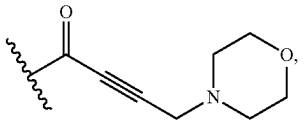
[79] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [78], wherein W has the structure of Formula IIIb:

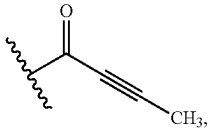
Formula IIIb

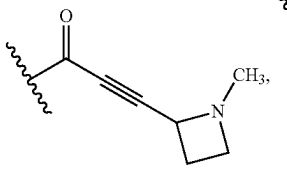


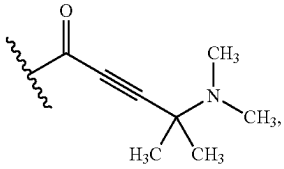
wherein R^{17} is hydrogen, —C_1-C_3 alkyl optionally substituted with one or more substituents independently selected from —OH, —O—C_1-C_3 alkyl, —NH_2, —NH (C_1-C_3 alkyl), —N(C_1-C_3 alkyl)$_2$, or a 4 to 7-membered saturated cycloalkyl, or a 4 to 7-membered saturated heterocycloalkyl.

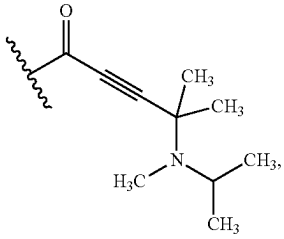
[80] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [79], wherein W is:

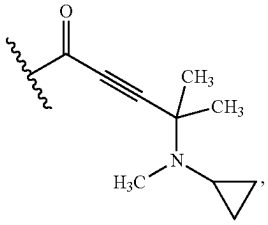


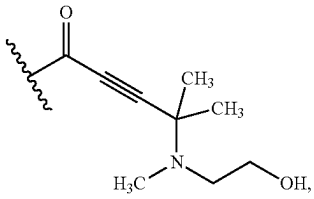












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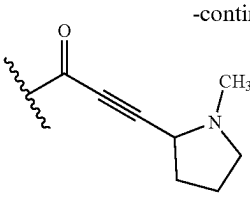

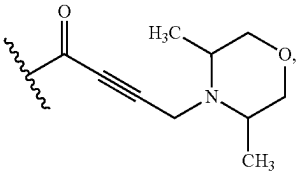

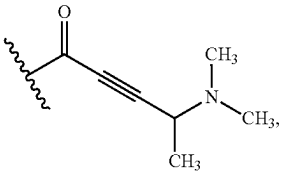

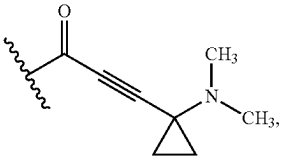

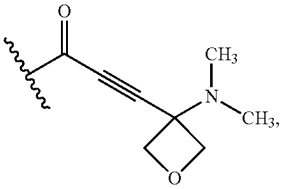

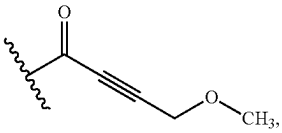

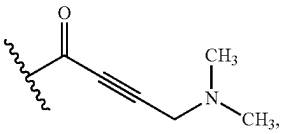

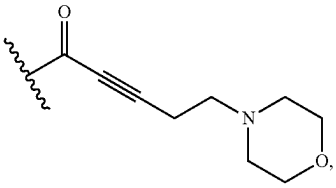

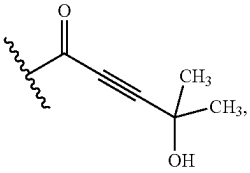

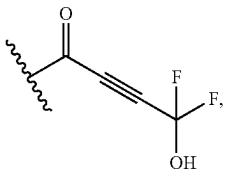

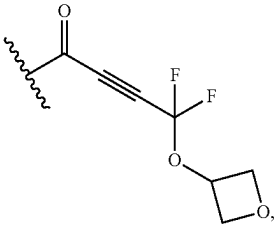

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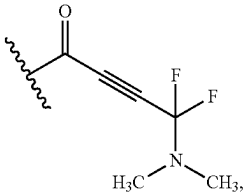

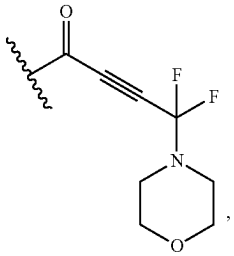

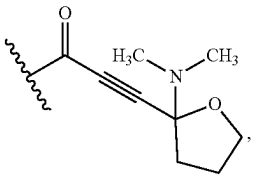

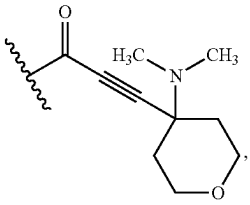

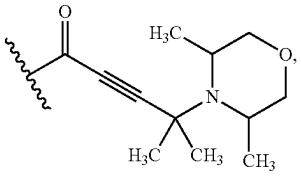

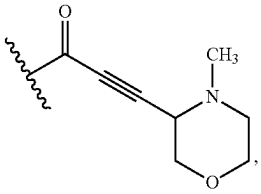

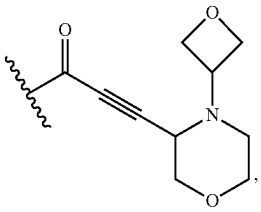

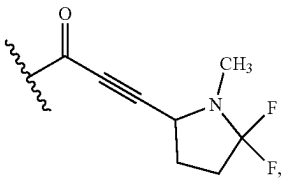

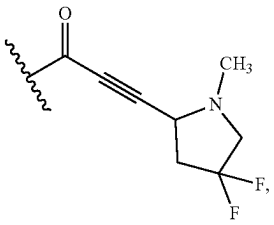

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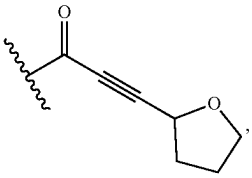

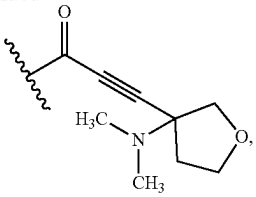

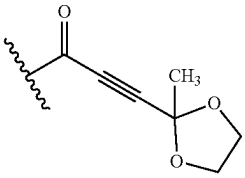

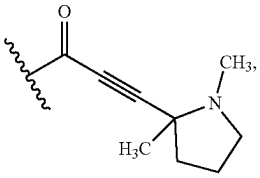

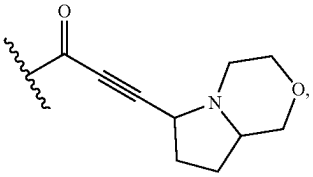

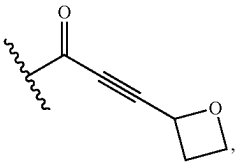

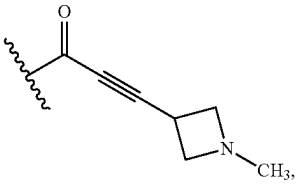

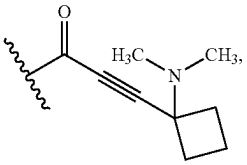

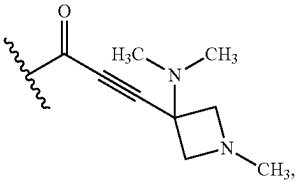

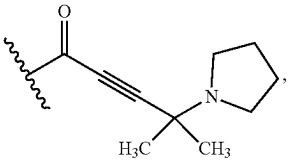

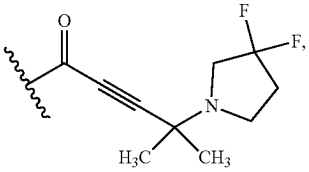

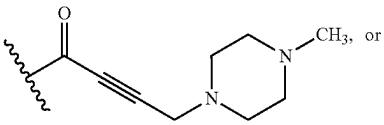

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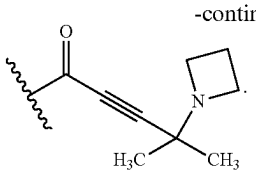
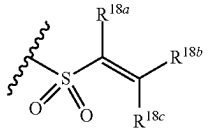


[81] The compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [74], wherein W is a cross-linking group comprising a vinyl sulfone.

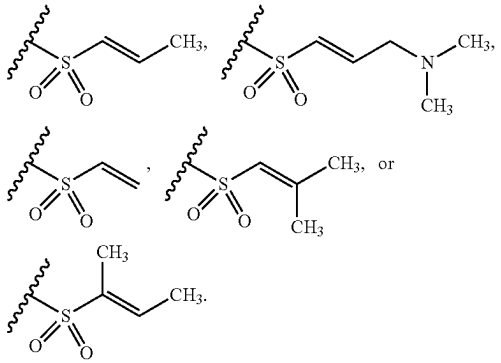
[82] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [81], wherein W has the structure of Formula IIIc:

Formula IIIc



wherein R^{18a}, R^{18b}, and R^{18c} are, independently, hydrogen, —CN, or —C_1-C_3 alkyl optionally substituted with one or more substituents independently selected from —OH, —O—C_1-C_3 alkyl, —NH_2, —NH(C_1-C_3 alkyl), —N(C_1-C_3 alkyl)$_2$, or a 4 to 7-membered saturated heterocycloalkyl.

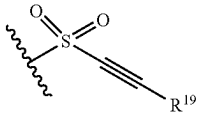
[83] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [82], wherein W is:



[84] The compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [74], wherein W is a cross-linking group comprising an alkynyl sulfone.

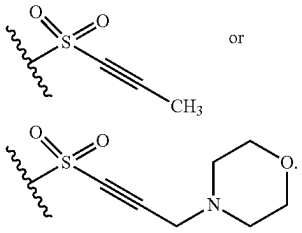
[85] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [84], wherein W has the structure of Formula IIId:

Formula IIId



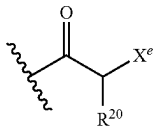
wherein R^{19} is hydrogen, —C_1-C_3 alkyl optionally substituted with one or more substituents independently selected from —OH, —O—C_1-C_3 alkyl, —NH_2, —NH(C_1-C_3 alkyl), —N(C_1-C_3 alkyl)$_2$, or a 4 to 7-membered saturated heterocycloalkyl, or a 4 to 7-membered saturated heterocycloalkyl.

[86] The compound, or a pharmaceutically acceptable salt thereof, of paragraph [85], wherein W is:



[87] The compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [74], wherein W has the structure of Formula IIIe:

Formula IIIe



wherein X^e is a halogen; and

R^{20} is hydrogen, —C_1-C_3 alkyl optionally substituted with one or more substituents independently selected from —OH, —O—C_1-C_3 alkyl, —NH_2, —NH(C_1-C_3 alkyl), —N(C_1-C_3 alkyl)$_2$, or a 4 to 7-membered saturated heterocycloalkyl.

[88] A compound, or a pharmaceutically acceptable salt thereof, selected from Table 1 or Table 2.

[89] A pharmaceutical composition comprising a compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [88], and a pharmaceutically acceptable excipient.

[90] A conjugate, or salt thereof, comprising the structure of Formula IV:

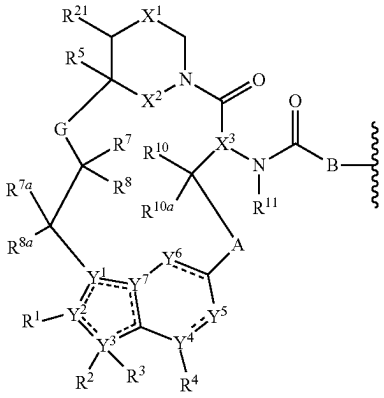
M-L-P Formula IV

wherein L is a linker;

P is a monovalent organic moiety; and

M has the structure of Formula V:

Formula V



wherein the dotted lines represent zero, one, two, three, or four non-adjacent double bonds;

A is —N(H or CH_3)C(O)—(CH_2)— where the amino nitrogen is bound to the carbon atom of —CH(R^{10})—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is absent, —CH(R^9)—, >C=C$R^9R^{9'}$, or >C$R^9R^{9'}$ where the carbon is bound to the carbonyl carbon of —N(R^{11})C(O)—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

G is optionally substituted C_1-C_4 alkylene, optionally substituted C_1-C_4 alkenylene, optionally substituted C_1-C_4 heteroalkylene, —C(O)O—CH(R^6)— where C is bound to —C(R^7R^8)—, —C(O)NH—CH(R^6)— where C is bound to —C(R^7R^8)—, optionally substituted C_1-C_4 heteroalkylene, or 3 to 8-membered heteroarylene;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or $S(O)_n$;

X^2 is O or NH;

X^3 is N or CH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

Y^1 is C, CH, or N;

Y^2, Y^3, Y^4, and Y^7 are, independently, C or N;

Y^5 is CH, CH_2, or N;

Y^6 is C(O), CH, CH_2, or N;

R^1 is cyano, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 6-membered cycloalkenyl, optionally substituted 3 to 6-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted 5 to 10-membered heteroaryl, or

R^1 and R^2 combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

R^2 is absent, hydrogen, optionally substituted C_1-C_6 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 6-membered cycloalkyl, optionally substituted 3 to 7-membered heterocycloalkyl, optionally substituted 6-membered aryl, optionally substituted 5 or 6-membered heteroaryl; R^3 is absent, or

R^2 and R^3 combine with the atom to which they are attached to form an optionally substituted 3 to 8-membered cycloalkyl or optionally substituted 3 to 14-membered heterocycloalkyl;

R^4 is absent, hydrogen, halogen, cyano, or methyl optionally substituted with 1 to 3 halogens;

R^5 is hydrogen, C_1-C_4 alkyl optionally substituted with halogen, cyano, hydroxy, or C_1-C_4 alkoxy, cyclopropyl, or cyclobutyl;

R^6 is hydrogen or methyl; R^7 is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl, or

R^6 and R^7 combine with the carbon atoms to which they are attached to form an optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^8 is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

R^7 and R^8 combine with the carbon atom to which they are attached to form C=C$R^{7'}R^{8'}$; C=N(OH), C=N(O—C_1-C_3 alkyl), C=O, C=S, C=NH, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

R^{7a} and R^{8a} are, independently, hydrogen, halo, optionally substituted C_1-C_3 alkyl, or combine with the carbon to which they are attached to form a carbonyl;

$R^{7'}$ is hydrogen, halogen, or optionally substituted C_1-C_3 alkyl; $R^{8'}$ is hydrogen, halogen, hydroxy, cyano, optionally substituted C_1-C_3 alkoxy, optionally substituted C_1-C_3 alkyl, optionally substituted C_2-C_6 alkenyl, optionally substituted C_2-C_6 alkynyl, optionally substituted 3 to 8-membered cycloalkyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 5 to 10-membered heteroaryl, or optionally substituted 6 to 10-membered aryl, or

$R^{7'}$ and $R^{8'}$ combine with the carbon atom to which they are attached to form optionally substituted 3 to 6-membered cycloalkyl or optionally substituted 3 to 7-membered heterocycloalkyl;

R^9 is H, F, optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl, or

R^9 and L combine with the atoms to which they are attached to form an optionally substituted 3 to 14-membered heterocycloalkyl;

$R^{9'}$ is hydrogen or optionally substituted C_1-C_6 alkyl; or

R^9 and $R^{9'}$, combined with the atoms to which they are attached, form a 3 to 6-membered cycloalkyl or a 3 to 6-membered heterocycloalkyl;

R^{10} is hydrogen, halo, hydroxy, C_1-C_3 alkoxy, or C_1-C_3 alkyl;

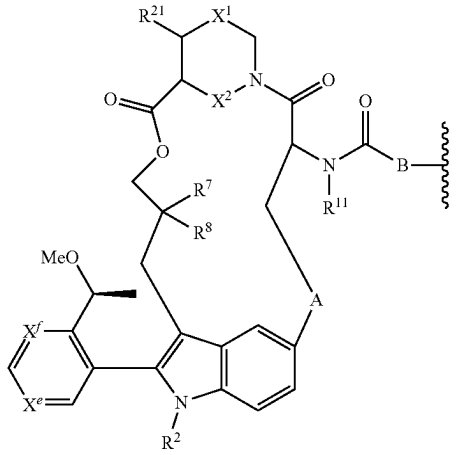
R^{10a} is hydrogen or halo;

R^{11} is hydrogen or C_1-C_3 alkyl; and

R^{21} is H or C_1-C_3 alkyl.

[91] The conjugate of paragraph [90], or salt thereof, wherein M has the structure of Formula Vd:

Formula Vd



wherein A is optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is —CH(R^9)— where the carbon is bound to the carbonyl carbon of —NHC(O)—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene;

X^1 is optionally substituted C_1-C_2 alkylene, NR, O, or $S(O)_n$;

X^2 is O or NH;

n is 0, 1, or 2;

R is hydrogen, cyano, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, C(O)R', C(O)OR', C(O)N(R')$_2$, S(O)R', S(O)$_2$R', or S(O)$_2$N(R')$_2$;

each R' is, independently, H or optionally substituted C_1-C_4 alkyl;

R^2 is C_1-C_6 alkyl, C_1-C_6 fluoroalkyl, or 3 to 6-membered cycloalkyl;

R^7 is C_1-C_3 alkyl;

R^8 is C_1-C_3 alkyl; and

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl;

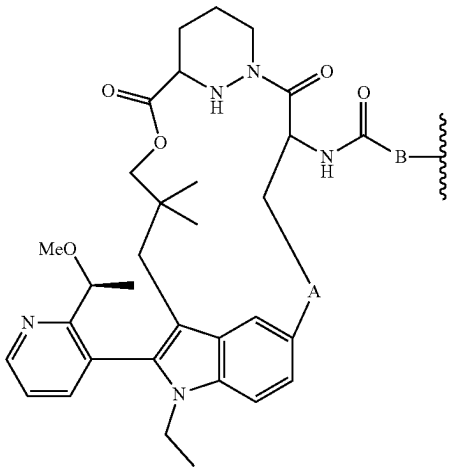
X^e and X^1 are, independently, N or CH;

R^{11} is hydrogen or C_1-C_3 alkyl; and

R^{21} is hydrogen or C_1-C_3 alkyl.

[92] The conjugate of paragraph [91], or salt thereof, wherein M has the structure of Formula Ve:

Formula Ve



wherein A is optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or optionally substituted 5 to 6-membered heteroarylene;

B is —CH(R^9)— where the carbon is bound to the carbonyl carbon of —NHC(O)—, optionally substituted 3 to 6-membered cycloalkylene, optionally substituted 3 to 6-membered heterocycloalkylene, optionally substituted 6-membered arylene, or 5 to 6-membered heteroarylene; and

R^9 is optionally substituted C_1-C_6 alkyl, optionally substituted C_1-C_6 heteroalkyl, optionally substituted 3 to 6-membered cycloalkyl, or optionally substituted 3 to 7-membered heterocycloalkyl.

[93] The conjugate, or salt thereof, of any one of paragraphs [90] to [92], wherein the linker has the structure of Formula II:

$$A^1\text{-}(B^1)_f\text{—}(C^1)_g\text{—}(B^2)_h\text{-}(D^1)\text{-}(B^3)_i\text{—}(C^2)_j\text{—}(B^4)_k\text{-}A^2 \qquad \text{Formula II}$$

where A^1 is a bond between the linker and B; A^2 is a bond between W and the linker; B^1, B^2, B^3, and B^4 each, independently, is selected from optionally substituted C_1-C_2 alkylene, optionally substituted C_1-C_3 heteroalkylene, O, S, and NR^N; R^N is hydrogen, optionally substituted C_1-C_4 alkyl, optionally substituted C_2-C_4 alkenyl, optionally substituted C_2-C_4 alkynyl, optionally substituted 3 to 14-membered heterocycloalkyl, optionally substituted 6 to 10-membered aryl, or optionally substituted C_1-C_7 heteroalkyl; C^1 and C^2 are each, independently, selected from carbonyl, thiocarbonyl, sulphonyl, or phosphoryl; f, g, h, i, j, and k are each, independently, 0 or 1; and D^1 is optionally substituted C_1-C_{10} alkylene, optionally substituted C_2-C_{10} alkenylene, optionally substituted C_2-C_{10} alkynylene, optionally substituted 3 to 14-membered heterocycloalkylene, optionally substituted 5 to 10-membered heteroarylene, optionally substituted 3 to 8-membered cycloalkylene, optionally substituted 6 to 10-membered arylene, optionally substituted C_2-C_{10} polyethylene glycolene, or optionally substituted C_1-C_{10} heteroalkylene, or a chemical bond linking A^1-$(B^1)_f$—$(C^1)_g$—$(B^2)_h$— to —$(B^3)_i$—$(C^2)_j$—$(B^4)_k$-A^2.

[94] The conjugate, or salt thereof, of any one of paragraphs [90] to [93], wherein the monovalent organic moiety is a protein.

[95] The conjugate, or salt thereof, of paragraph [94], wherein the protein is a Ras protein.

[96] The conjugate, or salt thereof, of paragraph [95], wherein the Ras protein is K-Ras G12C, K-Ras G13C, H-Ras G12C, H-Ras G13C, N-Ras G12C, or N-Ras G13C.

[97] The conjugate, or salt thereof, of any one of paragraphs [93] to [96], wherein the linker is bound to the monovalent organic moiety through a bond to a sulfhydryl group of an amino acid residue of the monovalent organic moiety.

[98] A method of treating cancer in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [88] or a pharmaceutical composition of paragraph [89].

[99] The method of paragraph [98], wherein the cancer is pancreatic cancer, colorectal cancer, non-small cell lung cancer, or endometrial cancer.

[100] The method of paragraph [98] or [99], wherein the cancer comprises a Ras mutation.

[101] The method of paragraph [100], wherein the Ras mutation is K-Ras G12C, K-Ras G13C, H-Ras G12C, H-Ras G13C, N-Ras G12C, or N-Ras G13C.

[102] A method of treating a Ras protein-related disorder in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a compound, or a pharmaceutically accept-

able salt thereof, of any one of paragraphs [1] to [88] or a pharmaceutical composition of paragraph [89].

[103] A method of inhibiting a Ras protein in a cell, the method comprising contacting the cell with an effective amount of a compound, or a pharmaceutically acceptable salt thereof, of any one of paragraphs [1] to [88] or a pharmaceutical composition of paragraph [89].

[104] The method of paragraph [102] or [103], wherein the Ras protein is K-Ras G12C, K-Ras G13C, H-Ras G12C, H-Ras G13C, N-Ras G12C, or N-Ras G13C.

[105] The method of paragraph [103] or [104], wherein the cell is a cancer cell.

[106] The method of paragraph [105], wherein the cancer cell is a pancreatic cancer cell, a colorectal cancer cell, a non-small cell lung cancer cell, or an endometrial cancer cell.

[107] The method or use of any one of paragraphs [98] to [106], wherein the method or use further comprises administering an additional anti-cancer therapy.

[108] The method of paragraph [107], wherein the additional anti-cancer therapy is an EGFR inhibitor, a second Ras inhibitor, a SHP2 inhibitor, a SOS1 inhibitor, a Raf inhibitor, a MEK inhibitor, an ERK inhibitor, a PI3K inhibitor, a PTEN inhibitor, an AKT inhibitor, an mTORC1 inhibitor, a BRAF inhibitor, a PD-L1 inhibitor, a PD-1 inhibitor, a CDK4/6 inhibitor, a HER2 inhibitor, or a combination thereof.

[109] The method of paragraph [107] or [108], wherein the additional anti-cancer therapy is a SHP2 inhibitor.

EXAMPLES

The disclosure is further illustrated by the following examples and synthesis examples, which are not to be construed as limiting this disclosure in scope or spirit to the specific procedures herein described. It is to be understood that the examples are provided to illustrate certain embodiments and that no limitation to the scope of the disclosure is intended thereby. It is to be further understood that resort may be had to various other embodiments, modifications, and equivalents thereof which may suggest themselves to those skilled in the art without departing from the spirit of the present disclosure or scope of the appended claims.

Chemical Syntheses

Definitions used in the following examples and elsewhere herein are:

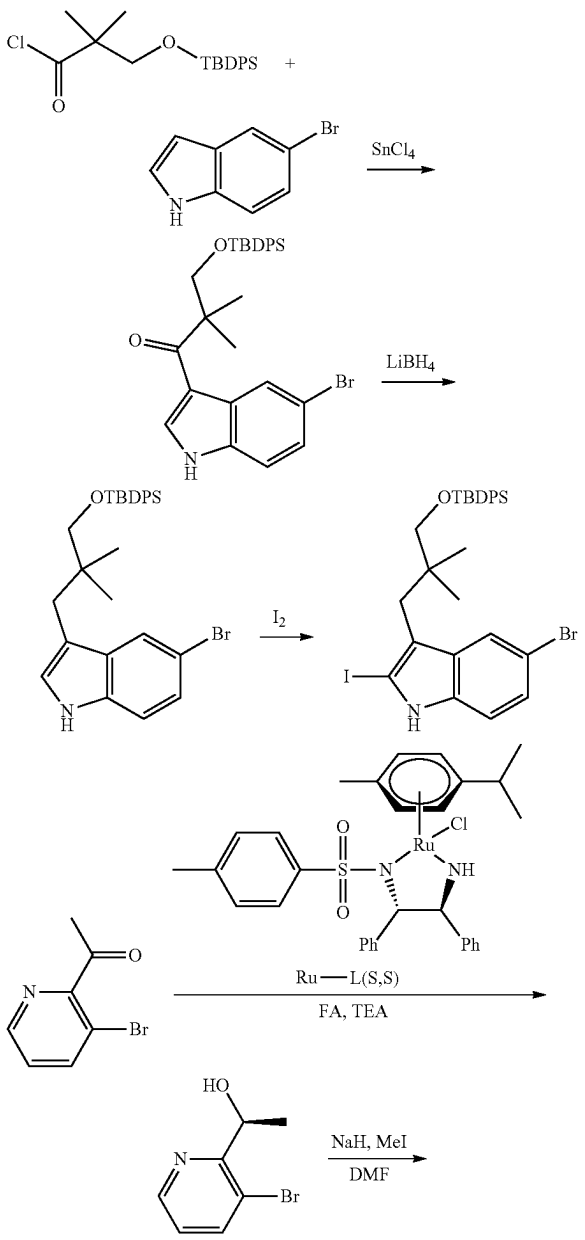
CH_2Cl_2, DCM	Methylene chloride, Dichloromethane
CH_3CN, MeCN	Acetonitrile
CuI	Copper (I) iodide
DIPEA	Diisopropylethyl amine
DMF	N,N-Dimethylformamide
EtOAc	Ethyl acetate
h	hour
H_2O	Water
HCl	Hydrochloric acid
K_3PO_4	Potassium phosphate (tribasic)
MeOH	Methanol
Na_2SO_4	Sodium sulfate
NMP	N-methyl pyrrolidone
$Pd(dppf)Cl_2$	[1,1'-Bis(diphenylphosphino)ferrocene]dichloropalladium(II)

Instrumentation

Mass spectrometry data collection took place with a Shimadzu LCMS-2020, an Agilent 1260LC-6120/6125MSD, a Shimadzu LCMS-2010EV, or a Waters Acquity UPLC, with either a QDa detector or SQ Detector 2. Samples were injected in their liquid phase onto a C-18 reverse phase. The compounds were eluted from the column using an acetonitrile gradient and fed into the mass analyzer. Initial data analysis took place with either Agilent ChemStation, Shimadzu LabSolutions, or Waters MassLynx. NMR data was collected with either a Bruker AVANCE III HD 400 MHz, a Bruker Ascend 500 MHz instrument, or a Varian 400 MHz, and the raw data was analyzed with either TopSpin or Mestrelab Mnova.

Synthesis of Intermediates

Intermediate 1. Synthesis of 3-(5-bromo-1-ethyl-2-[2-[(1S)-1-methoxyethyl]pyridin-3-yl]indol-3-yl)-2,2-dimethylpropan-1-ol



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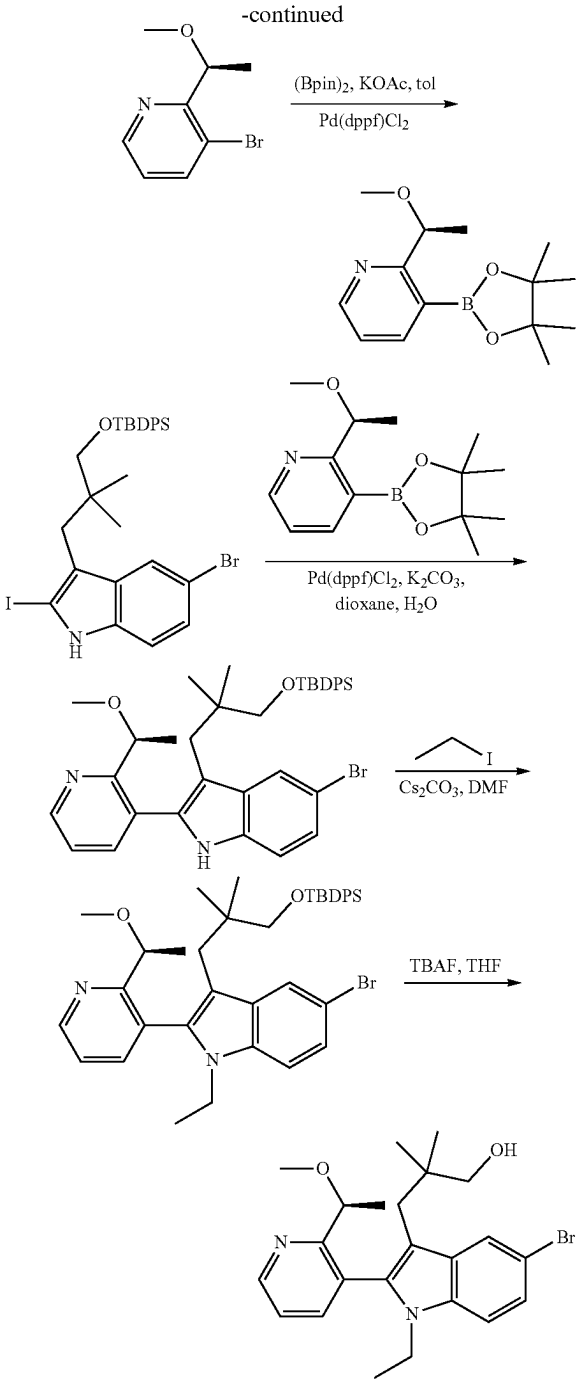


Intermediate 1.

Step 1.

To a mixture of 3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropanoyl chloride (65 g, 137 mmol, crude) in DCM (120 mL) at 0° C. under an atmosphere of N_2 was added 1M $SnCl_4$ in DCM (137 mL, 137 mmol) slowly. The mixture was stirred at 0° C. for 30 min, then a solution of 5-bromo-1H-indole (26.8 g, 137 mmol) in DCM (40 mL) was added dropwise. The mixture was stirred at 0° C. for 45 min, then diluted with EtOAc (300 mL), washed with brine (100 mL×4), dried over Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 1-(5-bromo-1H-indol-3-yl)-3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropan-1-one (55 g, 75% yield). LCMS (ESI): m/z [M+Na] calc'd for $C_{29}H_{32}BrNO_2SiNa$ 556.1; found 556.3.

Step 2.

To a mixture of 1-(5-bromo-1H-indol-3-yl)-3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropan-1-one (50 g, 93.6 mmol) in THF (100 mL) at 0° C. under an atmosphere of N_2 was added $LiBH_4$ (6.1 g, 281 mmol). The mixture was heated to 60° C. and stirred for 20 h, then MeOH (10 mL) and EtOAc (100 mL) were added and the mixture washed with brine (50 mL), dried over Na_2SO_4, filtered, and the filtrate concentrated under reduced pressure. The residue was diluted with DCM (50 mL), cooled to 10° C. and diludine (9.5 g, 37.4 mmol) and TsOH·H_2O (890 mg, 4.7 mmol) added. The mixture was stirred at 10° C. for 2 h, filtered, the filtrate concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 1-(5-bromo-1H-indol-3-yl)-3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropan-1-one (41 g, 84% yield). LCMS (ESI): m/z [M+H] calc'd for $C_{29}H_{34}BrNOSi$ 519.2; found 520.1; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.96 (s, 1H), 7.75-7.68 (m, 5H), 7.46-7.35 (m, 6H), 7.23-7.19 (m, 2H), 6.87 (d, J=2.1 Hz, 1H), 3.40 (s, 2H), 2.72 (s, 2H), 1.14 (s, 9H), 0.89 (s, 6H).

Step 3.

To a mixture of 1-(5-bromo-1H-indol-3-yl)-3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropan-1-one (1.5 g, 2.9 mmol) and 12 (731 mg, 2.9 mmol) in THF (15 mL) at rt was added AgOTf (888 mg, 3.5 mmol). The mixture was stirred at rt for 2 h, then diluted with EtOAc (200 mL) and washed with saturated $Na_2S_2O_3$ (100 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 5-bromo-3-(3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-iodo-1H-indole (900 mg, 72% yield) as a solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.70 (s, 1H), 7.68 (d, J=1.3 Hz, 1H), 7.64-7.62 (m, 4H), 7.46-7.43 (m, 6H), 7.24-7.22 (d, 1H), 7.14-7.12 (dd, J=8.6, 1.6 Hz, 1H), 3.48 (s, 2H), 2.63 (s, 2H), 1.08 (s, 9H), 0.88 (s, 6H).

Step 4.

To a stirred mixture of HCOOH (66.3 g, 1.44 mol) in TEA (728 g, 7.2 mol) at 0° C. under an atmosphere of Ar was added (4S,5S)-2-chloro-2-methyl-1-(4-methylbenzenesulfonyl)-4,5-diphenyl-1,3-diaza-2-ruthenacyclopentane cymene (3.9 g, 6.0 mmol) portion-wise. The mixture was heated to 40° C. and stirred for 15 min, then cooled to rt and 1-(3-bromopyridin-2-yl)ethanone (120 g, 600 mmol) added in portions. The mixture was heated to 40° C. and stirred for an additional 2 h, then the solvent was concentrated under reduced pressure. Brine (2 L) was added to the residue, the mixture was extracted with EtOAc (4×700 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give (1 S)-1-(3-bromopyridin-2-yl)ethanol (100 g, 74% yield) a an oil. LCMS (ESI): m/z [M+H] calc'd for C_7H_8BrNO 201.1; found 201.9.

Step 5.

To a stirred mixture of (1 S)-1-(3-bromopyridin-2-yl) ethanol (100 g, 495 mmol) in DMF (1 L) at 0° C. was added NaH, 60% dispersion in oil (14.25 g, 594 mmol) in portions. The mixture was stirred at 0° C. for 1 h. MeI (140.5 g, 990 mmol) was added dropwise at 0° C. and the mixture was allowed to warm to rt and stirred for 2 h. The mixture was cooled to 0° C. and saturated NH_4Cl (5 L) was added. The mixture was extracted with EtOAc (3×1.5 L), dried over

anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 3-bromo-2-[(1S)-1-methoxyethyl]pyridine (90 g, 75% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_8H_{10}BrNO$ 215.0; found 215.9.

Step 6.

To a stirred mixture of 3-bromo-2-[(1S)-1-methoxyethyl]pyridine (90 g, 417 mmol) and $Pd(dppf)Cl_2$ (30.5 g, 41.7 mmol) in toluene (900 mL) at rt under an atmosphere of Ar was added bis(pinacolato)diboron (127 g, 500 mmol) and KOAc (81.8 g, 833 mmol) in portions. The mixture was heated to 100° C. and stirred for 3 h. The filtrate was concentrated under reduced pressure and the residue was purified by Al_2O_3 column chromatography to give 2-[(1 S)-1-methoxyethyl]-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (100 g, 63% yield) as a semi-solid. LCMS (ESI): m/z [M+H] calc'd for $C_{14}H_{22}BNO_3$ 263.2; found 264.1.

Step 7.

To a stirred mixture of 5-bromo-3-[3-[(tert-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-2-iodo-1H-indole (140 g, 217 mmol) and 2-[(1 S)-1-methoxyethyl]-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (100 g, 380 mmol) in 1,4-dioxane (1.4 L) at rt under an atmosphere of Ar was added K_2CO_3 (74.8 g, 541 mmol), $Pd(dppf)Cl_2$ (15.9 g, 21.7 mmol), and H_2O (280 mL) in portions. The mixture was heated to 85° C. and stirred for 4 h, then cooled, H_2O (5 L) added, and the mixture extracted with EtOAc (3×2 L). The combined organic layers were washed with brine (2×1 L), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 5-bromo-3-[3-[(tert-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-2-[2-[(1 S)-1-methoxyethyl]pyridin-3-yl]-1H-indole (71 g, 45% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{37}H_{43}BrN_2O_2Si$ 654.2; found 655.1.

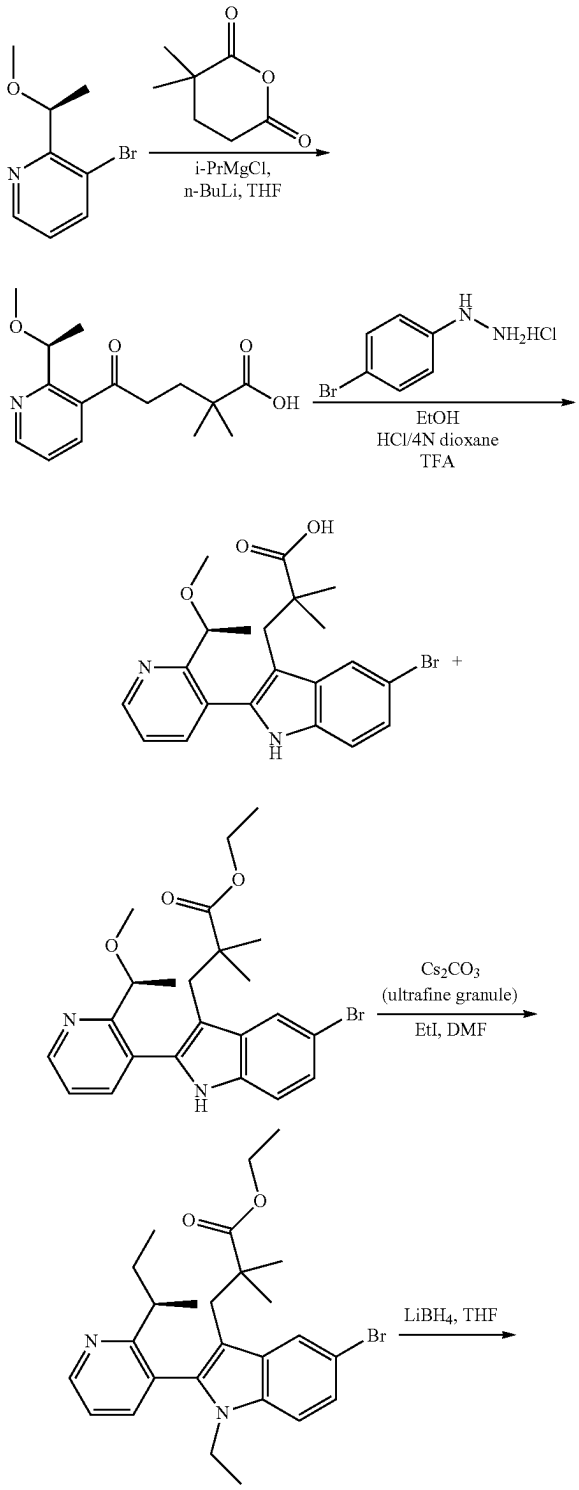
Step 8.

To a stirred mixture of 5-bromo-3-[3-[(tert-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-2-[2-[(1S)-1-methoxyethyl]pyridin-3-yl]-1H-indole (71 g, 108 mmol) in DMF (0.8 L) at 0° C. under an atmosphere of N_2 was added Cs_2CO_3 (70.6 g, 217 mmol) and EtI (33.8 g, 217 mmol) in portions. The mixture was warmed to rt and stirred for 16 h then H_2O (4 L) added and the mixture extracted with EtOAc (3×1.5 L). The combined organic layers were washed with brine (2×1 L), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 5-bromo-3-[3-[(tert-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-1-ethyl-2-[2-[(1S)-1-methoxyethyl]pyridin-3-yl]indole (66 g, 80% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{39}H_{47}BrN_2O_2Si$ 682.3; found 683.3.

Step 9.

To a stirred mixture of TBAF (172.6 g, 660 mmol) in THF (660 mL) at rt under an atmosphere of N_2 was added 5-bromo-3-[3-[(tert-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-1-ethyl-2-[2-[(1 S)-1-methoxyethyl]pyridin-3-yl]indole (66 g, 97 mmol) in portions. The mixture was heated to 50° C. and stirred for 16 h, cooled, diluted with H_2O (5 L), and extracted with EtOAc (3×1.5 L). The combined organic layers were washed with brine (2×1 L), dried over anhydrous Na_2SO_4, and filtered. After filtration, the filtrate was concentrated under reduced pressure. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 3-(5-bromo-1-ethyl-2-[2-[(1 S)-1-methoxyethyl]pyridin-3-yl]indol-3-yl)-2,2-dimethylpropan-1-ol (30 g, 62% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{23}H_{29}BrN_2O_2$ 444.1; found 445.1.

Intermediate 1. Alternative Synthesis Through Fisher Indole Route



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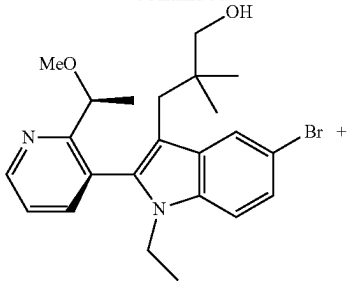
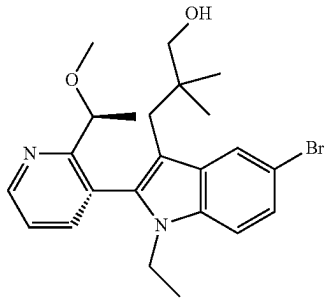


Intermediate 1



Step 1.

To a mixture of i-PrMgCl (2M in in THF, 0.5 L) at −10° C. under an atmosphere of N_2 was added n-BuLi, 2.5 M in hexane (333 mL, 833 mmol) dropwise over 15 min. The mixture was stirred for 30 min at −10° C. then 3-bromo-2-[(1 S)-1-methoxyethyl]pyridine (180 g, 833 mmol) in THF (0.5 L) added dropwise over 30 min at −10° C. The resulting mixture was warmed to −5° C. and stirred for 1 h, then 3,3-dimethyloxane-2,6-dione (118 g, 833 mmol) in THF (1.2 L) was added dropwise over 30 min at −5° C. The mixture was warmed to 0° C. and stirred for 1.5 h, then quenched with the addition of pre-cooled 4M HCl in 1,4-dioxane (0.6 L) at 0° C. to adjust pH~5. The mixture was diluted with ice-water (3 L) and extracted with EtOAc (3×2.5 L). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, the filtrate was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography to give 5-[2-[(1 S)-1-methoxyethyl]pyridin-3-yl]-2,2-dimethyl-5-oxopentanoic acid (87 g, 34% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{15}H_{21}NO_4$ 279.2; found 280.1.

Step 2.

To a mixture of 5-[2-[(1 S)-1-methoxyethyl]pyridin-3-yl]-2,2-dimethyl-5-oxopentanoic acid (78 g, 279 mmol) in EtOH (0.78 L) at rt under an atmosphere of N_2 was added (4-bromophenyl)hydrazine HCl salt (68.7 g, 307 mmol) in portions. The mixture was heated to 85° C. and stirred for 2 h, cooled to rt, then 4M HCl in 1,4-dioxane (69.8 mL, 279 mmol) added dropwise. The mixture was heated to 85° C. and stirred for an additional 3 h, then concentrated under reduced pressure, and the residue was dissolved in TFA (0.78 L). The mixture was heated to 60° C. and stirred for 1.5 h, concentrated under reduced pressure, and the residue adjusted to pH~5 with saturated $NaHCO_3$, then extracted with EtOAc (3×1.5 L). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, the filtrate concentrated under reduced pressure, and the residue was purified by silica gel column chromatography to give 3-(5-bromo-2-[2-[(1 S)-1-methoxyethyl]pyridin-3-yl]-1H-indol-3-yl)-2,2-dimethylpropanoic acid and ethyl (S)-3-(5-bromo-2-(2-(1-methoxyethyl)pyridin-3-yl)-1H-indol-3-yl)-2,2-dimethylpropanoate (78 g, crude). LCMS (ESI): m/z [M+H] calc'd for $C_{21}H_{23}BrN_2O_3$ 430.1 and $C_{23}H_{27}BrN_2O_3$ 458.1; found 431.1 and 459.1.

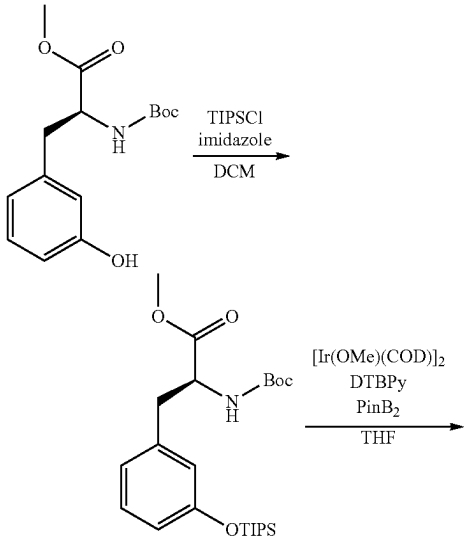
Step 3.

To a mixture of 3-(5-bromo-2-[2-[(1 S)-1-methoxyethyl]pyridin-3-yl]-1H-indol-3-yl)-2,2-dimethylpropanoic acid and ethyl (S)-3-(5-bromo-2-(2-(1-methoxyethyl)pyridin-3-yl)-1H-indol-3-yl)-2,2-dimethylpropanoate (198 g, 459 mmol) in DMF (1.8 L) at 0° C. under an atmosphere of N_2 was added Cs_2CO_3 (449 g, 1.38 mol) in portions. EtI (215 g, 1.38 mmol) in DMF (200 mL) was then added dropwise at 0° C. The mixture was warmed to rt and stirred for 4 h then diluted with brine (5 L) and extracted with EtOAc (3×2.5 L). The combined organic layers were washed with brine (2×1.5 L), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give ethyl 3-(5-bromo-1-ethyl-2-[2-[(1 S)-1-methoxyethyl]pyridin-3-yl]indol-3-yl)-2,2-dimethylpropanoate (160 g, 57% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{25}H_{31}BrN_2O_3$ 486.2; found 487.2.

Step 4.

To a mixture of ethyl 3-(5-bromo-1-ethyl-2-[2-[(1 S)-1-methoxyethyl]pyridin-3-yl]indol-3-yl)-2,2-dimethylpropanoate (160 g, 328 mmol) in THF (1.6 L) at 0° C. under an atmosphere of N_2 was added $LiBH_4$ (28.6 g, 1.3 mol). The mixture was heated to 60° C. for 16 h, cooled, and quenched with pre-cooled (0° C.) aqueous NH_4Cl (5 L). The mixture was extracted with EtOAc (3×2 L) and the combined organic layers were washed with brine (2×1 L), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give to two atropisomers (as single atropisomers) of 3-(5-bromo-1-ethyl-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1H-indol-3-yl)-2,2-dimethylpropan-1-ol (60 g, 38% yield) and (40 g, 26% yield) both as solids. LCMS (ESI): m/z [M+H] calc'd for $C_{23}H_{29}BrN_2O_2$ 444.1; found 445.2.

Intermediate 2 and Intermediate 4. Synthesis of (S)-1-((S)-2-((tert-butoxycarbonyl)amino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5-((triisopropylsilyl)oxy)phenyl)propanoyl)hexahydropyridazine-3-carboxylate



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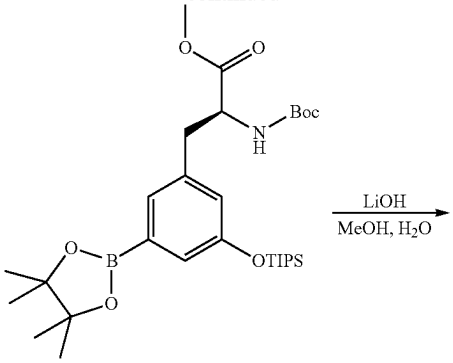
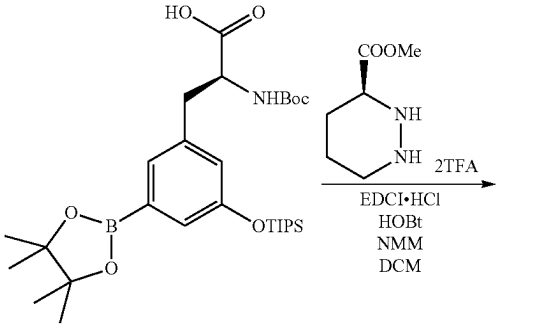
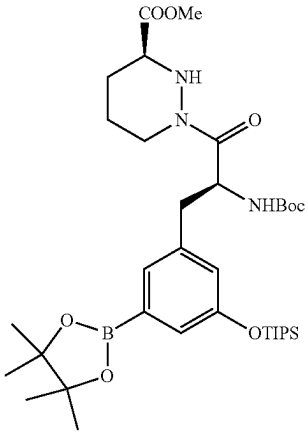


Intermediate 4.





Intermediate 2.

Step 1.

To a mixture of (S)-methyl 2-(tert-butoxycarbonylamino)-3-(3-hydroxyphenyl)propanoate (10.0 g, 33.9 mmol) in DCM (100 mL) was added imidazole (4.6 g, 67.8 mmol) and TIPSCI (7.8 g, 40.7 mmol). The mixture was stirred at rt overnight then diluted with DCM (200 mL) and washed with H_2O (150 mL×3). The organic layer was dried over anhydrous Na_2SO_4, filtered, concentrated under reduced pressure, and the residue was purified by silica gel column chromatography to give (S)-methyl 2-(tert-butoxycarbonylamino)-3-(3-(triisopropylsilyloxy)phenyl)-propanoate (15 g, 98% yield) as an oil. LCMS (ESI): m/z [M+Na] calc'd for $C_{24}H_{41}NO_5SiNa$ 474.3; found 474.2.

Step 2.

A mixture of (S)-methyl 2-(tert-butoxycarbonylamino)-3-(3-(triisopropylsilyloxy)phenyl)-propanoate (7.5 g, 16.6 mmol), $PinB_2$ (6.3 g, 24.9 mmol), $[Ir(OMe)(COD)]_2$ (1.1 g, 1.7 mmol), and 4-tert-butyl-2-(4-tert-butyl-2-pyridyl)pyridine (1.3 g, 5.0 mmol) was purged with Ar (×3), then THF (75 mL) was added and the mixture placed under an atmosphere of Ar and sealed. The mixture was heated to 80° C. and stirred for 16 h, concentrated under reduced pressure, and the residue was purified by silica gel column chromatography to give (S)-methyl 2-(tert-butoxycarbonylamino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5-(triisopropylsilyloxy)phenyl)-propanoate (7.5 g, 78% yield) as a solid. LCMS (ESI): m/z [M+Na] calc'd for $C_{30}H_{52}BNO_7SiNa$ 600.4; found 600.4; ^1H NMR (300 MHz, CD_3OD) δ 7.18 (s, 1H), 7.11 (s, 1H), 6.85 (s, 1H), 4.34 (m, 1H), 3.68 (s, 3H), 3.08 (m, 1H), 2.86 (m, 1H), 1.41-1.20 (m, 26H), 1.20-1.01 (m, 22H), 0.98-0.79 (m, 4H).

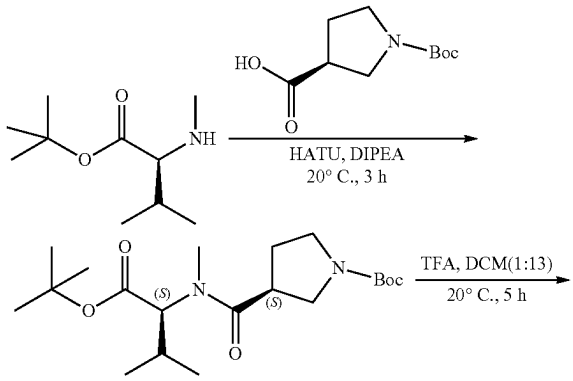
Step 3.

To a mixture of triisopropylsilyl (S)-2-((tert-butoxycarbonyl)amino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5-((triisopropylsilyl)oxy)phenyl)propanoate (4.95 g, 6.9 mmol) in MeOH (53 mL) at 0° C. was added LiOH (840 mg, 34.4 mmol) in H_2O (35 mL). The mixture was stirred at 0° C. for 2 h, then acidified to pH~5 with 1M HCl and extracted with EtOAc (250 mL×2). The combined organic layers were washed with brine (100 mL×3), dried over anhydrous Na_2SO_4, filtered, and the filtrate concentrated under reduced pressure to give (S)-2-((tert-butoxycarbonyl)amino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5-((triisopropylsilyl)oxy)phenyl)propanoic acid (3.7 g, 95% yield), which was used directly in the next step without further purification. LCMS (ESI): m/z [M+NH_4] calc'd for $C_{29}H_{50}BNO_7SiNH_4$ 581.4; found 581.4.

Step 4.

To a mixture of methyl (S)-hexahydropyridazine-3-carboxylate (6.48 g, 45.0 mmol) in DCM (200 mL) at 0° C. was added NMM (41.0 g, 405 mmol), (S)-2-((tert-butoxycarbonyl)amino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5-((triisopropylsilyl)oxy)phenyl)propanoic acid (24 g, 42.6 mmol) in DCM (50 mL) then HOBt (1.21 g, 9.0 mmol) and EDCI HCl salt (12.9 g, 67.6 mmol). The mixture was warmed to rt and stirred for 16 h, then diluted with DCM (200 mL) and washed with H_2O (3×150 mL). The organic layer was dried over anhydrous Na_2SO, filtered, the filtrate concentrated under reduced pressure, and the residue was purified by silica gel column chromatography to give methyl (S)-1-((S)-2-((tert-butoxycarbonyl)amino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5-((triisopropylsilyl)oxy)phenyl)propanoyl)hexahydropyridazine-3-carboxylate (22 g, 71% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{35}H_{60}BN_3O_8Si$ 689.4; found 690.5.

Intermediate 3. Synthesis of N—((S)-1-acryloylpyrrolidine-3-carbonyl)-N-methyl-L-valine



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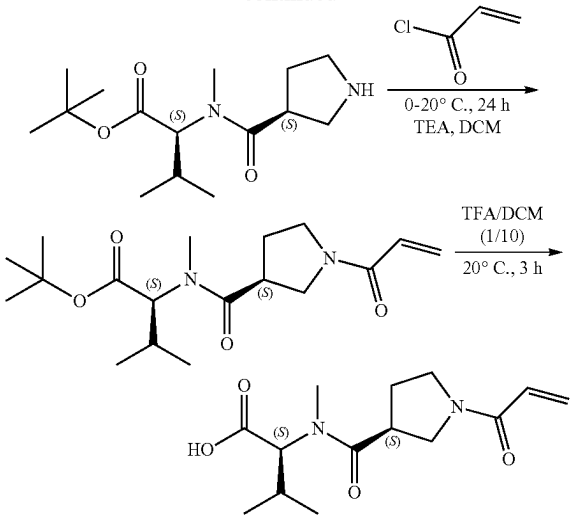


Intermediate 3

Step 1.

To a mixture of (S)-1-(tert-butoxycarbonyl)pyrrolidine-3-carboxylic acid (2.2 g, 10.2 mmol) in DMF (10 mL) at rt was added HATU (7.8 g, 20.4 mmol) and DIPEA (5 mL). After stirring at rt for 10 min, tert-butyl methyl-L-valinate (3.8 g, 20.4 mmol) in DMF (10 mL) was added. The mixture was stirred at rt for 3 h, then diluted with DCM (40 mL) and H_2O (30 mL). The aqueous and organic layers were separated and the organic layer was washed with H_2O (3×30 mL), brine (30 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give (S)-tert-butyl 3-(((S)-1-(tert-butoxy)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)pyrrolidine-1-carboxylate (3.2 g, 82% yield) as an oil. LCMS (ESI): m/z [M+Na] calc'd for $C_{20}H_{36}N_2O_5Na$ 407.3; found 407.2.

Step 2.

A mixture of (S)-tert-butyl 3-(((S)-1-(tert-butoxy)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)pyrrolidine-1-carboxylate (3.2 g, 8.4 mmol) in DCM (13 mL) and TFA (1.05 g, 9.2 mmol) was stirred at rt for 5 h. The mixture was concentrated under reduced pressure to give (S)-tert-butyl 3-methyl-2-((S)—N-methylpyrrolidine-3-carboxamido)butanoate (2.0 g, 84% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{15}H_{28}N_2O_3$ 284.2; found 285.2.

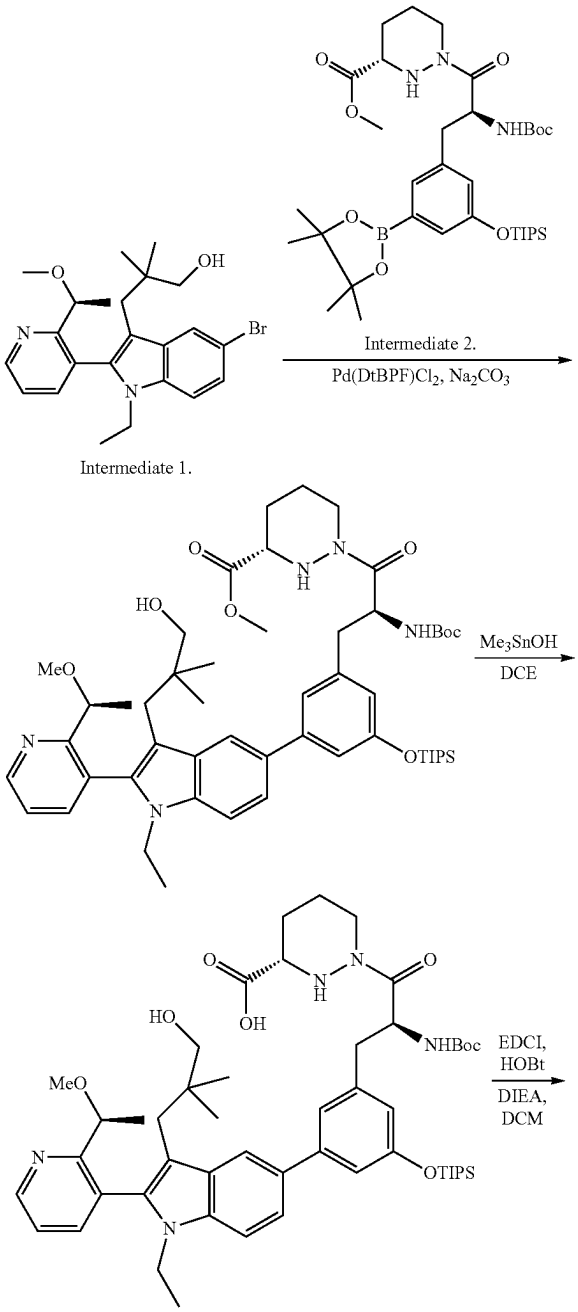
Step 3.

To a mixture of (S)-tert-butyl 3-methyl-2-((S)—N-methylpyrrolidine-3-carboxamido)butanoate (600 mg, 2.1 mmol) in DCM (6 mL) at 0° C. was added TEA (342 mg, 3.36 mmol). After stirring at 0° C. for 10 mins, acryloyl chloride (284 mg, 3.2 mmol) in DCM (10 mL) was added. The mixture was warmed to rt and stirred for 24 h, then diluted with DCM (30 mL) and H_2O (30 mL). The aqueous and organic layers were separated and the organic layer was washed with H_2O (3×30 mL), brine (30 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give tert-butyl N—((S)-1-acryloylpyrrolidine-3-carbonyl)-N-methyl-L-valinate (500 mg, 70% yield) as an oil.

Step 4.

To a mixture of tert-butyl N—((S)-1-acryloylpyrrolidine-3-carbonyl)-N-methyl-L-valinate (100 mg, 0.29 mmol) in DCM (3.0 mL) at 15° C. was added TFA (0.3 mL). The mixture was warmed to rt and stirred for 5 h, then the mixture was concentrated under reduced pressure to give N—((S)-1-acryloylpyrrolidine-3-carbonyl)-N-methyl-L-valine (150 mg) as a solid. The crude product was used directly in the next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{14}H_{22}N_2O_4$ 282.2; found 283.2.

Intermediate 5. Synthesis of tert-butyl ((63S,4S)-11-ethyl-12-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-25-((triisopropylsilyl)oxy)-61,62,63,64,65,66-hexahydro-11H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate



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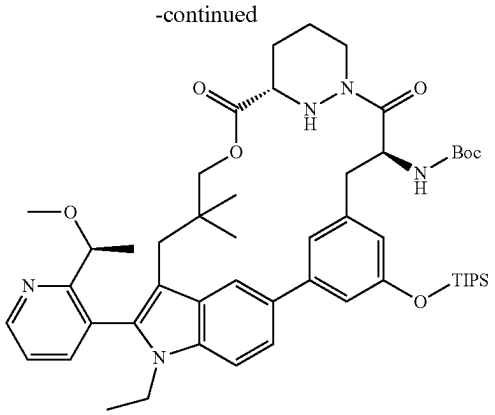


Intermediate 5.

Step 1.

To a stirred mixture of 3-(5-bromo-1-ethyl-2-[2-[(1 S)-1-methoxyethyl]pyridin-3-yl]indol-3-yl)-2,2-dimethylpropan-1-ol (30 g, 67 mmol) and methyl (3S)-1-[(2S)-2-[(tert-butoxycarbonyl)amino]-3-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5-[(triisopropylsilyl)oxy]phenyl] propanoyl]-1,2-diazinane-3-carboxylate (55.8 g, 80.8 mmol) in 1,4-dioxane (750 mL) at rt under an atmosphere of Ar was added Na_2CO_3 (17.9 g, 168.4 mmol), Pd(DtBPF)Cl_2 (4.39 g, 6.7 mmol), and H_2O (150.00 mL) in portions. The mixture was heated to 85° C. and stirred for 3 h, cooled, diluted with H_2O (2 L), and extracted with EtOAc (3×1 L). The combined organic layers were washed with brine (2×500 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl (3S)-1-[(2S)-2-[(tert-butoxycarbonyl)amino]-3-[3-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-[(1 S)-1-methoxyethyl]pyridin-3-yl]indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylate (50 g, 72% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{52}H_{77}N_5O_8Si$ 927.6; found 928.8.

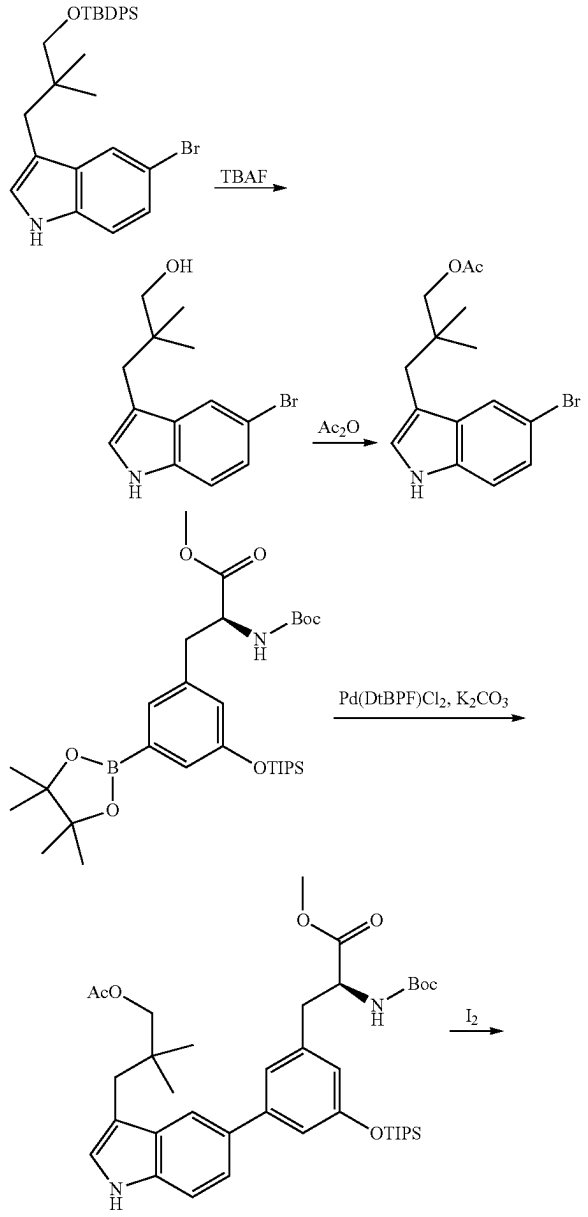
Step 2.

To a stirred mixture of methyl (3S)-1-[(2S)-2-[(tert-butoxycarbonyl)amino]-3-[3-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-[(1 S)-1-methoxyethyl]pyridin-3-yl]indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylate (50 g, 54 mmol) in DCE (500 mL) at rt was added trimethyltin hydroxide (48.7 g, 269 mmol) in portion. The mixture was heated to 65° C. and stirred for 16 h, then filtered and the filter cake washed with DCM (3×150 mL). The filtrate was concentrated under reduced pressure to give (3S)-1-[(2S)-2-[(tert-butoxycarbonyl) amino]-3-[3-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-[(1 S)-1-methoxyethyl]pyridin-3-yl]indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylic acid (70 g, crude), which was used directly in the next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{51}H_{75}N_5O_8Si$ 913.5; found 914.6.

Step 3.

To a stirred mixture of (3S)-1-[(2S)-2-[(tert-butoxycarbonyl)amino]-3-[3-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-[(1 S)-1-methoxyethyl]pyridin-3-yl]indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylic acid (70 g) in DCM (5 L) at 0° C. under an atmosphere of N_2 was added DIPEA (297 g, 2.3 mol), HOBT (51.7 g, 383 mmol) and EDCI (411 g, 2.1 mol) in portions. The mixture was warmed to rt and stirred for 16 h, then diluted with DCM (1 L), washed with brine (3×1 L), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give tert-butyl ((6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl) carbamate (36 g, 42% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{51}H_{73}N_5O_7Si$ 895.5; found 896.5.

Intermediate 6. Synthesis of tert-butyl N-[(8S,14S)-21-iodo-18,18-dimethyl-9,15-dioxo-4-[(triisopropylsilyl)oxy]-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]carbamate



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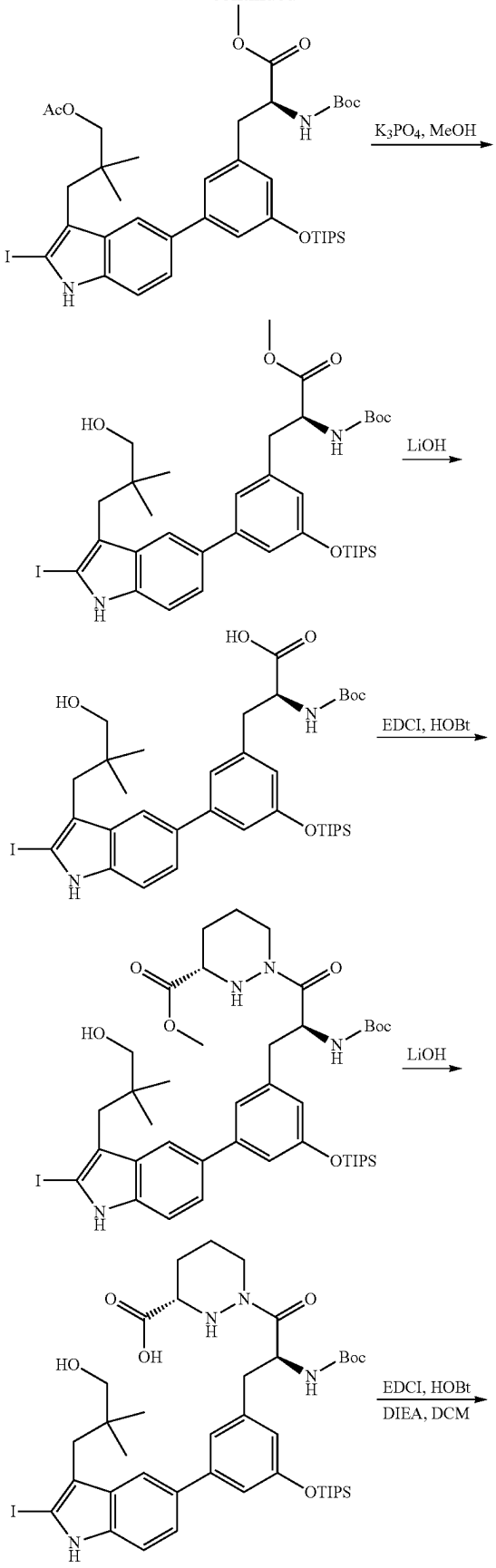

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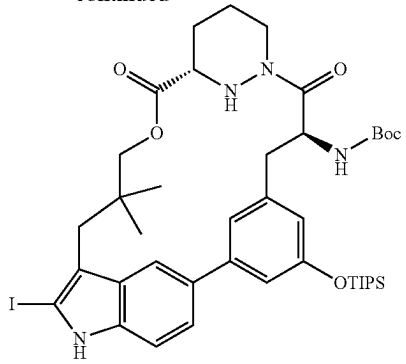


Intermediate 6.

Step 1.

This reaction was undertaken on 5-batches in parallel on the scale illustrated below.

Into a 2 L round-bottom flasks each were added 5-bromo-3-[3-[(tert-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl]-1H-indole (100 g, 192 mmol) and TBAF (301.4 g, 1.15 mol) in THF (1.15 L) at rt. The resulting mixture was heated to 50° C. and stirred for 16 h, then the mixture was concentrated under reduced pressure. The combined residues were diluted with H_2O (5 L) and extracted with EtOAc (3×2 L). The combined organic layers were washed with brine (2×1.5 L), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 3-(5-bromo-1H-indol-3-yl)-2,2-dimethylpropan-1-ol (310 g, crude) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{13}H_{16}BrNO$ 281.0 and 283.0; found 282.1 and 284.1.

Step 2.

This reaction was undertaken on two batches in parallel on the scale illustrated below.

To a stirred mixture of 3-(5-bromo-1H-indol-3-yl)-2,2-dimethylpropan-1-ol (135 g, 478 mmol) and TEA (145.2 g, 1.44 mol) in DCM (1.3 L) at 0° C. under an atmosphere of N_2 was added Ac_2O (73.3 g, 718 mmol) and DMAP (4.68 g, 38.3 mmol) in portions. The resulting mixture was stirred for 10 min at 0° C., then washed with H_2O (3×2 L). The organic layers from each experiment were combined and washed with brine (2×1 L), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography to give 3-(5-bromo-1H-indol-3-yl)-2,2-dimethylpropyl acetate (304 g, 88% yield) as a solid. ^1H NMR (400 MHz, DMSO-d_6) δ 11.16-11.11 (m, 1H), 7.69 (d, J=2.0 Hz, 1H), 7.32 (d, J=8.6 Hz, 1H), 7.19-7.12 (m, 2H), 3.69 (s, 2H), 2.64 (s, 2H), 2.09 (s, 3H), 0.90 (s, 6H).

Step 3.

This reaction was undertaken on four batches in parallel on the scale illustrated below.

Into a 2 L round-bottom flasks were added methyl (2S)-2-[(tert-butoxycarbonyl)amino]-3-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-5-[(triisopropylsilyl)oxy]phenyl] propanoate (125 g, 216 mmol), 1,4-dioxane (1 L), H_2O (200 mL), 3-(5-bromo-1H-indol-3-yl)-2,2-dimethylpropyl acetate (73.7 g, 227 mmol), $K2CO_3$ (59.8 g, 433 mmol), and $Pd(DtBPF)Cl_2$ (7.05 g, 10.8 mmol) at rt under an atmosphere of Ar. The resulting mixture was heated to 65° C. and stirred for 2 h, then diluted with H_2O (10 L) and extracted with EtOAc (3×3 L). The combined organic layers were washed with brine (2×2 L), dried over anhydrous Na_2SO_4, and

filtered. The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography to give methyl (2S)-3-(3-[3-[3-(acetyloxy)-2,2-dimethylpropyl]-1H-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl)-2-[(tert-butoxycarbonyl)amino]propanoate (500 g, 74% yield) as an oil. LCMS (ESI): m/z [M+Na] calc'd for $C_{39}H_{58}N_2O_7SiNa$ 717.4; found 717.3.

Step 4.

This reaction was undertaken on three batches in parallel on the scale illustrated below.

To a stirred mixture of methyl (2S)-3-(3-[3-[3-(acetyloxy)-2,2-dimethylpropyl]-1H-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl)-2-[(tert-butoxycarbonyl)amino]propanoate (150 g, 216 mmol) and $NaHCO_3$ (21.76 g, 259 mmol) in THF (1.5 L) was added AgOTf (66.5 g, 259 mmol) in THF dropwise at 0° C. under an atmosphere of nitrogen. 12 (49.3 g, 194 mmol) in THF was added dropwise over 1 h at 0° C. and the resulting mixture was stirred for an additional 10 min at 0° C. The combined experiments were diluted with aqueous $Na_2S_2O_3$ (5 L) and extracted with EtOAc (3×3 L). The combined organic layers were washed with brine (2×1.5 L), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography to give methyl (2S)-3-(3-[3-[3-(acetyloxy)-2,2-dimethylpropyl]-2-iodo-1H-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl)-2-[(tert-butoxycarbonyl)amino]propanoate (420 g, 71% yield) as an oil. LCMS (ESI): m/z [M+Na] calc'd for $C_{39}H_{57}IN_2O_7SiNa$, 843.3; found 842.9.

Step 5.

This reaction was undertaken on three batches in parallel on the scale illustrated below.

To a 2 L round-bottom flask were added methyl (2S)-3-(3-[3-[3-(acetyloxy)-2,2-dimethylpropyl]-2-iodo-1H-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl)-2-[(tert-butoxycarbonyl)amino]propanoate (140 g, 171 mmol), MeOH (1.4 L) and K_3PO_4 (108.6 g, 512 mmol) at 0° C. The mixture was warmed to rt and stirred for 1 h, then the combined experiments were diluted with H_2O (9 L) and extracted with EtOAc (3×3 L). The combined organic layers were washed with brine (2×2 L), dried over anhydrous Na_2SO_4, filtered, and the filtrate was concentrated under reduced pressure to give methyl (2S)-2-[(tert-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1H-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoate (438 g, crude) as a solid. LCMS (ESI): m/z [M+Na] calc'd for $C_{37}H_{55}IN_2O_6SiNa$ 801.3; found 801.6.

Step 6.

This reaction was undertaken on three batches in parallel on the scale illustrated below.

To a stirred mixture of methyl (2S)-2-[(tert-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1H-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoate (146 g, 188 mmol) in THF (1.46 L) was added LiOH (22.45 g, 937 mmol) in H_2O (937 mL) dropwise at 0° C. The resulting mixture was warmed to rt and stirred for 1.5 h [note: LCMS showed 15% de-TIPS product]. The mixture was acidified to pH 5 with 1M HCl (1M) and the combined experiments were extracted with EtOAc (3×3 L). The combined organic layers were washed with brine (2×2 L), dried over anhydrous Na_2SO_4, filtered, and the filtrate was concentrated under reduced pressure to give (2S)-2-[(tert-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1H-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoic acid (402 g, crude) as a solid. LCMS (ESI): m/z [M+Na] calc'd for $C_{36}H_{53}IN_2O_6SiNa$ 787.3; found 787.6.

Step 7.

To a stirred mixture of (2S)-2-[(tert-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1H-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoic acid (340 g, 445 mmol) and methyl (3S)-1,2-diazinane-3-carboxylate (96.1 g, 667 mmol) in DCM (3.5 L) was added NMM (225 g, 2.2 mol), EDCI (170 g, 889 mmol), and HOBT (12.0 g, 88.9 mmol) portionwise at 0° C. The mixture was warmed to rt and stirred for 16 h, then washed with H_2O (3×2.5 L), brine (2×1 L), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography to give methyl (3S)-1-[(2S)-2-[(tert-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1H-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylate (310 g, 62% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{42}H_{63}IN_4O_7Si$ 890.4; found 890.8.

Step 8.

This reaction was undertaken on three batches in parallel on the scale illustrated below.

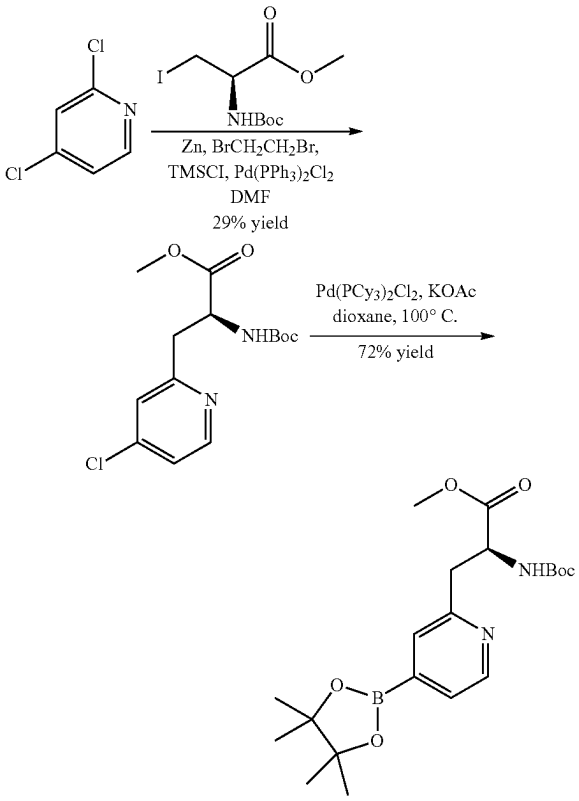
To a stirred mixture of methyl (3S)-1-[(2S)-2-[(tert-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1H-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylate (85.0 g, 95.4 mmol) in THF (850 mL) each added LiOH (6.85 g, 286 mmol) in H_2O (410 mL) dropwise at 0° C. under an atmosphere of N_2. The mixture was stirred at 0° C. for 1.5 h [note: LCMS showed 15% de-TIPS product], then acidified to pH 5 with 1M HCl and the combined experiments extracted with EtOAc (3×2 L). The combined organic layers were washed with brine (2×1.5 L), dried over anhydrous Na_2SO_4, filtered, and the filtrate was concentrated under reduced pressure to give (3S)-1-[(2S)-2-[(tert-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1H-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylic acid (240 g, crude) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{41}H_{61}IN_4O_7Si$ 876.3; found 877.6.

Step 9.

This reaction was undertaken on two batches in parallel on the scale illustrated below.

To a stirred mixture of (3S)-1-[(2S)-2-[(tert-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1H-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylic acid (120 g, 137 mmol) in DCM (6 L) was added DIPEA (265 g, 2.05 mol), EDCI (394 g, 2.05 mol), and HOBT (37 g, 274 mmol) in portions at 0° C. under an atmosphere of N_2. The mixture was warmed to rt and stirred overnight, then the combined experiments were washed with H_2O (3×6 L), brine (2×6 L), dried over anhydrous Na_2SO_4, and filtered. After filtration, the filtrate was concentrated under reduced pressure. The filtrate was concentrated under reduced pressure and the residue was purified by column chromatography to give tert-butyl N-[(8S,14S)-21-iodo-18,18-dimethyl-9,15-dioxo-4-[(triisopropylsilyl)oxy]-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]carbamate (140 g, 50% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{41}H_{59}IN_4O_6Si$ 858.9; found 858.3.

Intermediate 7. Synthesis of (S)-methyl 2-((tert-butoxycarbonyl)amino)-3-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-yl)propanoate

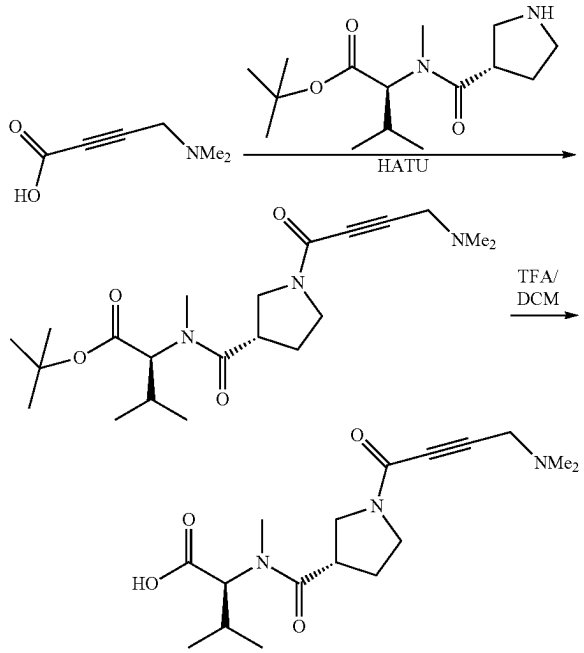


Step 1. Zn dust (28 g, 428 mmol) was added to a 1 L, three necked, round bottomed flask, purged with N_2, and heated with a heat gun for 10 min under vacuum. The mixture was cooled to rt, and a solution of 1,2-dibromoethane (1.85 mL, 21.5 mmol) in DMF (90 mL) was added dropwise over 10 min. The mixture was heated at 90° C. for 30 min and re-cooled to rt. TMSCl (0.55 mL, 4.3 mmol) was added, and the mixture was stirred for 30 min at rt, then a mixture of (R)-methyl 2-((tert-butoxycarbonyl)amino)-3-iodopropanoate (22.5 g, 71.4 mmol) in DMF (200 mL) was added dropwise over a period of 10 min. The mixture was heated at 35° C. and stirred for 2 h, then cooled to rt, and 2,4-dichloropyridine (16 g, 109 mmol) and $Pd(PPh_3)_2Cl_2$ (4 g, 5.7 mmol) added. The mixture was heated at 45° C. and stirred for 2 h, cooled, and filtered, then H_2O (1 L) and EtOAc (0.5 L) were added to the filtrate. The organic and aqueous layers were separated, and the aqueous layer was extracted with EtOAc (2×500 mL). The organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography to give (S)-methyl 2-((tert-butoxycarbonyl)amino)-3-(4-chloropyridin-2-yl)propanoate (6.5 g, 29% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{14}H_{19}ClN_2O_4$ 314.1; found 315.1.

Step 2.

To a mixture of (S)-methyl 2-((tert-butoxycarbonyl)amino)-3-(4-chloropyridin-2-yl)propanoate (6.5 g, 20.6 mmol) in 1,4-dioxane (80 mL) at rt under an atmosphere of N_2 was added bis(pinacolato)diboron (6.3 g, 24.7 mmol), KOAc (8.1 g, 82.4 mmol), and $Pd(PCy_3)_2Cl_2$ (1.9 g, 2.5 mmol). The mixture was heated to 100° C. and stirred for 3 h, then H_2O (100 mL) added and the mixture extracted with EtOAc (3×200 mL). The organic layers were combined, washed with brine (2×100 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give (S)-methyl 2-((tert-butoxycarbonyl)amino)-3-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-yl)propanoate (6 g, 72% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{20}H_{31}BN_2O_6$ 406.2; found 407.3.

Synthesis of Intermediate 8.



Step 1.

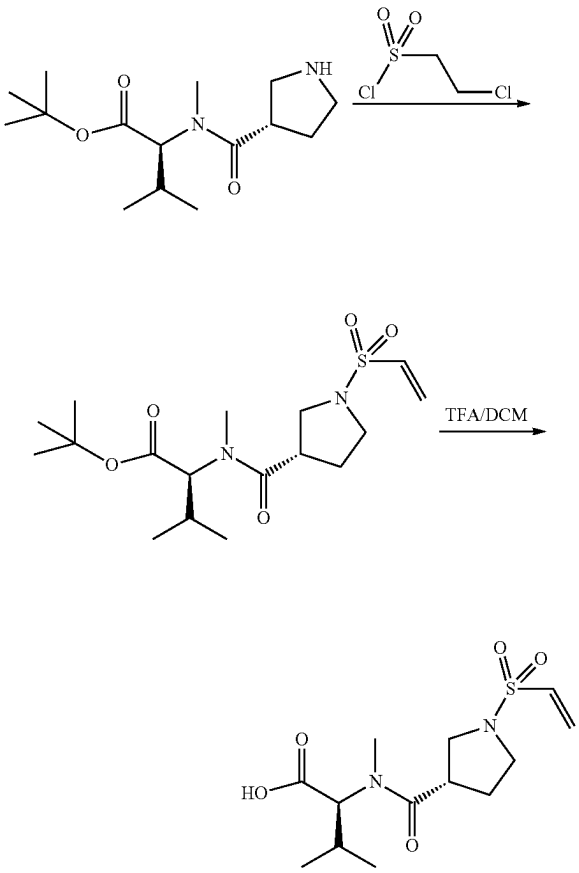
To a mixture of 4-(dimethylamino)but-2-ynoic acid (900 mg, 7.0 mmol) in DMF (20 mL) at −5° C. was added tert-butyl N-methyl-N—((S)-pyrrolidine-3-carbonyl)-L-valinate (1.0 g, 3.5 mmol), DIPEA (2.2 g, 17.6 mmol) and HATU (2.7 g, 7.0 mmol) in portions. The mixture was stirred between −5 to 5° C. for 1 h, then diluted with EtOAc (100 mL) and ice-H_2O (100 mL). The aqueous and organic layers were separated and the organic layer was washed with H_2O (3×100 mL), brine (100 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give tert-butyl N—((S)-1-(4-(dimethylamino)but-2-ynoyl)pyrrolidine-3-carbonyl)-N-methyl-L-valinate (900 mg, 55% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{21}H_{35}N_3O_4$ 393.5; found 394.3.

Step 2.

To a mixture of tert-butyl N—((S)-1-(4-(dimethylamino)but-2-ynoyl)pyrrolidine-3-carbonyl)-N-methyl-L-valinate (260 mg, 0.66 mmol) in DCM (6 mL) was added TFA (3 mL) at rt. The mixture was stirred at rt for 2 h, then the solvent was concentrated under reduced pressure to give (2S)-2-{1-[(3S)-1-[4-(dimethylamino)but-2-ynoyl]pyrrolidin-3-yl]-N-methylformamido}-3-methylbutanoic acid (280

mg) as an impure oil. The crude product was used directly in the next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{17}H_{27}N_3O_4$ 337.2; found 338.3.

Synthesis of Intermediate 9.



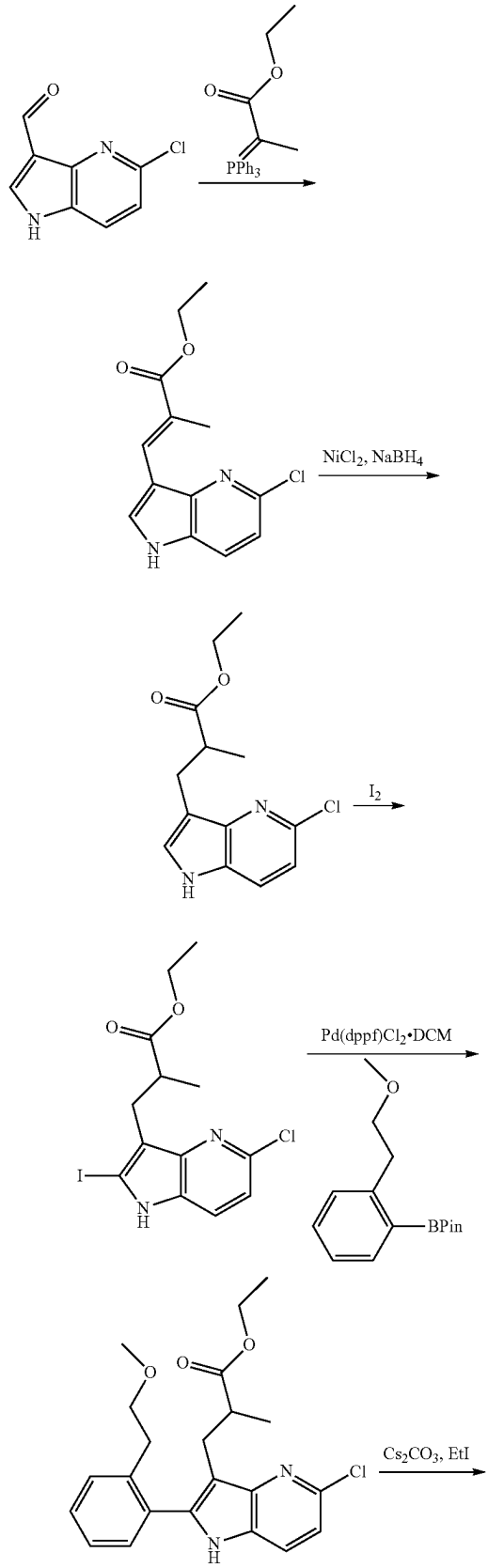
Step 1.

To a mixture of tert-butyl N-methyl-N—((S)-pyrrolidine-3-carbonyl)-L-valinate (500 mg, 1.8 mmol) in DCM (8 mL) at 5° C. was added TEA (533 mg, 5.3 mmol) followed by dropwise addition of 2-chloroethane-1-sulfonyl chloride (574 mg, 3.5 mmol) in DCM (2 mL) The mixture was stirred at 5° C. for 1 h, then diluted with H_2O (20 mL) and extracted with EtOAC (3×10 mL). The combined organic layers were washed with brine (10 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give tert-butyl N-methyl-N—((S)-1-(vinylsulfonyl)pyrrolidine-3-carbonyl)-L-valinate (300 mg, 45% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{17}H_{30}N_2O_5S$ 374.2; found 375.2.

Step 2.

To a mixture of tert-butyl N-methyl-N—((S)-1-(vinylsulfonyl)pyrrolidine-3-carbonyl)-L-valinate (123 mg, 0.33 mmol) in DCM (3 mL) at rt was added TFA (1 mL). The mixture was stirred at rt for 1 h, then concentrated under reduced pressure to give N-methyl-N—((S)-1-(vinylsulfonyl)pyrrolidine-3-carbonyl)-L-valine (130 mg, crude) as a solid, which was used directly in the next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{13}H_{22}N_2O_5S$ 318.1; found 319.1.

Synthesis of Intermediate 10.



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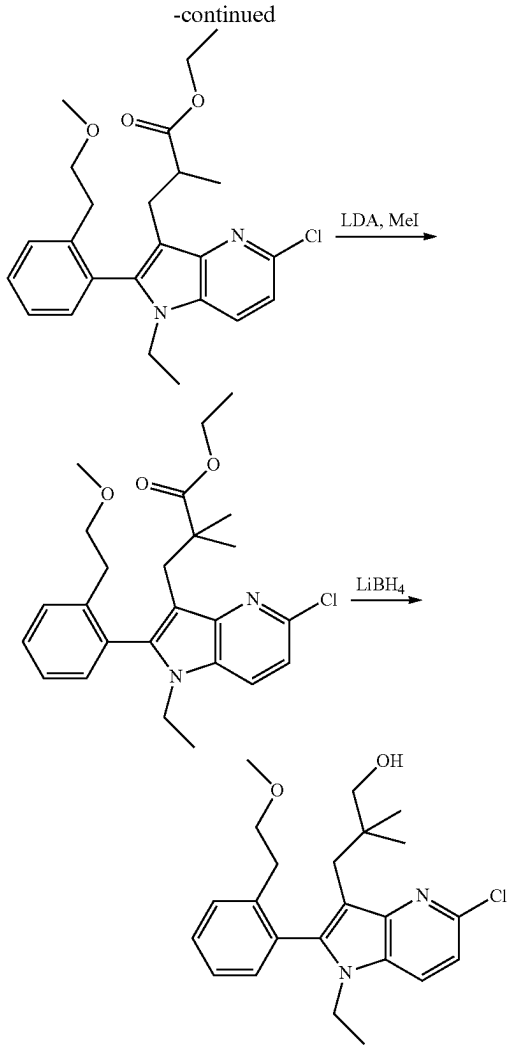


Step 1.

A mixture of 5-chloro-1H-pyrrolo[3,2-b]pyridine-3-carbaldehyde (8.5 g, 47.1 mmol) and ethyl 2-(triphenylphosphoranylidene)propionate (2.56 g, 70.7 mmol) in 1,4-dioxane (120 mL) was stirred at reflux for 4 h, then concentrated under reduced pressure. EtOAc (200 mL) was added and the mixture was washed with brine, dried over Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give ethyl (E)-3-(5-chloro-1H-pyrrolo[3,2-b]pyridin-3-yl)-2-methylacrylate (7.5 g, 60% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{13}H_{13}ClN_2O_2$ 264.1; found 265.1.

Step 2.

To a mixture of ethyl (E)-3-(5-chloro-1H-pyrrolo[3,2-b]pyridin-3-yl)-2-methylacrylate (7.5 g, 28.3 mmol) and $NiCl_2$ (4.8 g, 28.3 mmol) in 1:1 THF/MeOH (300 mL) was added $NaBH_4$ (21.5 g, 566 mmol) in 20 portions every 25 minutes. After complete addition, the mixture was stirred at rt for 30 min, then diluted with EtOAc (500 mL) and washed with brine, dried over Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give ethyl 3-(5-chloro-1H-pyrrolo[3,2-b]pyridin-3-yl)-2-methylpropanoate (3.4 g, 45% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{13}H_{15}ClN_2O_2$ 266.1; found 267.1.

Step 3.

To a mixture of ethyl 3-(5-chloro-1H-pyrrolo[3,2-b]pyridin-3-yl)-2-methylpropanoate (7.0 g, 26.2 mmol) and AgOTf (6.7 g, 26.2 mmol) in THF (50 mL) at 0° C. was added 12 (6.65 g, 26.2 mol). The mixture was stirred at 0° C. for 30 min then diluted with EtOAc (100 mL), washed with Na_2SO_3 (50 mL), brine (50 mL), dried over Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give ethyl 3-(5-chloro-2-iodo-1H-pyrrolo[3,2-b]pyridin-3-yl)-2-methylpropanoate (6 g, 58% yield) as white solid. LCMS (ESI): m/z [M+H] calc'd for $C_{13}H_{14}ClIN_2O_2$ 392.0; found 393.0.

Step 4.

To a mixture of ethyl 3-(5-chloro-2-iodo-1H-pyrrolo[3,2-b]pyridin-3-yl)-2-methylpropanoate (6.0 g, 15.3 mmol) and 2-(2-(2-methoxyethyl)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (5.6 g, 21.4 mmol) and $K2CO_3$ (6.3 g, 45.9 mmol) in 1,4-dioxane (150 mL) and H_2O (30 mL) under an atmosphere of N_2 was added $Pd(dppf)Cl_2$.DCM (1.3 g, 3.1 mmol). The mixture was heated to 80° C. and stirred for 4 h, then diluted with EtOAc (500 mL), washed with brine, dried over Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 3-(5-chloro-2-(2-(2-methoxyethyl)phenyl)-1H-pyrrolo[3,2-b]pyridin-3-yl)-2-methylpropanoate (5.5 g, 50% yield) as a viscous oil. LCMS (ESI): m/z [M+H] calc'd for $C_{22}H_{25}ClN_2O_3$ 400.2; found 401.2.

Step 5.

A mixture of ethyl 3-(5-chloro-2-(2-(2-methoxyethyl)phenyl)-1H-pyrrolo[3,2-b]pyridin-3-yl)-2-methylpropanoate (5.5 g, 13.8 mmol), Cs_2CO_3 (8.9 g, 27.5 mmol), and EtI (3.5 g, 27.5 mmol) in DMF (30 mL) at rt was stirred for 10 h. The mixture was diluted with EtOAc (100 mL), washed with brine (20 mL×4), dried over Na_2SO_4, filtered, and concentrated in vacuo. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give ethyl 3-(5-chloro-1-ethyl-2-(2-(2-methoxyethyl)phenyl)-1H-pyrrolo[3,2-b]pyridin-3-yl)-2-methylpropanoate (5.6 g, 95% yield) as a viscous oil. LCMS (ESI): m/z [M+H] calc'd for $C_{25}H_{31}ClN_2O_3$ 428.2; found 429.2.

Step 6.

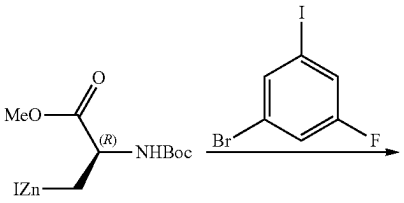
To a mixture of ethyl 3-(5-chloro-1-ethyl-2-(2-(2-methoxyethyl)phenyl)-1H-pyrrolo[3,2-b]pyridin-3-yl)-2-methylpropanoate (5.4 g, 12.6 mmol) in THF (50 mL) at −65° C. was added 2M LDA (25 mL, 50 mmol) and stirred at −65° C. for 1 h. MeI (3.6 g, 25 mmol) was added and the mixture was stirred at −65° C. for 2.5 h, then aqueous NH_4Cl and EtOAc (50 mL) were added. The aqueous and organic layers were separated and the organic layer was washed with brine (30 mL), dried over Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give ethyl 3-(5-chloro-1-ethyl-2-(2-(2-methoxyethyl)phenyl)-1H-pyrrolo[3,2-b]pyridin-3-yl)-2,2-dimethylpropanoate (3.2 g, 57% yield) as a viscous oil. LCMS (ESI): m/z [M+H] calc'd for $C_{25}H_{31}ClN_2O_3$ 442.2; found 443.2.

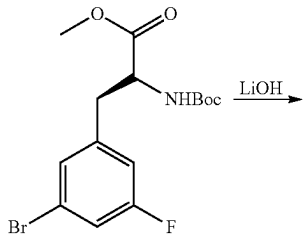
Step 7.

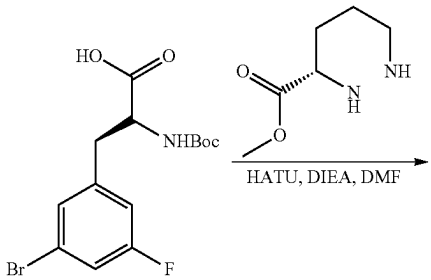
To a mixture of ethyl 3-(5-chloro-1-ethyl-2-(2-(2-methoxyethyl)phenyl)-1H-pyrrolo[3,2-b]pyridin-3-yl)-2,2-dimethylpropanoate (1.0 g, 2.3 mmol) in THF (10 mL) at 5° C. was added $LiBH_4$ (196 mg, 9.0 mmol). The mixture was heated to 65° C. and stirred for 2 h then aqueous NH_4Cl and EtOAc (50 mL) added. The aqueous and organic layers were separated and the organic layer was washed with brine (30

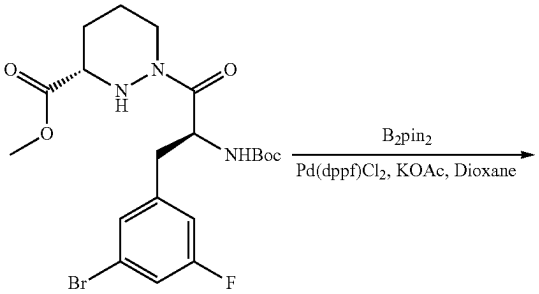
mL), dried over Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 3-(5-chloro-1-ethyl-2-(2-(2-methoxyethyl)phenyl)-1H-pyrrolo[3,2-b]pyridin-3-yl)-2,2-dimethylpropan-1-ol (0.75 g, 82% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{23}H_{29}ClN_2O_2$ 400.2; found 401.2.

Intermediate 11: Methyl (3S)-1-{(2S)-2-(tert-butoxycarbonyl)amino-3-[3-fluoro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]propanoyl}-1,2-diazinane-3-carboxylate









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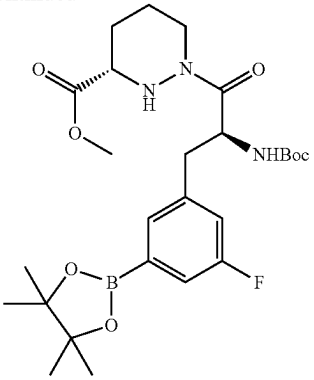


Step 1.

To a stirred solution of methyl (2R)-2-{[(tert-butoxy)carbonyl]amino}-3-(iodozincio)propanoate (12 g, 30 mmol, 1.2 eq) in DMF (100 mL) was added 1-bromo-3-fluoro-5-iodobenzene (7.5 g, 25 mmol, 1 eq) and $Pd(PPh_3)_2Cl_2$ (1.7 g, 2.5 mmol, 0.1 equiv) at 20° C. under N_2 atmosphere. The resulting mixture was stirred for 2 hrs at 65° C. under N_2 atmosphere. The reaction mixture was quenched with water and extracted with EA (200 mL×2). The organic phase was washed with water (200 mL×1) and brine (100 mL×1) and concentrated to dryness to give a residue. The residue was purified by prep-TLC (PE/EA=10/1) to afford methyl 3-(3-bromo-5-fluorophenyl)-2-{[(tert-butoxy)carbonyl]amino}propanoate (6 g, 58% yield) as a colorless oil. LCMS (ESI) m/z=398.1 $[M+Na]^+$, calculated for $C_{15}H_{19}BrFNO_4$: 375.0

Step 2.

To a solution of methyl 3-(3-bromo-5-fluorophenyl)-2-{[(tert-butoxy)carbonyl]amino}propanoate (3.2 g, 8.5 mmol, 1 eq) in THF (50 mL) was added Lithium hydroxide (610.7 mg, 25.5 mmol, 3 eq) in H_2O (10 mL). Then the reaction mixture was stirred at 20° C. for 1 h. The mixture was adjusted to pH=5.0 with 1 M HCl aqueous solution. The mixture was quenched with H_2O (150 mL) and extracted with EA (200 mL×3). The combined organic layers was washed bine (50 mL), dried over Na_2SO_4 and concentrated to afford 3-(3-bromo-5-fluorophenyl)-2-{[(tert-butoxy)carbonyl]amino}propanoic acid (2.65 g, 68% yield) as a white solid. LCMS (ESI) m/z=384.1 $[M+Na]^+$, calculated for $C_{14}H_{15}BrFNO4$ MW: 361.0

Step 3.

To a mixture of 3-(3-bromo-5-fluorophenyl)-2-{[(tert-butoxy)carbonyl]amino}propanoic acid (2.3 g, 6.4 mmol, 1 eq) and methyl (3S)-1,2-diazinane-3-carboxylate (1.66 g, 11.5 mmol, 1.8 eq) in DMF (150 mL) was added HATU (4.9 g, 12.8 mmol, 2 eq) and DIEA (16.5 g, 128 mmol, 20 eq) in DMF (50 mL) at 0° C. Then the reaction mixture was stirred at 0° C. for 1 h. The mixture was quenched with H_2O (100 mL) and extracted with EA (300 mL×3). The combined organic layers was washed bine (50 mL), dried over Na_2SO_4 and concentrated to give the residue, which was purified by Pre-HPLC eluting with acetonitrile in water (0.1% FA) from 60% to 70% in 10 minutes to give methyl (3S)-1-[(2S)-3-(3-bromo-5-fluorophenyl)-2-{[(tert-butoxy)carbonyl]amino}propanoyl]-1,2-diazinane-3-carboxylate (2.7 g, 78% yield) as a pale yellow solid. LCMS (ESI) m/z=510.1 $[M+Na]^+$,calculated for $C_{20}H_{27}BrFNO_5$: 487.1.

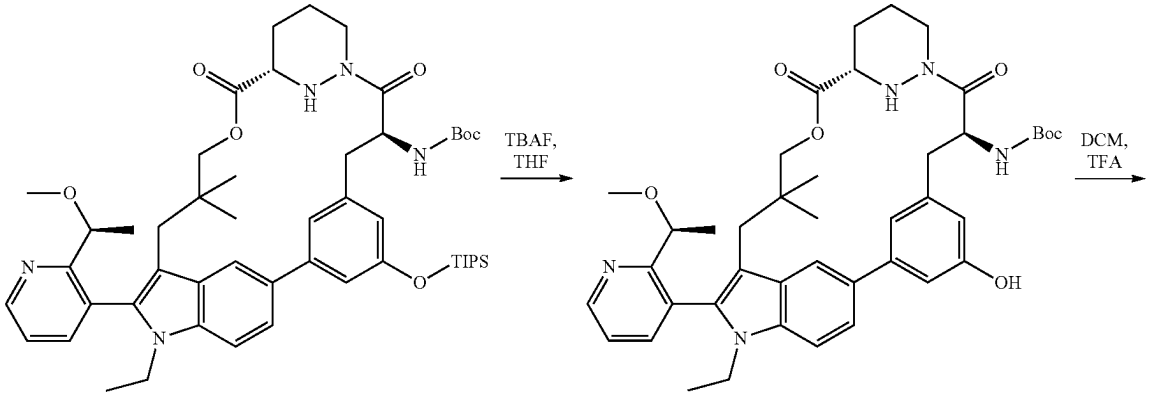
Step 4.

A mixture of methyl (S)-1-((S)-3-(3-bromo-5-fluorophenyl)-2-((tert-butoxycarbonyl)amino)propanoyl)hexahydro-

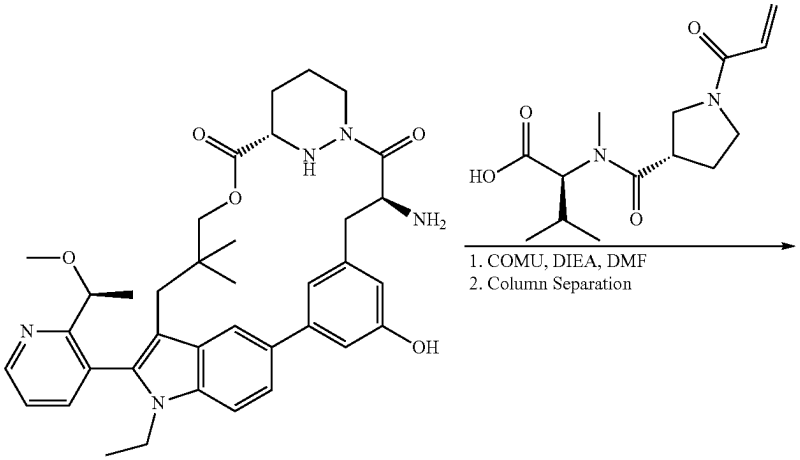
pyridazine-3-carboxylate (3 g, 6.16 mmol, 1 eq), 4,4,5,5-tetramethyl-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,2-dioxaborolane (1.9 g, 7.4 mmol, 1.2 eq), KOAc (900 mg, 9.24 mmol, 1.5 eq) and Pd(dppf)Cl_2DCM (0.3 g, 0.37 mmol, 0.05 eq) in dioxane (50 mL) was heated at 100° C. for 17 h under N_2 atmosphere. The mixture was concentrated and purified by column chromatography (DCM/MeOH=100/1 to 40/1) to give methyl (3S)-1-(2S)-2-{(tert-butoxycarbonyl)amino-3-[3-fluoro-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]propanoyl}-1,2-diazinane-3-carboxylate (2.6 g, 79% yield) as a yellow oil. LCMS (ESI) m/z=536.2 [M+H]+, calculated for $C_{26}H_{39}BFNO_7$: 535.3.

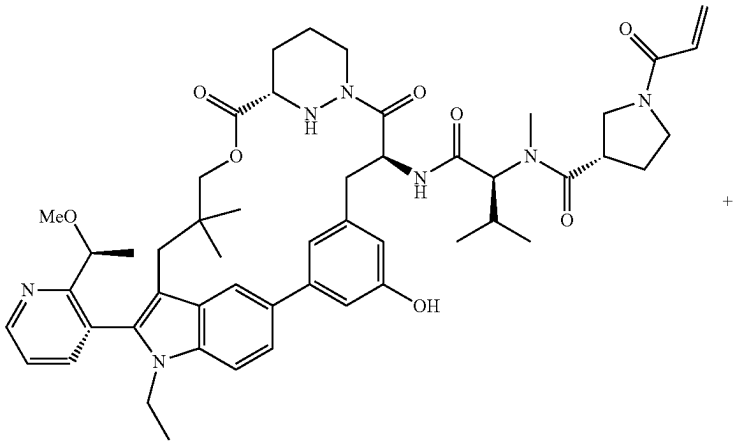
Compounds A341 and A342 may be prepared using methods disclosed herein via Intermediate 11.

Example A75. Synthesis of two atropisomers of (2S)—N-[(8S,14S,20M)-22-ethyl-4-hydroxy-21-{2-[(1S)-1-methoxyethyl]pyridin-3-yl}-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methyl-2-{N-methyl-1-[(3S)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido}butanamide



Intermediate 5.





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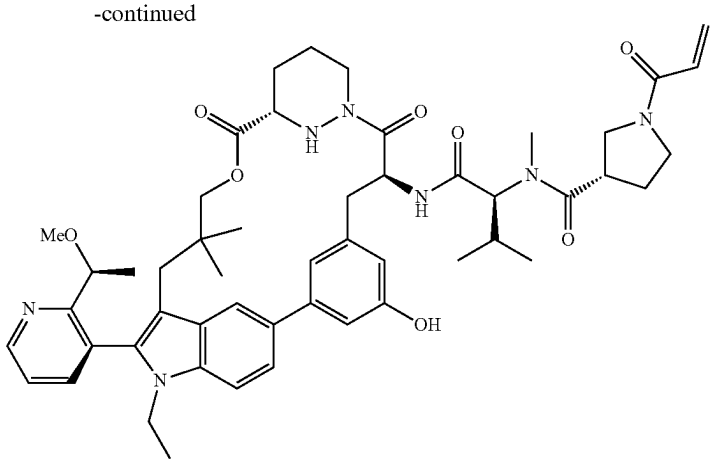


Step 1.

To a stirred mixture of tert-butyl ((6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (18.0 g, 20.1 mmol) in THF (180 mL) at 0° C. was added a 1M solution of TBAF in THF (24.1 mL, 24.1 mmol). The mixture was stirred at 0° C. for 1 h, then diluted with brine (1.5 L) and extracted with EtOAc (3×1 L). The combined organic layers were washed with brine (2×500 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give tert-butyl ((6^{3}S,4S)-1^1-ethyl-2^5-hydroxy-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (11.5 g, 69% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{42}H_{53}N_5O_7$ 739.4; found 740.4.

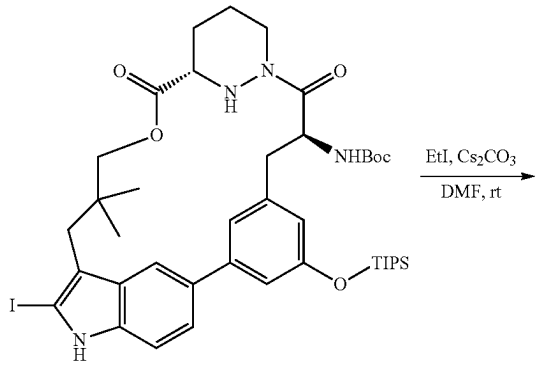
Step 2.

To a stirred mixture of tert-butyl ((6^{3}S,4S)-1^1-ethyl-2^5-hydroxy-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (11.5 g, 15.5 mmol) in DCM (120 mL) at 0° C. was added TFA (60 mL, 808 mmol). The mixture was stirred at 0° C. for 1 h, then concentrated under reduced pressure and the residue again concentrated under reduced pressure with toluene (20 mL; repeated ×3) to give (6^{3}S,4S)-4-amino-1^1-ethyl-2^5-hydroxy-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (12 g, crude), which was used directly in the next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{37}H_{45}N_5O_5$ 639.3; found 640.6.

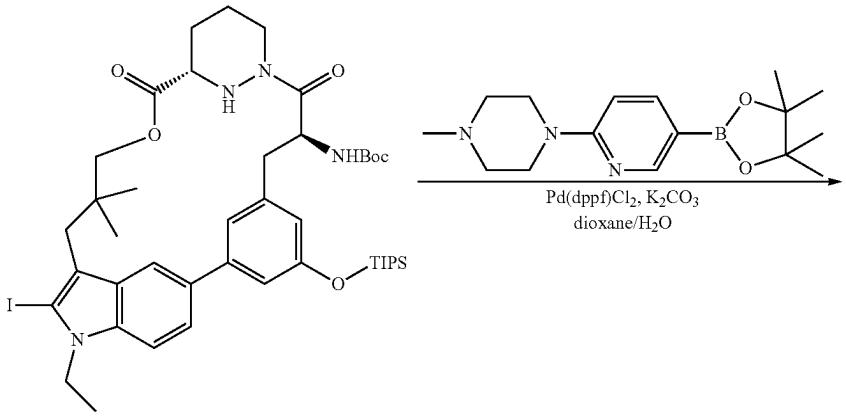
Step 3.

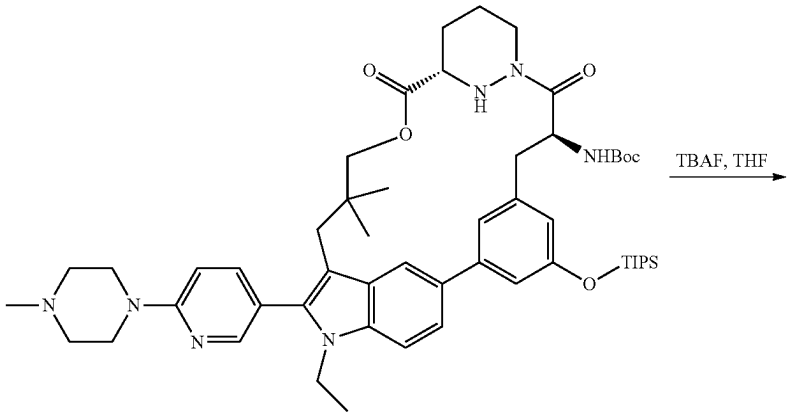
To a stirred mixture of (6^{3}S,4S)-4-amino-1^1-ethyl-2^5-hydroxy-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (11.9 g, 18.6 mmol) in DMF (240 mL) at 0° C. under an atmosphere of N_2 was added DIPEA (48.1 g, 372 mmol), (2S)-3-methyl-2-[N-methyl-1-[(3S)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido]butanoic acid (9.45 g, 33.5 mmol) and COMU (11.95 g, 27.9 mmol) in portions. The mixture was stirred ay 0° C. for 90 min, then diluted with brine (1.5 L) and extracted with EtOAc (3×1 L). The combined organic layers were washed with brine (2×500 mL), dried over anhydrous Na_2SO_4, and filtered. After filtration, the filtrate was concentrated under reduced pressure. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography (×2) to give two atropisomers of (2S)—N-[(8S,14S,20M)-22-ethyl-4-hydroxy-21-{2-[(1 S)-1-methoxyethyl]pyridin-3-yl}-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methyl-2-{N-methyl-1-[(3S)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido}butanamide (2.7 g, 15.5%, yield) and (4.2 g, 24.7% yield) both as solids. LCMS (ESI): m/z [M+H] calc'd for $C_{51}H_{65}N_7O_8$ 903.5; found 904.7; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 9.35-9.27 (m, 1H), 8.77 (dd, J=4.7, 1.7 Hz, 1H), 7.95 (dq, J=6.2, 2.0 Hz, 2H), 7.55 (ddd, J=28.0, 8.2, 4.3 Hz, 3H), 7.08 (dd, J=37.9, 6.2 Hz, 2H), 6.69-6.48 (m, 2H), 6.17 (ddt, J=16.7, 7.2, 2.3 Hz, 1H), 5.74-5.62 (m, 1H), 5.43-5.34 (m, 1H), 5.12-5.00 (m, 1H), 4.25 (d, J=12.3 Hz, 1H), 4.17-3.99 (m, 3H), 3.89-3.65 (m, 4H), 3.66-3.45 (m, 3H), 3.12 (s, 4H), 2.95-2.70 (m, 6H), 2.41-2.06 (m, 5H), 1.99-1.88 (m, 1H), 1.82 (d, J=12.1 Hz, 2H), 1.54 (t, J=12.0 Hz, 1H), 1.21 (dd, J=6.3, 2.5 Hz, 3H), 1.11 (t, J=7.1 Hz, 3H), 0.99-0.88 (m, 6H), 0.79 (ddd, J=27.8, 6.7, 2.1 Hz, 3H), 0.48 (d, J=3.7 Hz, 3H) and LCMS (ESI): m/z [M+H] calc'd for $C_{51}H_{65}N_7O_8$ 903.5; found 904.7; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 9.34-9.27 (m, 1H), 8.77 (dd, J=4.7, 1.7 Hz, 1H), 8.17-7.77 (m, 3H), 7.64-7.43 (m, 3H), 7.33 (d, J=13.7 Hz, 1H), 7.05-6.94 (m, 1H), 6.69-6.41 (m, 2H), 6.26-5.94 (m, 1H), 5.73-5.63 (m, 1H), 5.50-5.20 (m, 2H), 4.40-4.15 (m, 3H), 4.00-3.40 (m, 9H), 3.11 (d, J=4.4 Hz, 3H), 2.93-2.60 (m, 8H), 2.29-2.01 (m, 3H), 1.99 (s, 1H), 1.87-1.75 (m, 2H), 1.73-1.47 (m, 2H), 1.40 (d, J=6.0 Hz, 3H), 1.01-0.88 (m, 6H), 0.85-0.65 (m, 7H), 0.56 (s, 3H).

Example A89. Synthesis of (2S)—N-[(8S,14S)-22-ethyl-4-hydroxy-18,18-dimethyl-21-[6-(4-methylpiperazin-1-yl)pyridin-3-yl]-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methyl-2-{N-methyl-1-[(3S)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido}butanamide



Intermediate 6





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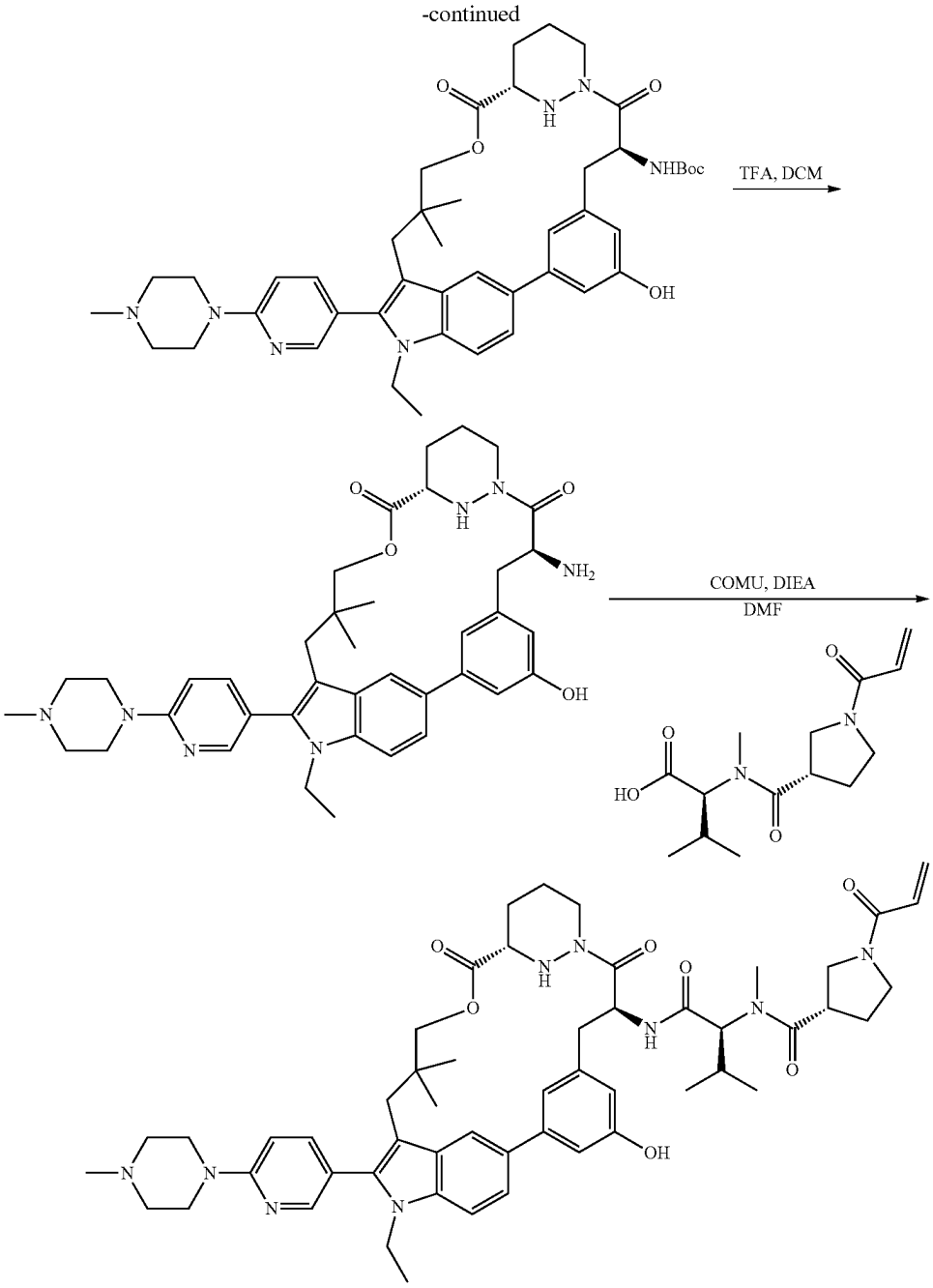


Step 1.

To a mixture of tert-butyl ((6³S,4S)-1²-iodo-10,10-dimethyl-5,7-dioxo-2⁵-((triisopropylsilyl)oxy)-6¹,6²,6³,6⁴,6⁵,6⁶-hexahydro-1¹H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (240.00 mg, 0.279 mmol, 1.00 equiv) and Cs_2CO_3 (182 mg, 0.558 mmol, 2 equiv) in DMF (5.00 mL) was added ethyl iodide (113.45 mg, 0.727 mmol, 2.60 equiv) dropwise at 0° C. The reaction was stirred for 16 h at 25° C. The resulting mixture was diluted with water (10 mL) and extracted with EtOAc (3×10 mL). The combined organic layers were washed with brine (3×10 mL), and dried over anhydrous Na_2SO_4. The filtrate was concentrated under reduced pressure and the remaining residue was purified by silica gel column chromatography to afford tert-butyl ((6³S,4S)-1¹-ethyl-1²-iodo-10,10-dimethyl-5,7-dioxo-2⁵-((triisopropylsilyl)oxy)-6¹,6²,6³,6⁴,6⁵,6⁶-hexahydro-1¹H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (190 mg, 77% yield) as a yellow solid.

Step 2.

A mixture of tert-butyl ((6³S,4S)-1¹-ethyl-1²-iodo-10,10-dimethyl-5,7-dioxo-2⁵-((triisopropylsilyl)oxy)-6¹,6²,6³,6⁴,6⁵,6⁶-hexahydro-1¹H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (500 mg, 0.54 mmol), 1-methyl-4-[5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-yl]piperazine (257 mg, 0.8 mmol), $Pd(dppf)Cl_2$ (83 mg, 0.11 mmol) and K_2CO_3 (156 mg, 1.1 mmol) in 1,4-dioxane (25 mL) and H_2O (5 mL)

under an atmosphere of Ar was stirred at 80° C. for 2 h. The mixture was concentrated under reduced pressure and the residue was purified by prep-TLC to afford tert-butyl ((6^{3}S,4S)-1^1-ethyl-10,10-dimethyl-1^2-(6-(4-methylpiperazin-1-yl)pyridin-3-yl)-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl) carbamate (400 mg, 76% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{53}H_{77}N_7O_6Si$ 935.6; found 936.6.

Step 3.

A mixture of tert-butyl ((6^{3}S,4S)-1^1-ethyl-10,10-dimethyl-1^2-(6-(4-methylpiperazin-1-yl)pyridin-3-yl)-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (350 mg, 0.36 mmol) and 1M TBAF in THF (0.4 mL, 0.4 mmol) in THF (5 mL) was stirred at 0° C. for 1 h. The mixture was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give tert-butyl ((6^{3}S,4S)-1^1-ethyl-2^5-hydroxy-10,10-dimethyl-1^2-(6-(4-methylpiperazin-1-yl)pyridin-3-yl)-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl) carbamate (290 mg, 100% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{44}H_{57}N_7O_6$ 779.4; found 780.4.

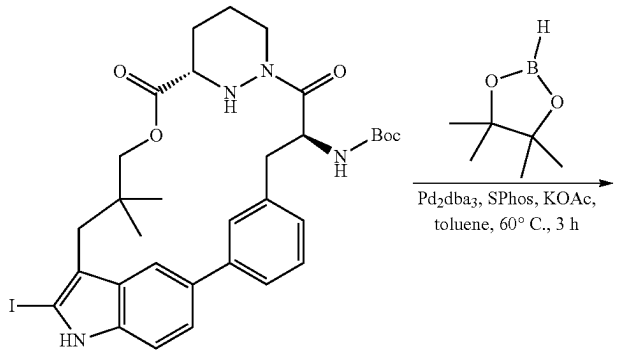
Step 4.

A mixture of tert-butyl ((6^{3}S,4S)-1^1-ethyl-2^5-hydroxy-10,10-dimethyl-1^2-(6-(4-methylpiperazin-1-yl)pyridin-3-yl)-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (300 mg, 0.37 mmol) in TFA (5 mL) and DCM (5 mL) was stirred at rt for 1 h. The mixture was concentrated under reduced pressure to give (6^{3}S,4S)-4-amino-1^1-ethyl-2^5-hydroxy-10,10-dimethyl-1^2-(6-(4-methylpiperazin-1-yl)pyridin-3-yl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (300 mg, crude) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{39}H_{49}N_7O_4$ 679.4; found 680.3.

Step 5.

To a mixture of (6^{3}S,4S)-4-amino-1^1-ethyl-2^5-hydroxy-10,10-dimethyl-1^2-(6-(4-methylpiperazin-1-yl)pyridin-3-yl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (300 mg, 0.36 mmol) in DMF (3 mL) at 0° C. under an atmosphere of N_2 was added DIPEA (0.96 mL, 5.4 mmol) and (2S)-3-methyl-2-[N-methyl-1-[(3S)-1-(prop-2-enoyl) pyrrolidin-3-yl]formamido]butanoic acid (213 mg, 0.72 mmol), followed by dropwise addition of COMU (243 mg, 0.56 mmol). H_2O was added at 0° C. and the mixture was extracted with EtOAc (3×10 mL). The combined organic layers were washed with brine (3×10 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the crude residue was purified by Prep-HPLC to give (2S)—N-[(8S,14S)-22-ethyl-4-hydroxy-18,18-dimethyl-21-[6-(4-methylpiperazin-1-yl) pyridin-3-yl]-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methyl-2-{N-methyl-1-[(3S)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido}butanamide (45 mg, 13.2% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{53}H_{69}N_9O_7$ 943.5; found 944.8; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 9.39-9.23 (m, 1H), 8.64-8.60 (m, 1H), 8.19-8.16 (m, 1H), 8.15 (d, J=6.2 Hz, 1H), 7.86 (s, 1H), 7.66-7.62 (m, 1H), 7.56-7.54 (m, 1H), 7.50-7.43 (m, 1H), 7.13-7.11 (m, 1H), 7.03-6.95 (m, 1H), 6.70-6.47 (m, 2H), 6.17 (ddt, J=16.8, 6.4, 2.8 Hz, 1H), 5.76-5.63 (m, 1H), 5.45-5.33 (m, 1H), 5.11 (m, 1H), 4.75-4.72 (m, 1H), 4.28-4.24 (m, 1H), 4.11-3.98 (m, 4H), 3.91-3.76 (m, 1H), 3.73-3.71 (m, 1H), 3.59-3.56 (m, 7H), 3.51-3.40 (m, 2H), 3.08-2.94 (m, 1H), 2.94-2.92 (m, 2H), 2.92-2.87 (m, 2H), 2.86-2.83 (m, 2H), 2.80-2.65 (m, 2H), 2.83-2.82 (m, 3H), 2.28-2.25 (m, 3H), 2.08-2.05 (m, 2H), 2.02-1.96 (m, 1H), 1.87-1.78 (m, 1H), 1.74-1.66 (m, 1H), 1.56-1.48 (m, 1H), 1.11-1.08 (m, 4H), 0.99-0.92 (m, 2H), 0.89-0.87 (m, 5H), 0.82-0.73 (m, 2H).

Example A115. Synthesis of two atropisomers of (2S)—N-[(8S,14S,20P)-22-ethyl-21-{4-[(1S)-1-methoxyethyl]pyridin-3-yl}-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methyl-2-{N-methyl-1-[(3S)-1-(prop-2-enoyl)pyrrolidin-3-yl] formamido}butanamide



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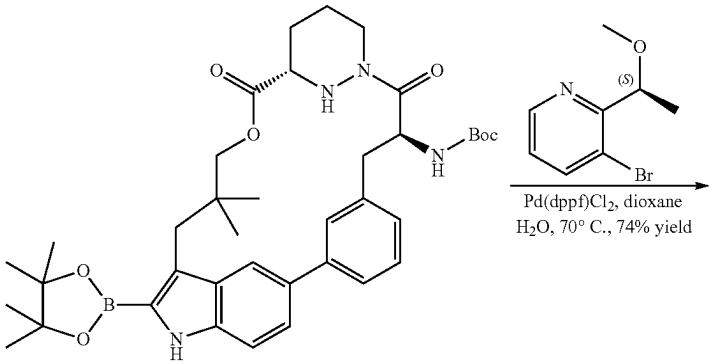
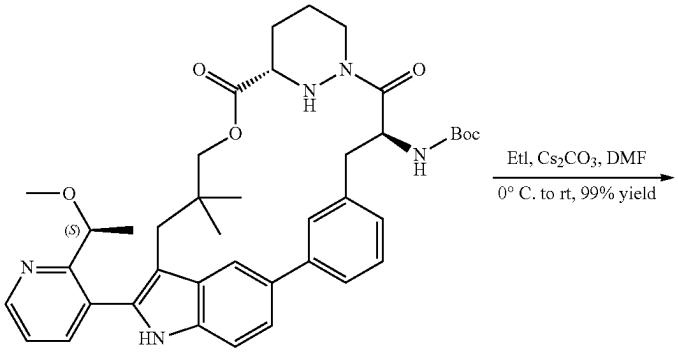
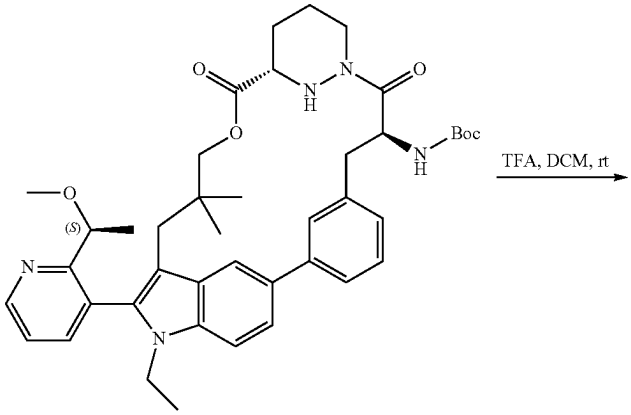
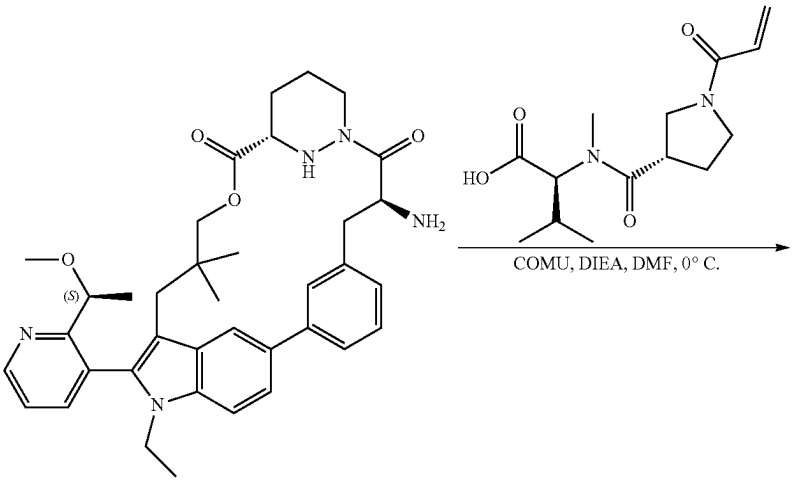

Pd(dppf)Cl2, dioxane
H2O, 70° C., 74% yield


EtI, Cs2CO3, DMF
0° C. to rt, 99% yield


TFA, DCM, rt


COMU, DIEA, DMF, 0° C.

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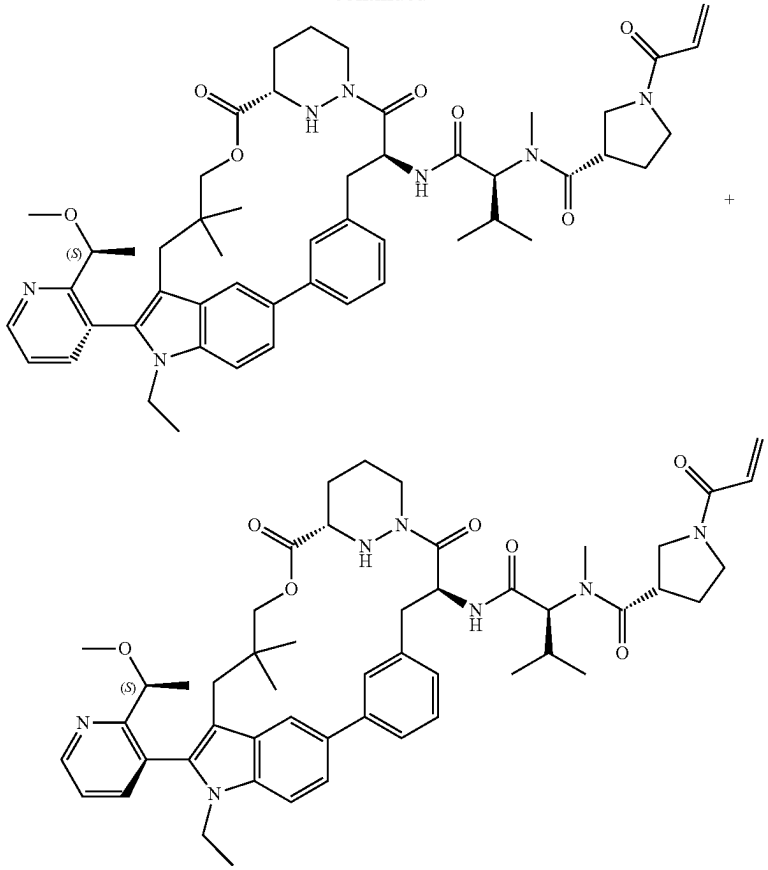


Step 1.

A 1 L round-bottom flask was charged with tert-butyl ((6^{3}S,4S)-1^2-iodo-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (22.00 g, 32.042 mmol, 1.00 equiv), toluene (300.00 mL), $Pd_2(dba)_3$ (3.52 g, 3.845 mmol, 0.12 equiv), S-Phos (3.95 g, 9.613 mmol, 0.30 equiv), and KOAc (9.43 g, 96.127 mmol, 3.00 equiv) at room temperature. To the mixture was added 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (26.66 g, 208.275 mmol, 6.50 equiv) dropwise with stirring at room temperature. The resulting solution was stirred for 3 h at 60° C. The resulting mixture was filtered, and the filter cake was washed with EtOAc. The filtrate was concentrated under reduced pressure and the remaining residue was purified by silica gel column chromatography to afford tert-butyl ((6^{3}S,4S)-10,10-dimethyl-5,7-dioxo-12-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (22 g, 90%) as a light yellow solid. ESI-MS m/z=687.3 $[M+H]^+$; Calculated MW: 686.4

Step 2.

A mixture of tert-butyl ((6^{3}S,4S)-10,10-dimethyl-5,7-dioxo-1^2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1 ^{1}H-8-oxa-1 (5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl) carbamate (2.0 g, 2.8 mmol), 3-bromo-2-[(1S)-1-methoxyethyl]pyridine (0.60 g, 2.8 mmol), $Pd(dppf)Cl_2$ (0.39 g, 0.5 mmol), and K_3PO_4 (1.2 g, 6.0 mmol) in 1,4-dioxane (50 mL) and H_2O (10 mL) under an atmosphere of N_2 was heated to 70° C. and stirred for 2 h. The mixture was diluted with H_2O (50 mL) and extracted with EtOAc (3×50 mL). The combined organic layers were washed with brine (3×50 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give tert-butyl ((6^{3}S,4S)-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (1.5 g, 74% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{40}H_{49}N_5O_6$695.4; found 696.5.

Step 3.

A mixture of tert-butyl ((6^{3}S,4S)-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl) carbamate (1.5 g, 2.1 mmol), Cs_2CO_3 (2.1 g, 6.3 mmol), and ethyl iodide (0.43 mL, 5.1 mmol) in DMF (50 mL) was stirred at 0° C. for 16 h. The mixture was quenched at 0° C. with H_2O and extracted with EtOAc (3×50 mL). The combined organic layers were washed with brine (3×50 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give tert-butyl ((6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (1.5 g, 99%

yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{42}H_{53}N_5O_6$ 723.4; found 724.6.

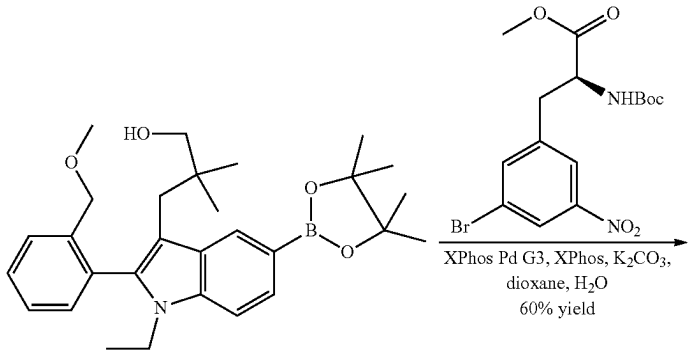
Step 4.

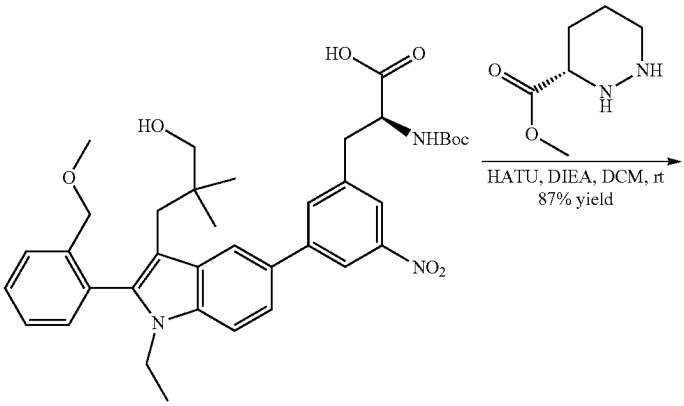
A mixture of tert-butyl ((6[3]S,4S)-1[1]-ethyl-1[2]-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6[1],6[2],6[3],6[4],6[5],6[6]-hexahydro-1 [1]H-8-oxa-1 (5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl) carbamate (1.3 g, 1.7 mmol) in TFA (10 mL) and DCM (20 mL) was stirred at 0° C. for 2 h. The mixture was concentrated under reduced pressure to afford (6[3]S,4S)-4-amino-1[1]-ethyl-1[2]-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6[1],6[2],6[3],6[4],6[5],6[6]-hexahydro-1[1]H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (1.30 g, crude) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{37}H_{45}N_5O_4$ 623.3; found 624.4.

Step 5.

Into a 40-mL vial purged and maintained with an inert atmosphere of Ar, was placed (6[3]S,4S)-4-amino-1[1]-ethyl-1[2]-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6[1],6[2],6[3],6[4],6[5],6[6]-hexahydro-1[1]H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (250 mg, 0.4 mmol), (2S)-3-methyl-2-[N-methyl-1-[(3S)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido]butanoic acid (226 mg, 0.8 mmol), DIPEA (774 mg, 6.0 mmol), and DMF (3 mL). A solution of COMU (257 mg, 0.6 mmol) in DMF (2 mL) was added at 0° C. and the resulting mixture was stirred at 0° C. for 1 h. The mixture was filtered, the filtrate was concentrated under reduced pressure, and the residue was purified by prep-HPLC to give two atropisomers of (2S)—N-[(8S,14S,20P)-22-ethyl-21-{4-[(1 S)-1-methoxyethyl]pyridin-3-yl}-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1[2],[6].1[10],[14].0[23],[27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methyl-2-{N-methyl-1-[(3S)-1-(prop-2-enoyl)pyrrolidin-3-yl] formamido}butanamide (56 mg, 15% yield) and (46 mg, 13% yield) both as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{51}H_{65}N_7O_7$ 887.5; found 888.4; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.81 (s, 1H), 8.07 (s, 1H), 8.05-7.96 (m, 1H), 7.78-7.45 (m, 5H), 7.41-7.08 (m, 2H), 6.66-6.58 (m, 1H), 6.18 (d, J=17.0 Hz, 1H), 5.75-5.67 (m, 1H), 5.46-5.31 (m, 1H), 5.16-5.04 (m, 1H), 4.75 (dd, J=10.9, 4.5 Hz, 1H), 4.31-4.21 (m, 2H), 4.11-3.95 (m, 3H), 3.87-3.71 (m, 5H), 3.74-3.54 (m, 3H), 3.11 (s, 4H), 2.95 (d, J=9.7 Hz, 2H), 2.85-2.72 (m, 3H), 2.31-2.04 (m, 3H), 1.88-1.47 (m, 2H), 1.24-1.21 (m, 3H), 1.16-1.08 (m, 3H), 1.03-0.91 (m, 6H), 0.85-0.74 (m, 3H), 0.51-0.46 (m, 3H) and LCMS (ESI): m/z [M+H] calc'd for $C_{51}H_{65}N_7O_7$ 887.5; found 888.4; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.77 (s, 1H), 8.71-8.63 (m, 0.5H), 8.23-8.17 (m, 0.5H), 8.00 (s, 1H), 7.85 (t, J=9.9 Hz, 2H), 7.77-7.62 (m, 3H), 7.57-7.50 (m, 1H), 7.33-7.22 (m, 1H), 7.15-7.06 (m, 1H), 6.73-6.56 (m, 1H), 6.17 (ddd, J=16.7, 6.1, 2.7 Hz, 1H), 5.76-5.64 (m, 1H), 5.49-5.29 (m, 2H), 4.70 (dd, J=10.8, 3.5 Hz, 1H), 4.33-4.22 (m, 3H), 4.14-3.95 (m, 2H), 3.86-3.77 (m, 1H), 3.72-3.65 (m, 2H), 3.61 (t, J=10.6 Hz, 3H), 3.46-3.42 (m, 1H), 3.13 (d, J=4.8 Hz, 3H), 2.99 (d, J=14.4 Hz, 1H), 2.95-2.70 (m, 6H), 2.24-1.99 (m, 4H), 1.95-1.44 (m, 4H), 1.40 (d, J=6.1 Hz, 3H), 0.98-0.87 (m, 6H), 0.86-0.64 (m, 6H), 0.64-0.54 (m, 3H).

Example A2. Synthesis of (2S)—N-[(8S,14S)-4-amino-22-ethyl-21-[2-(2-methoxyethyl)phenyl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1[2],[6].1[10],[14].0[23],[27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methyl-2-{N-methyl-1-[(3S)-1-(prop-2-enoyl)pyrrolidin-3-yl] formamido}butanamide





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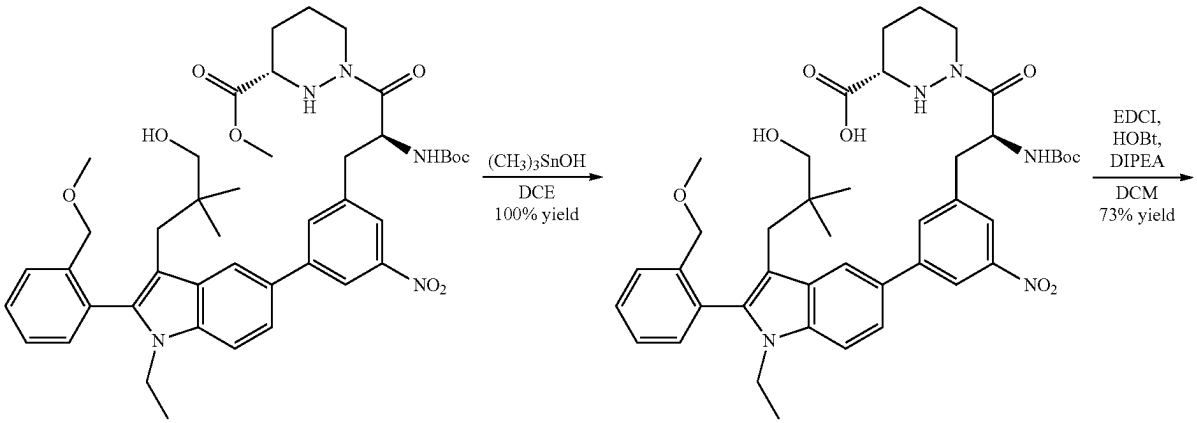

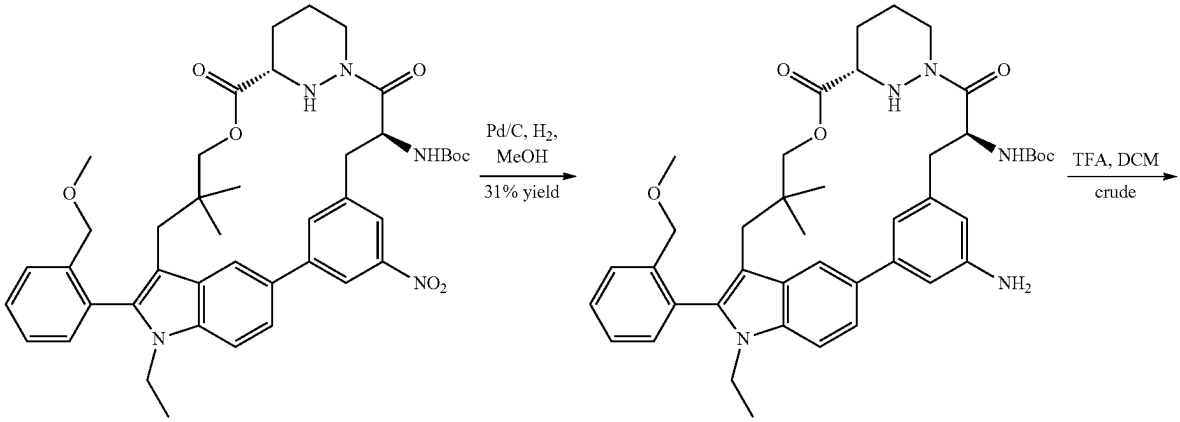

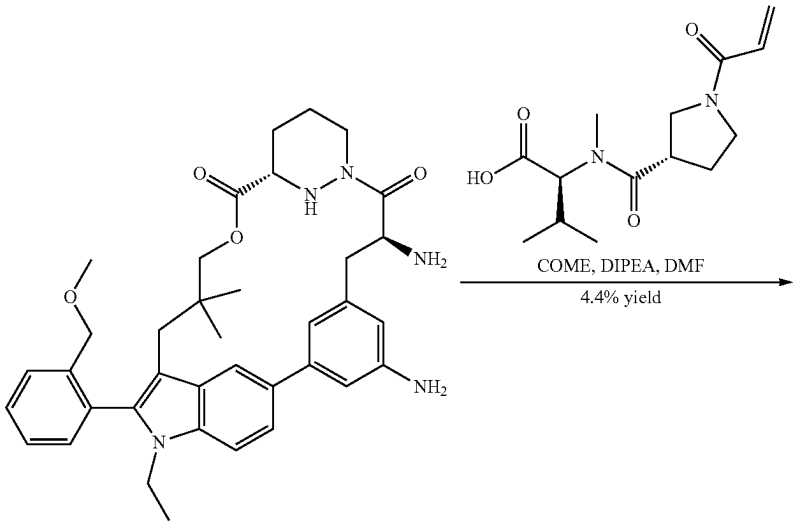

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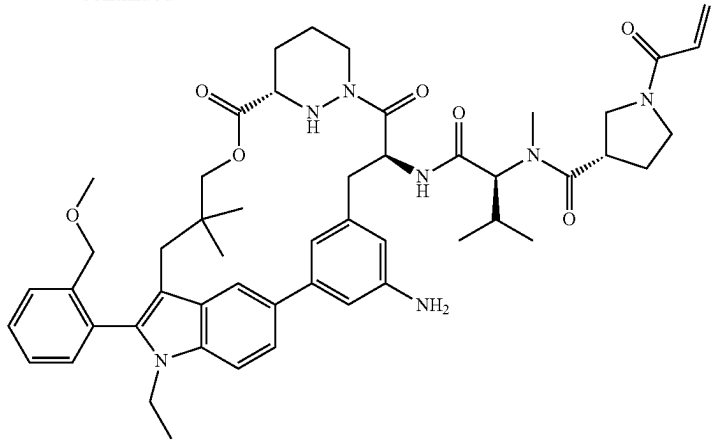


Step 1.

Into a 25 mL sealed tube were added 3-[1-ethyl-2-[2-(methoxymethyl)phenyl]-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)indol-3-yl]-2,2-dimethylpropan-1-ol (590 mg, 1.2 mmol), methyl (2S)-3-(3-bromo-5-nitrophenyl)-2-[(tert-butoxycarbonyl)amino]propanoate (747 mg, 1.9 mmol), XPhos Pd G3 (105 mg, 0.12 mmol), XPhos (71 mg, 0.15 mmol), K_2CO_3 (427 mg, 3.1 mmol), and 1,4-dioxane (2 mL) under an atmosphere of N_2 at rt. The mixture was heated to 60° C. and stirred overnight, then cooled and H_2O added. The mixture was extracted with EtOAc (3×20 mL) and the combined organic layers were washed with brine (1×20 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give (2S)-2-[(tert-butoxycarbonyl)amino]-3-[3-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-(methoxymethyl)phenyl]indol-5-yl]-5-nitrophenyl]propanoic acid (500 mg, 61% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{37}H_{45}N_3O_8$ 659.3; found 660.4.

Step 2.

A mixture of (2S)-2-[(tert-butoxycarbonyl)amino]-3-[3-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-(methoxymethyl)phenyl]indol-5-yl]-5-nitrophenyl]propanoic acid (500 mg, 0.79 mmol), methyl (3S)-1,2-diazinane-3-carboxylate (164 mg, 1.1 mmol), DCM (6 mL), DIPEA (294 mg, 2.3 mmol) and HATU (432 mg, 1.1 mmol) was stirred at 0° C. for 1 h under an atmosphere of air. H_2O was added and the mixture was extracted with DCM (3×20 mL), then the combined organic layers were dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl (3S)-1-[(2S)-2-[(tert-butoxycarbonyl)amino]-3-[3-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-(methoxymethyl)phenyl]indol-5-yl]-5-nitrophenyl]propanoyl]-1,2-diazinane-3-carboxylate (520 mg, 87% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{43}H_{55}N_5O_9$ 785.4; found 786.8.

Step 3.

Into a 40 mL sealed tube were added methyl (3S)-1-[(2S)-2-[(tert-butoxycarbonyl)amino]-3-[3-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-(methoxymethyl)phenyl]indol-5-yl]-5-nitrophenyl]propanoyl]-1,2-diazinane-3-carboxylate (510 mg, 0.65 mmol), DCE (5 mL) and trimethyltin hydroxide (587 mg, 3.3 mmol) at rt under an atmosphere of air. The mixture was heated to 60° C. and stirred overnight, cooled, and diluted with DCM (20 mL). The mixture was washed with 0.1 N $KHSO_4$ (3×20 mL), dried over anhydrous Na_2SO_4, filtered, and the filtrate was concentrated under reduced pressure to give (3S)-1-[(2S)-2-[(tert-butoxycarbonyl)amino]-3-[3-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-(methoxymethyl)phenyl]indol-5-yl]-5-nitrophenyl]propanoyl]-1,2-diazinane-3-carboxylic acid (500 mg, 100%) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{42}H_{53}N_5O_9$ 771.4; found 772.7.

Step 4.

A mixture of (3S)-1-[(2S)-2-[(tert-butoxycarbonyl)amino]-3-[3-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-(methoxymethyl)phenyl]indol-5-yl]-5-nitrophenyl]propanoyl]-1,2-diazinane-3-carboxylic acid (490 mg, 0.64 mmol), DCM (100 mL), DIPEA (2.5 g, 19.0 mmol), HOBT (429 mg, 3.2 mmol), and EDCI (3.65 g, 19.0 mmol) at room temperature was stirred at rt overnight under an atmosphere of air. H_2O was added and the mixture was extracted with DCM (3×60 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give tert-butyl ((6^{3}S,4S)-1^1-ethyl-1^2-(2-(methoxymethyl)phenyl)-10,10-dimethyl-2^5-nitro-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (350 mg, 73% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{42}H_{51}N_5O_8$ 753.4; found 754.2.

Step 5.

A mixture of tert-butyl ((6^{3}S,4S)-1^1-ethyl-1^2-(2-(methoxymethyl)phenyl)-10,10-dimethyl-2^5-nitro-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)- benzenacycloundecaphane-4-yl)carbamate (200 mg, 0.27 mmol), MeOH (4 mL), and Pd on carbon (20 mg) was stirred at rt for 2 h under an atmosphere of H_2. The mixture was filtered, the filter cake was washed with MeOH (3×5 mL), and the filtrate was concentrated under reduced pressure to give tert-butyl ((6^{3}S,4S)-2^5-amino-1^1-ethyl-1^2-(2-(methoxymethyl)phenyl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (60 mg, 31% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{42}H_{53}N_5O_6$ 723.4; found 724.4.

Step 6.

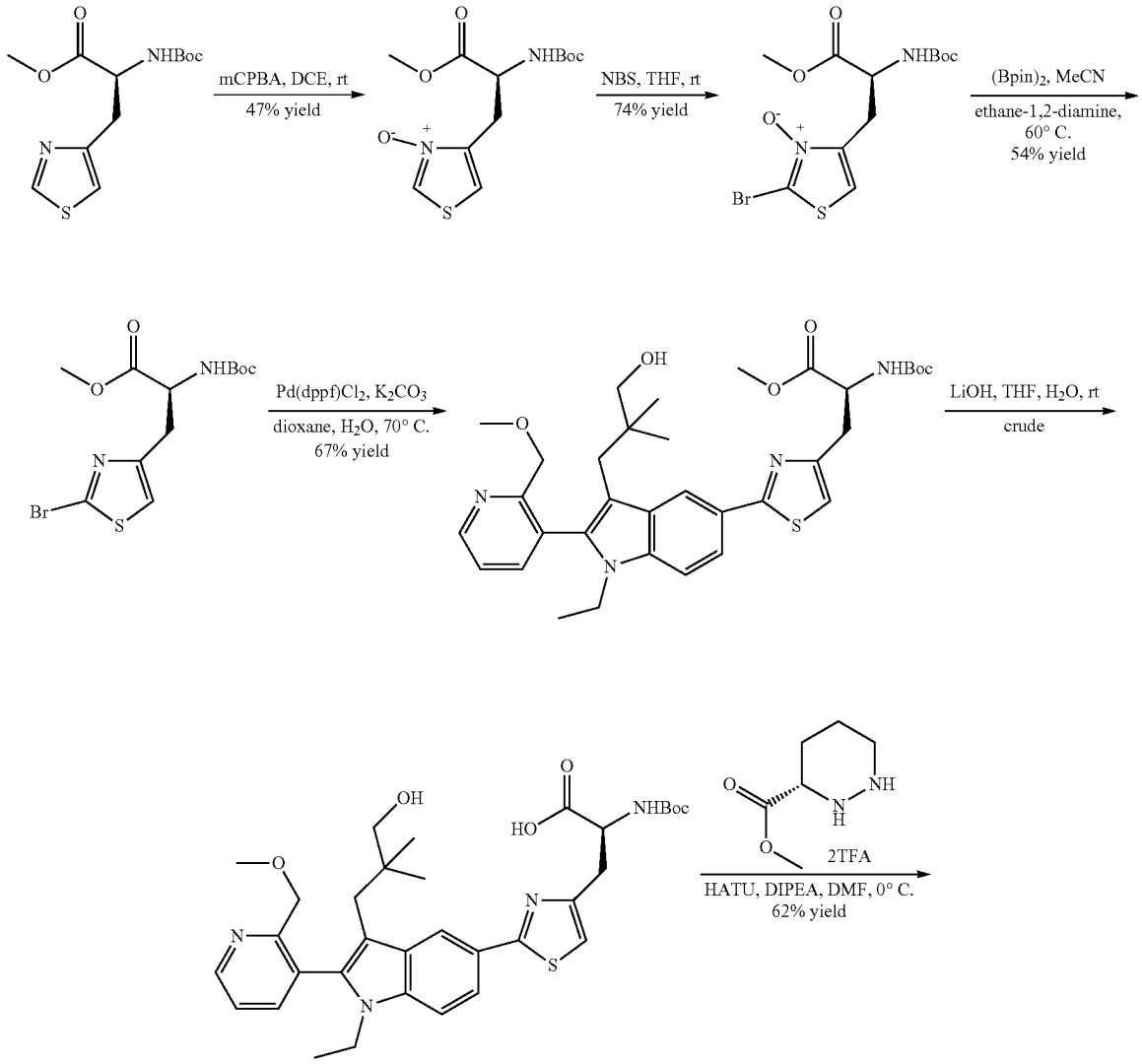
Into an 8 mL vial were added tert-butyl ((6^{3}S,4S)-2^5-amino-1^1-ethyl-1^2-(2-(methoxymethyl)phenyl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacyclounde-

caphane-4-yl)carbamate (50 mg, 0.07 mmol), DCM (1 mL), and TFA (158 mg, 1.4 mmol) at 0° C. under an atmosphere of air. The mixture was stirred for at 0° C. for 2 h then concentrated under reduced pressure to give (6^3S,4S)-2^5,4-diamino-1^1-ethyl-1^2-(2-(methoxymethyl)phenyl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (45 mg) as a solid, which was used directly in the next step directly without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{37}H_{45}N_5O_4$ 623.3; found 624.4.

Step 7.

Into an 8 mL vial were added (6^3S,4S)-2^5,4-diamino-1^1-ethyl-1^2-(2-(methoxymethyl)phenyl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1 ^{1}H-8-oxa-1 (5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (40 mg, 0.06 mmol), DMF (1 mL), DIPEA (75 mg, 0.58 mmol), and COMU (41 mg, 0.1 mmol) at 0° C. under an atmosphere of air. The mixture was stirred at 0° C. for 1 h, then H_2O added. The mixture was extracted with EtOAc (3×30 mL), the combined organic layers were concentrated under reduced pressure, and purified by prep-HPLC to give (2S)—N-[(8S,14S)-4-amino-22-ethyl-21-[2-(2-methoxyethyl)phenyl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.$1^{2,6}$.$1^{10,14}$.$0^{23,27}$]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methyl-2-{N-methyl-1-[(3S)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido}butanamide (2.5 mg, 4.4% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{51}H_{65}N_7O_7$ 887.5; found 888.6; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.74-8.55 (m, 1H), 7.89 (d, J=9.6 Hz, 1H), 7.66-7.53 (m, 1H), 7.57-7.47 (m, 6H), 7.32 (t, J=6.4 Hz, 1H), 6.85 (d, J=8.4 Hz, 2H), 6.70-6.55 (m, 1H), 6.24-6.12 (m, 1H), 5.69 (ddd, J=14.8, 8.0, 3.9 Hz, 1H), 5.41 (s, 1H), 5.09-4.80 (m, 2H), 4.26 (d, J=10.1 Hz, 2H), 4.19 (s, 2H), 4.17-4.06 (m, 1H), 4.02 (dd, J=12.0, 3.9 Hz, 1H), 3.92 (d, J=8.0 Hz, 3H), 3.78 (d, J=8.7 Hz, 5H), 3.29 (s, 2H), 3.14 (d, J=1.9 Hz, 1H), 2.98-2.92 (m, 1H), 2.87-2.68 (m, 3H), 2.62 (d, J=12.5 Hz, 3H), 2.15-1.99 (m, 4H), 1.80 (s, 1H), 1.68-1.53 (m, 2H), 1.08 (t, J=7.1 Hz, 1H), 0.98-0.88 (m, 6H), 0.82 (dd, J=23.3, 16.4 Hz, 3H), 0.74 (t, J=7.2 Hz, 3H), 0.44 (s, 2H), 0.43 (s, 3H).

Example A118. Synthesis of (2S)—N-[(7S,13S)-21-ethyl-20-[2-(methoxymethyl)pyridin-3-yl]-17,17-dimethyl-8,14-dioxo-15-oxa-3-thia-9,21,27,28-tetraazapentacyclo[17.5.2.$1^{2,5}$.$1^{9,13}$.$0^{22,26}$]octacosa-1(25),2(28),4,19,22(26),23-hexaen-7-yl]-3-methyl-2-{N-methyl-1-[(3S)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido}butanamide



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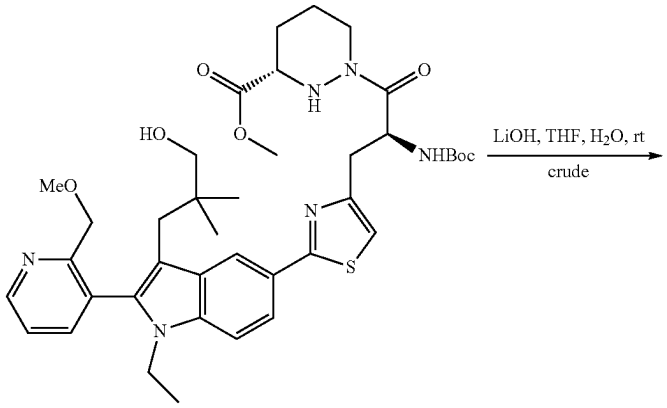
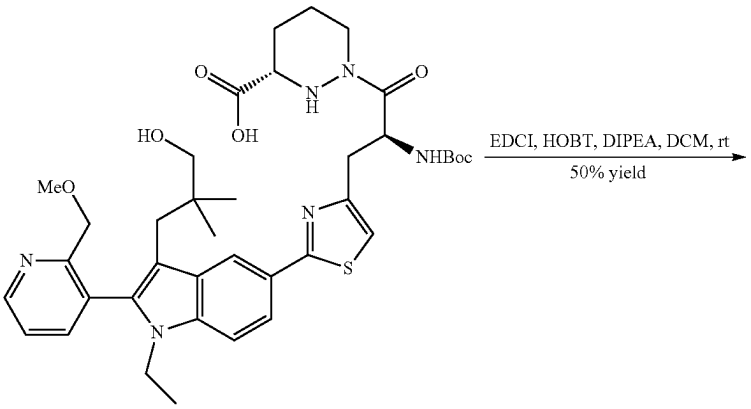
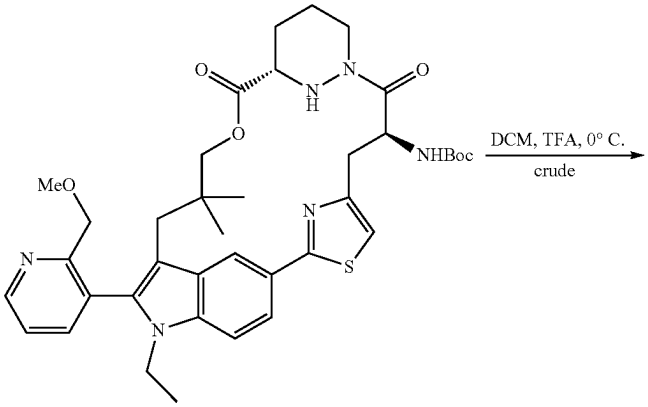
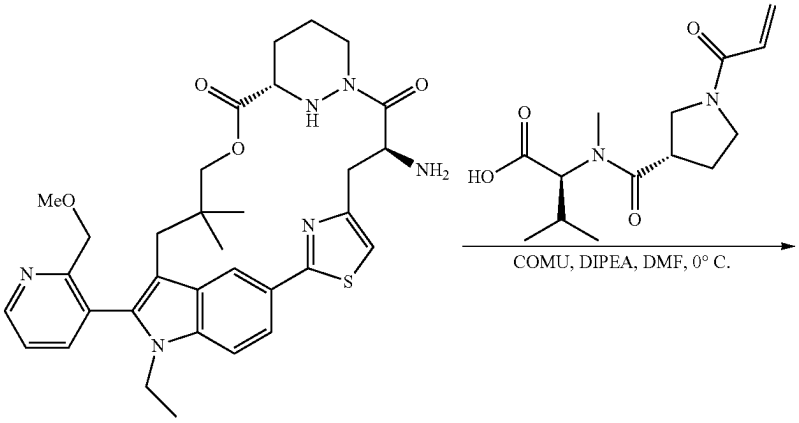

LiOH, THF, H_2O, rt
crude


EDCI, HOBT, DIPEA, DCM, rt
50% yield


DCM, TFA, 0° C.
crude


COMU, DIPEA, DMF, 0° C.

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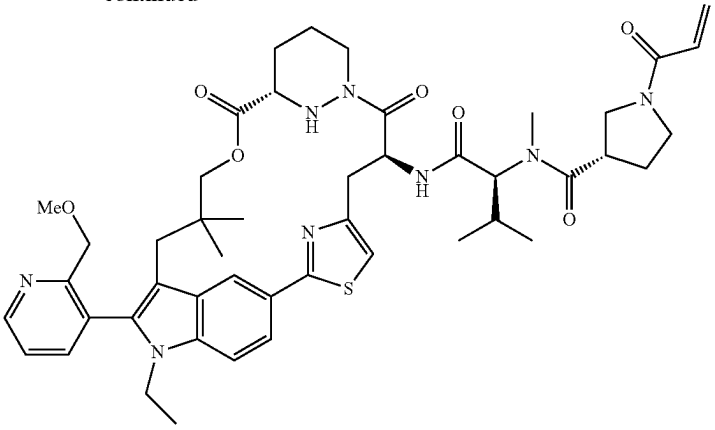


Step 1.

A mixture of methyl (2S)-2-[(tert-butoxycarbonyl)amino]-3-(1,3-thiazol-4-yl)propanoate (2.08 g, 7.26 mmol) and mCPBA (1.88 g, 10.9 mmol) in DCE (15 mL) at 0° C. under an atmosphere of N_2 was diluted with DCM (100 mL). The mixture was allowed to warm to rt and stirred for 16 h, then diluted with DCM, washed with H_2O (1×30 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 4-[(2S)-2-[(tert-butoxycarbonyl)amino]-3-methoxy-3-oxopropyl]-1,3-thiazol-3-ium-3-olate (1.15 g, 47% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{12}H_{18}N_2O_5S$ 302.1; found 303.2.

Step 2.

To a mixture of 4-[(2S)-2-[(tert-butoxycarbonyl)amino]-3-methoxy-3-oxopropyl]-1,3-thiazol-3-ium-3-olate (1.15 g, 3.8 mmol) in THF at 0° C. under an atmosphere of N_2 was added NBS (0.74 g, 4.2 mmol) dropwise. The mixture was allowed to warm to rt and stirred for 2 h, then diluted with H_2O (500 mL) and extracted with EtOAc (3×500 mL). The combined organic layers were washed with water (2×30 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 2-bromo-4-[(2S)-2-[(tert-butoxycarbonyl)amino]-3-methoxy-3-oxopropyl]-1,3-thiazol-3-ium-3-olate (1.2 g, 74% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{12}H_{17}BrN_2O_5S$ 380.0; found 381.0.

Step 3.

To a stirred mixture of 2-bromo-4-[(2S)-2-[(tert-butoxycarbonyl)amino]-3-methoxy-3-oxopropyl]-1,3-thiazol-3-ium-3-olate (1.2 g, 3.2 mmol) and 4,4,5,5-tetramethyl-2-(tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,2-dioxaborolane (1.04 g, 4.1 mmol) in MeCN at 70° C. under an atmosphere of N_2 was added ethane-1,2-diamine (1.89 g, 31.5 mmol) in portions. The mixture was cooled to 60° C. and the mixture was stirred overnight, then diluted with water (500 mL) and extracted with EtOAc (3×400 mL). The combined organic layers were washed with brine (1×50 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl (2S)-3-(2-bromo-1,3-thiazol-4-yl)-2-[(tert-butoxycarbonyl)amino]propanoate (653 mg, 54% yield) as a solid.

Step 4.

A 50 mL sealed tube was charged with 3-[1-ethyl-2-[2-(methoxymethyl)pyridin-3-yl]-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)indol-3-yl]-2,2-dimethylpropan-1-ol (1.00 g, 2.1 mmol), K_2CO_3 (727 mg, 5.2 mmol), Pd(dppf)Cl_2 (153 mg, 0.21 mmol), and 2,4-dibromo-1,3-thiazole (1.0 g, 4.2 mmol) at rt under an atmosphere of N_2, then 1,4-dioxane (1.0 mL) and H_2O (0.20 mL) were added. The mixture was heated to 70° C. and stirred for 4 h, then cooled, diluted with H_2O (100 mL) and extracted with EtOAc (3×100 mL). The combined organic layers were washed with brine (3×100 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 3-[5-(4-bromo-1,3-thiazol-2-yl)-1-ethyl-2-[2-(methoxymethyl)pyridin-3-yl]indol-3-yl]-2,2-dimethylpropan-1-ol (727 mg, 67% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{34}H_{44}N_4O_6S$ 636.3; found 637.3.

Step 5.

To a stirred mixture of methyl (2S)-2-[(tert-butoxycarbonyl)amino]-3-[2-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-(methoxymethyl)pyridin-3-yl]indol-5-yl]-1,3-thiazol-4-yl]propanoate (636 mg, 1.0 mmol) and LiOH·H_2O (126 mg, 3.0 mmol) in THF at 0° C. under an atmosphere of N_2 was added H_2O (1.24 mL) portionwise. The mixture was allowed to warm to rt and stirred for 1 h, then diluted with water (300 mL) and extracted with EtOAc (3×300 mL). The combined organic layers were washed with brine (1×100 mL), dried over anhydrous Na_2SO_4, filtered, and the filtrate concentrated under reduced pressure to give (2S)-2-[(tert-butoxycarbonyl)amino]-3-[2-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-(methoxymethyl)pyridin-3-yl]indol-5-yl]-1,3-thiazol-4-yl]propanoic acid (622 mg, crude), which was used in the next step directly without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{33}H_{42}N_4O_6S$ 622.3; found 623.2.

Step 6.

To a stirred mixture of (2S)-2-[(tert-butoxycarbonyl)amino]-3-[2-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-(methoxymethyl)pyridin-3-yl]indol-5-yl]-1,3-thiazol-4-yl]propanoic acid (622 mg, 1.0 mmol) and methyl (3S)-1,2-diazinane-3-carboxylate (288 mg, 2.0 mmol) in DMF at 0° C. under an atmosphere of N_2 was added HATU (570 mg, 1.5 mmol). The mixture was stirred at 0° C. for 1 h, then diluted with EtOAc and washed with H_2O (1×10 mL), dried over anhydrous Na_2SO_4, filtered, and the filtrate concentrated under reduced pressure to give methyl (3S)-1-[(2S)-

2-[(tert-butoxycarbonyl)amino]-3-[2-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-(methoxymethyl)pyridin-3-yl]indol-5-yl]-1,3-thiazol-4-yl]propanoyl]-1,2-diazinane-3-carboxylate (550 mg, 62% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{39}H_{52}N_6O_7S$ 748.4; found 749.6.

Step 7.

(3S)-1-[(2S)-2-[(tert-butoxycarbonyl)amino]-3-[2-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-(methoxymethyl)pyridin-3-yl]indol-5-yl]-1,3-thiazol-4-yl]propanoyl]-1,2-diazinane-3-carboxylic acid was synthesized in a manner similar to (3S)-1-[(2S)-2-[(tert-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1H-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylic acid except methyl (3S)-1-[(2S)-2-[(tert-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1H-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylate was substituted with methyl (3S)-1-[(2S)-2-[(tert-butoxycarbonyl)amino]-3-[2-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-(methoxymethyl)pyridin-3-yl]indol-5-yl]-1,3-thiazol-4-yl]propanoyl]-1,2-diazinane-3-carboxylate. LCMS (ESI): m/z [M+H] calc'd for $C_{38}H_{50}N_6O_7S$ 734.3; found 735.3.

Step 8.

tert-butyl ((6³S,4S,Z)-1¹-ethyl-1²-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6¹,6²,6³,6⁴,6⁵,6⁶-hexahydro-1¹H-8-oxa-2(2,4)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate was synthesized in a manner similar to tert-butyl ((6³S,4S)-1¹-ethyl-1²-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2⁵-((triisopropylsilyl)oxy)-6¹,6²,6³,6⁴,6⁵,6⁶-hexahydro-1¹H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate except (3S)-1-[(2S)-2-[(tert-butoxycarbonyl)amino]-3-[3-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-[(1 S)-1-methoxyethyl]pyridin-3-yl]indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylic acid was substituted with (3S)-1-[(2S)-2-[(tert-butoxycarbonyl)amino]-3-[2-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-(methoxymethyl)pyridin-3-yl]indol-5-yl]-1,3-thiazol-4-yl]propanoyl]-1,2-diazinane-3-carboxylic acid. LCMS (ESI): m/z [M+H] calc'd for $C_{38}H_{48}N_6O_6S$ 716.3; found 717.4.

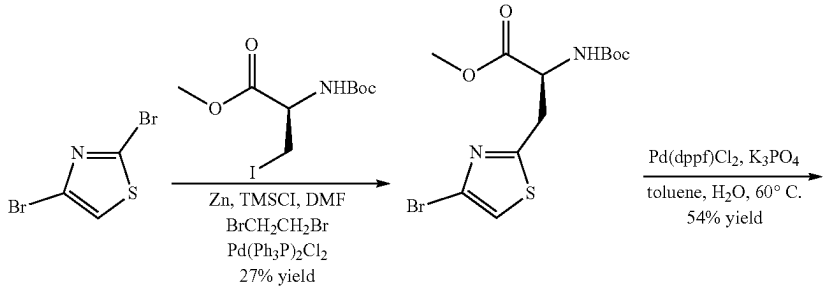
Step 9.

To a stirred mixture of tert-butyl ((6³S,4S,Z)-1¹-ethyl-1²-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6¹,6²,6³,6⁴,6⁵,6⁶-hexahydro-1¹H-8-oxa-2(2,4)-thiazola-1(5,3)-indola-6(1,3)- pyridazinacycloundecaphane-4-yl)carbamate (253 mg) in DCM at 0° C. under an atmosphere of N_2 was added TFA (1.0 mL) dropwise. The mixture was stirred at 0° C. for 1 h, then concentrated under reduced pressure and then repeated using toluene (20 mL×3) to give (6³S,4S,Z)-4-amino-1¹-ethyl-1²-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-6¹,6²,6³,6⁴,6⁵,6⁶-hexahydro-1¹H-8-oxa-2(2,4)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (253 mg, crude) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{33}H_{40}N_6O_4S$ 616.3; found 617.3.

Step 10.

(2S)—N-[(7S,13S)-21-ethyl-20-[2-(methoxymethyl)pyridin-3-yl]-17,17-dimethyl-8,14-dioxo-15-oxa-3-thia-9,21,27,28-tetraazapentacyclo[17.5.2.1²,⁵.1⁹,¹³.0²²,²⁶]octacosa-1(25),2(28),4,19,22(26),23-hexaen-7-yl]-3-methyl-2-{N-methyl-1-[(3S)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido}butanamide was synthesized in a manner similar to (2S)—N-[(8S,14S)-4-amino-22-ethyl-21-[2-(2-methoxyethyl)phenyl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1²,⁶.1¹⁰,¹⁴.0²³,²⁷]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methyl-2-{N-methyl-1-[(3S)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido}butanamide except (6³S,4S)-4-amino-1¹-ethyl-2⁵-hydroxy-1²-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6¹,6²,6³,6⁴,6⁵,6⁶-hexahydro-1¹H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione was substituted with (6³S,4S,Z)-4-amino-1¹-ethyl-1²-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-6¹,6²,6³,6⁴,6⁵,6⁶-hexahydro-1¹H-8-oxa-2(2,4)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione. LCMS (ESI): m/z [M+H] calc'd for $C_{47}H_{60}N_8O_7S$ 880.4; found 881.6; ¹H NMR (400 MHz, DMSO-d_6) δ 8.75 (m, 1H), 8.55 (d, J=6.7 Hz, 1H), 8.32 (d, J=8.3 Hz, 1H), 7.99 (d, J=7.7 Hz, 1H), 7.65-7.51 (m, 3H), 7.11-6.92 (m, 1H), 6.72-6.56 (m, 1H), 6.18 (dd, J=16.8, 2.9 Hz, 1H), 5.82-5.65 (m, 1H), 5.61-5.46 (m, 1H), 5.02 (dd, J=24.2, 12.2 Hz, 1H), 4.69 (d, J=10.9 Hz, 1H), 4.37-4.11 (m, 5H), 4.05-3.79 (m, 4H), 3.76-3.50 (m, 6H), 3.47 (s, 2H), 3.08 (s, 3H), 3.04 (s, 1H), 2.98 (d, J=1.9 Hz, 1H), 2.95 (d, J=3.6 Hz, 2H), 2.83 (d, J=2.0 Hz, 2H), 2.24-2.03 (m, 4H), 1.81 (s, 2H), 1.56 (s, 1H), 1.11 (t, J=7.0 Hz, 2H), 1.02-0.87 (m, 8H), 0.80 (dd, J=24.6, 6.6 Hz, 3H), 0.41 (s, 2H), 0.31 (s, 1H).

Example A194. Synthesis of (2S)—N-[(7S,13S)-21-ethyl-20-[2-(methoxymethyl)pyridin-3-yl]-17,17-dimethyl-8,14-dioxo-15-oxa-4-thia-9,21,27,28-tetraazapentacyclo[17.5.2.1²,⁵.1⁹,¹³.0²²,²⁶]octacosa-1(25),2,5(28),19,22(26),23-hexaen-7-yl]-3-methyl-2-{N-methyl-1-[(3S)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido}butanamide



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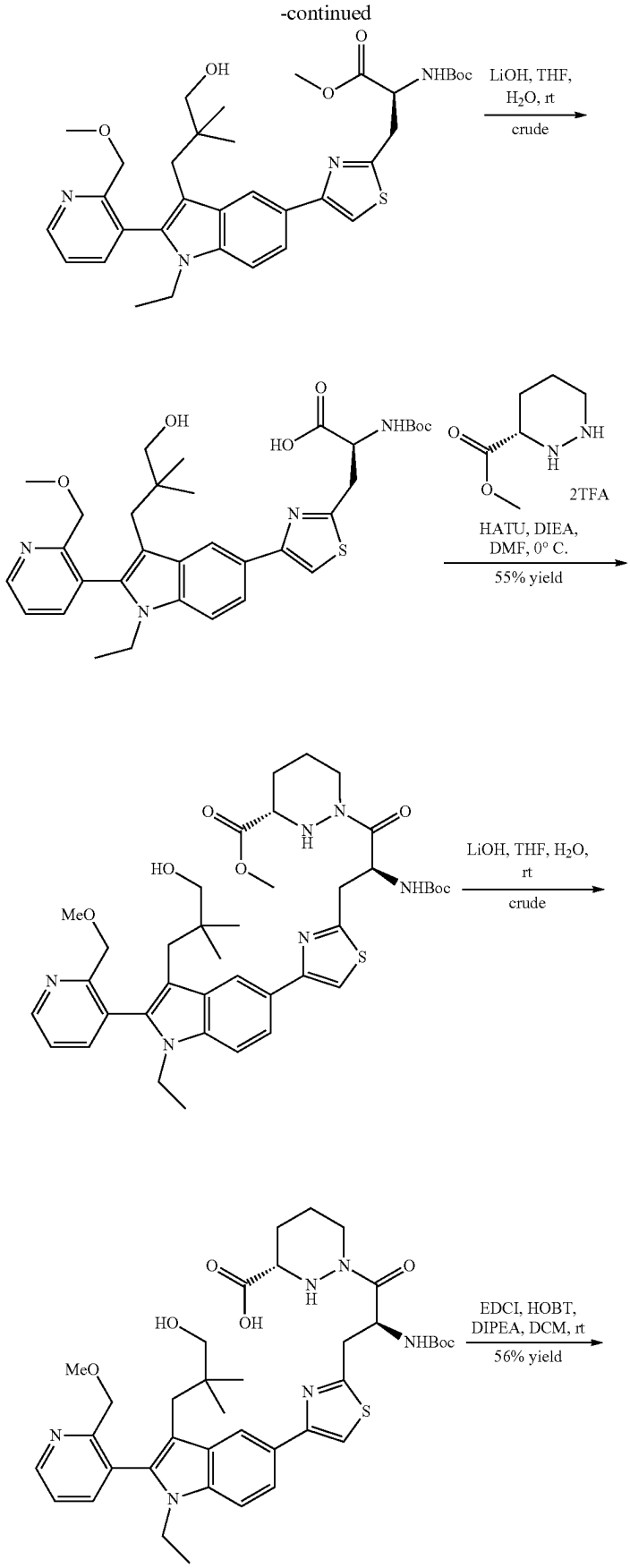
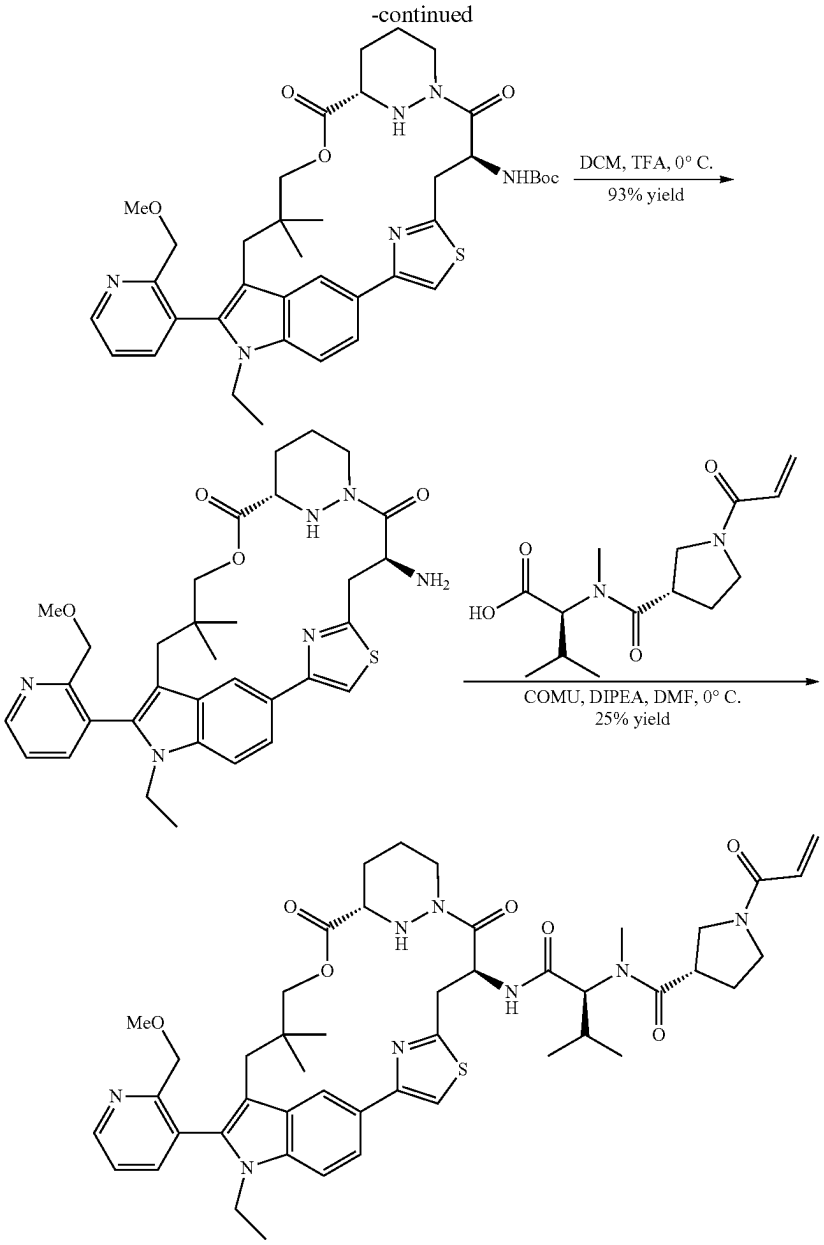

LiOH, THF, H_2O, rt
crude
HATU, DIEA, DMF, 0° C.
55% yield
2TFA
LiOH, THF, H_2O, rt
crude
EDCI, HOBT, DIPEA, DCM, rt
56% yield

-continued



Step 1.

A mixture of Zn (1.2 g, 182 mmol) and 1,2-dibromoethane (1.71 g, 9.1 mmol) and DMF (50 mL) was stirred for 30 min at 90° C. under an atmosphere of Ar. The mixture was allowed to rt, then TMSCI (198 mg, 1.8 mmol) was added dropwise over 30 min at rt. Methyl (2R)-2-[(tert-butoxycarbonyl) amino]-3-iodopropanoate (10.0 g, 30.4 mmol) in DMF (100 mL) was added dropwise over 10 min at rt. The mixture was heated to 35° C. and stirred for 2 h, then a mixture of 2,5-dibromo-1,3-thiazole (1.48 g, 60.8 mmol) and $Pd(PPh_3)_2Cl_2$ (2.1 g, 3.0 mmol) in DMF (100 mL) was added dropwise. The mixture was heated to 70° C. and stirred for 2 h, then filtered and the filtrate diluted with EtOAc (1 L) and washed with H_2O (3×1 L), dried with anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl (2S)-3-(5-bromo-1,3-thiazol-2-yl)-2-[(tert-butoxycarbonyl) amino]propanoate (3 g, 27% yield) as a semi-solid. LCMS (ESI): m/z [M+H] calc'd for $C_{12}H_{17}BrN_2O_4S$ 364.0; found 365.1.

Step 2.

Into a 20 mL sealed tube were added 3-[1-ethyl-2-[2-(methoxymethyl)pyridin-3-yl]-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)indol-3-yl]-2,2-dimethylpropan-1-ol (100 mg, 0.21 mmol), K_3PO_4 (111 mg, 0.52 mmol), $Pd(dppf)Cl_2$ (15 mg, 0.02 mmol), methyl (2S)-3-(4-bromo-1,3-thiazol-2-yl)-2-[(tert-butoxycarbonyl)amino]propanoate (153 mg, 0.42 mmol), toluene (1 mL), and H_2O (0.2 mL) at rt under an atmosphere of N_2. The mixture was heated to 60° C. and stirred for 3 h, cooled, diluted with H_2O (10 mL) and extracted with EtOAc (10 mL×3). The combined organic layers were washed with brine (3×10 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concen-

trated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl (2S)-2-[(tert-butoxycarbonyl)amino]-3-[4-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-(methoxymethyl)pyridin-3-yl]indol-5-yl]-1,3-thiazol-2-yl]propanoate (72 mg, 54% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{34}H_{44}N_4O_6S$ 636.3; found 637.2.

Step 3.

A mixture of methyl (2S)-2-[(tert-butoxycarbonyl)amino]-3-[4-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-(methoxymethyl)pyridin-3-yl]indol-5-yl]-1,3-thiazol-2-yl]propanoate (40 mg, 0.06 mmol) and $LiOH{\cdot}H_2O$ (unspecified) in THF (1 mL) and H_2O (0.2 mL) was stirred at rt under an atmosphere of N_2 for 2 h. The mixture was acidified to pH 5 with aqueous $NaHSO_4$ and extracted with EtOAc (3×10 mL). The combined organic layers were washed with brine (3×10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to give (2S)-2-((tert-butoxycarbonyl)amino)-3-(4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-(methoxymethyl)pyridin-3-yl)-1H-indol-5-yl)thiazol-2-yl)propanoic acid. The crude product was used in the next step directly without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{33}H_{42}N_4O_6S$ 622.3; found 623.3.

Step 4.

Methyl (3S)-1-((2S)-2-((tert-butoxycarbonyl)amino)-3-(4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-(methoxymethyl)pyridin-3-yl)-1H-indol-5-yl)thiazol-2-yl)propanoyl)hexahydropyridazine-3-carboxylate was synthesized in a manner similar to methyl (3S)-1-[(2S)-2-[(tert-butoxycarbonyl)amino]-3-[2-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-(methoxymethyl)pyridin-3-yl]indol-5-yl]-1,3-thiazol-4-yl]propanoyl]-1,2-diazinane-3-carboxylate except (2S)-2-[(tert-butoxycarbonyl)amino]-3-[2-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-(methoxymethyl)pyridin-3-yl]indol-5-yl]-1,3-thiazol-4-yl]propanoic acid was substituted with (2S)-2-((tert-butoxycarbonyl)amino)-3-(4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-(methoxymethyl)pyridin-3-yl)-1H-indol-5-yl)thiazol-2-yl)propanoic acid. LCMS (ESI): m/z [M+H] calc'd for $C_{39}H_{52}N_6O_7S$ 748.4; found 749.4.

Step 5.

(3S)-1-[(2S)-2-[(tert-butoxycarbonyl)amino]-3-[4-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-(methoxymethyl)pyridin-3-yl]indol-5-yl]-1,3-thiazol-2-yl]propanoyl]-1,2-diazinane-3-carboxylic acid was synthesized in a manner similar to (3S)-1-[(2S)-2-[(tert-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1H-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylic acid except methyl (3S)-1-[(2S)-2-[(tert-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1H-indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylate was substituted with methyl (3S)-1-((2S)-2-((tert-butoxycarbonyl)amino)-3-(4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-(methoxymethyl)pyridin-3-yl)-1H-indol-5-yl)thiazol-2-yl)propanoyl)hexahydropyridazine-3-carboxylate. LCMS (ESI): m/z [M+H] calc'd for $C_{38}H_{50}N_6O_7S$ 734.3; found 735.4.

Step 6.

Tert-butyl ((6^{3}S,4S,Z)-1^1-ethyl-12-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate was synthesized in a manner similar to tert-butyl ((6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^5-((triisopropylsilyl)oxy)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate except (3S)-1-[(2S)-2-[(tert-butoxycarbonyl)amino]-3-[3-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-[(1S)-1-methoxyethyl]pyridin-3-yl]indol-5-yl]-5-[(triisopropylsilyl)oxy]phenyl]propanoyl]-1,2-diazinane-3-carboxylic acid was substituted with (3S)-1-[(2S)-2-[(tert-butoxycarbonyl)amino]-3-[4-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-(methoxymethyl)pyridin-3-yl]indol-5-yl]-1,3-thiazol-2-yl]propanoyl]-1,2-diazinane-3-carboxylic acid. LCMS (ESI): m/z [M+H] calc'd for $C_{38}H_{48}N_6O_6S$ 716.3; found 717.3.

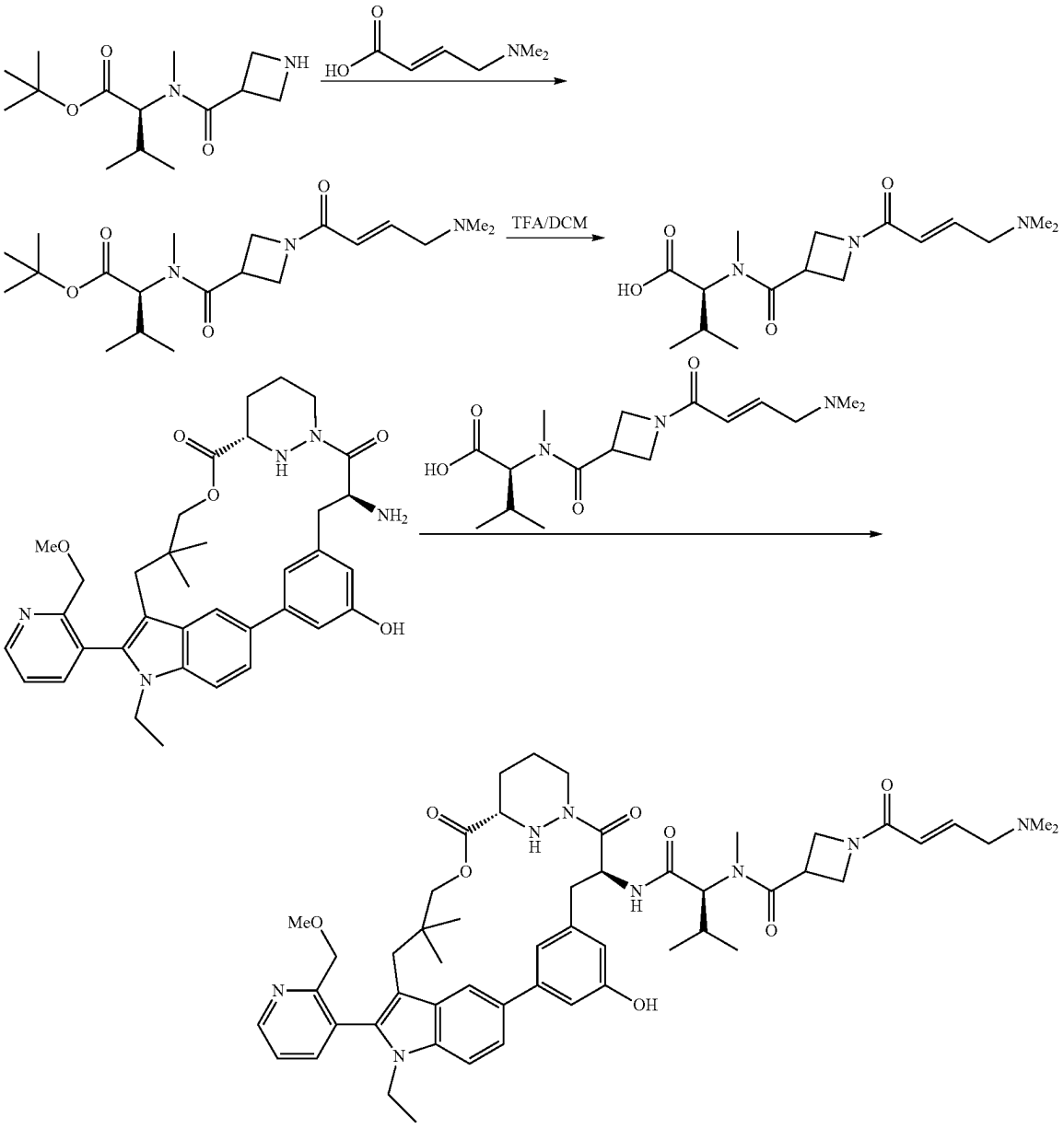
Step 7.

(6^{3}S,4S,Z)-4-amino-1^1-ethyl-1^2-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione was synthesized in a manner similar to (6^{3}S,4S,Z)-4-amino-1^1-ethyl-1^2-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(2,4)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione except tert-butyl ((6^{3}S,4S,Z)-1^1-ethyl-1^2-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(2,4)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate was substituted with tert-butyl ((6^{3}S,4S,Z)-1^1-ethyl-12-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate. LCMS (ESI): m/z [M+Na] calc'd for $C_{33}H_{40}N_6O_4SNa$ 639.3; found 640.3.

Step 8.

(2S)—N-[(7S,13S)-21-ethyl-20-[2-(methoxymethyl)pyridin-3-yl]-17,17-dimethyl-8,14-dioxo-15-oxa-4-thia-9,21,27,28-tetraazapentacyclo[17.5.2.1^{2,5}.1^{9,13}.0^{22,26}]octacosa-1(25),2,5(28),19,22(26),23-hexaen-7-yl]-3-methyl-2-{N-methyl-1-[(3S)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido}butanamide was synthesized in a manner similar to (2S)—N-[(7S,13S)-21-ethyl-20-[2-(methoxymethyl)pyridin-3-yl]-17,17-dimethyl-8,14-dioxo-15-oxa-3-thia-9,21,27,28-tetraazapentacyclo[17.5.2.1^{2,5}.1^{9,13}.0^{22,26}]octacosa-1(25),2(28),4,19,22(26),23-hexaen-7-yl]-3-methyl-2-{N-methyl-1-[(3S)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido}butanamide except (6^{3}S,4S)-4-amino-1^1-ethyl-2^5-hydroxy-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione was substituted with (6^{3}S,4S,Z)-4-amino-1^1-ethyl-1^2-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione. LCMS (ESI): m/z [M+H] calc'd for $C_{47}H_{60}N_8O_7S$ 880.4; found 881.5; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 8.70 (dt, J=16.2, 8.1 Hz, 1H), 8.54 (ddd, J=6.6, 4.7, 1.7 Hz, 1H), 8.50 (m, 1H), 7.96 (d, J=7.8 Hz, 1H), 7.88 (t, J=2.1 Hz, 2H), 6.70-6.57 (m, 2H), 6.24-6.13 (m, 2H), 5.75 (m, 1H), 5.55 (t, J=7.3 Hz, 1H), 5.46 (d, J=8.5 Hz, 1H), 5.14 (d, J=13.0 Hz, 1H), 4.84-4.75 (m, 1H), 4.35 (d, J=10.7 Hz, 1H), 4.28-4.19 (m, 4H), 3.91 (s, 3H), 3.87 (dd, J=10.4, 8.1 Hz, 1H), 3.78-3.70 (m, 2H), 3.63 (t, J=8.8 Hz, 2H), 3.61-3.49 (m, 2H), 2.87 (d, J=1.1 Hz, 2H), 2.79 (s, 1H), 2.38 (s, 1H), 2.18 (s, 1H), 2.13 (d, J=10.7 Hz, 4H), 1.96 (s, 2H), 1.81 (s, 1H), 1.53 (s, 2H), 1.11 (t, J=7.1 Hz, 2H), 0.99-0.89 (m, 7H), 0.93-0.81 (m, 2H), 0.78 (d, J=6.6 Hz, 2H), 0.28 (s, 3H).

Example A71. Synthesis of (2S)-2-(1-{1-[(2E)-4-(dimethylamino)but-2-enoyl]azetidin-3-yl}-N-methylformamido)-N-[(8S,14S)-22-ethyl-4-hydroxy-21-[2-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methylbutanamide



Step 1.

To a mixture of tert-butyl N-(azetidine-3-carbonyl)-N-methyl-L-valinate (350 mg, 1.3 mmol) and (2E)-4-(dimethylamino)but-2-enoic acid (201 mg, 1.56 mmol) in DCM (8 mL) at 5° C. was added a solution of T3P, 50% in EtOAc (827 mg, 2.6 mmol) and DIPEA (1.7 g, 13 mmol) in DCM (2 mL). The mixture was stirred for 1 h, then diluted with EtOAc (20 mL) and H_2O (20 mL). The aqueous and organic layers were separated and the organic layer was washed with H_2O (3×10 mL), brine (10 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by prep-HPLC to give tert-butyl (E)-N-(1-(4-(dimethylamino)but-2-enoyl)azetidine-3-carbonyl)-N-methyl-L-valinate (200 mg, 39% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{20}H_{35}N_3O_4$ 381.3; found 382.3.

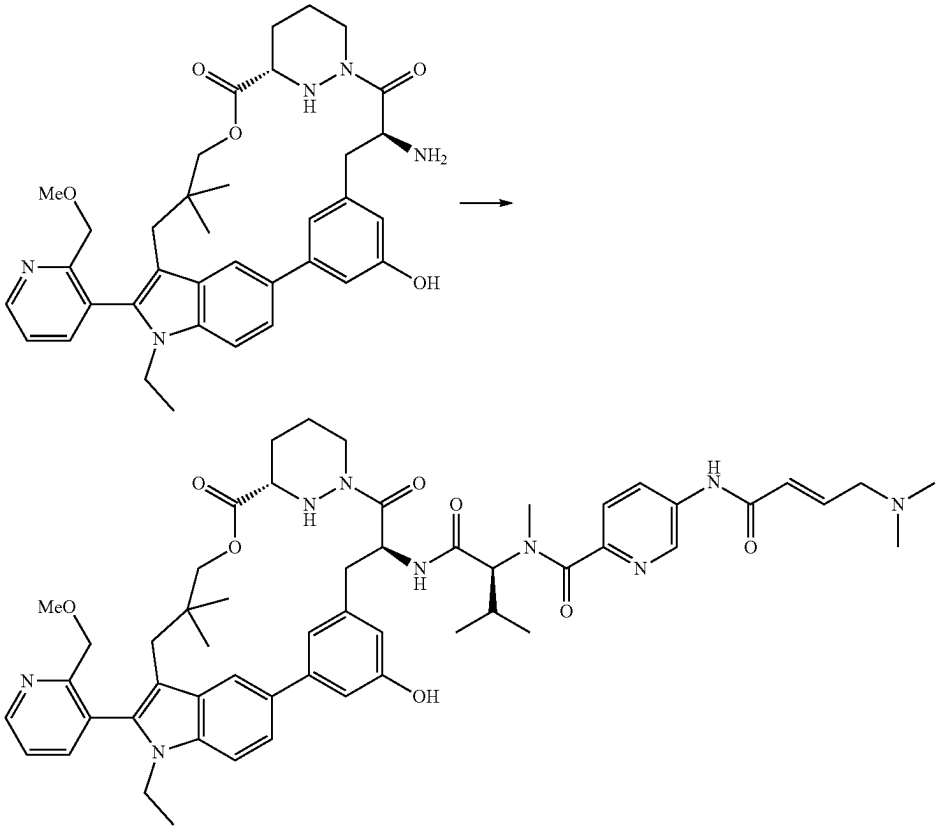
Step 2.

To a mixture of tert-butyl (E)-N-(1-(4-(dimethylamino)but-2-enoyl)azetidine-3-carbonyl)-N-methyl-L-valinate (190 mg, 0.32 mmol) in DCM (3 mL) at rt was added TFA (1 mL). The mixture was stirred at rt for 1 h, then concentrated under reduced pressure to give (E)-N-(1-(4-(dimethylamino)but-2-enoyl)azetidine-3-carbonyl)-N-methyl-L-valine (190 mg, 90%) as a solid, which was used directly in the next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{16}H_{27}N_3O_4$ 325.2; found 326.2.

Step 3.

To a mixture of (6^3S,4S)-4-amino-1^1-ethyl-2^5-hydroxy-1^2-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1 ^{1}H-8-oxa-1 (5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (172 mg, 0.27 mmol) and (E)-N-(1-(4-(dimethylamino)but-2-enoyl)azetidine-3-carbonyl)-N-methyl-L-valine (105 mg, 0.32 mmol) in DMF (2 mL) at 5° C. was added a mixture of HATU (133 mg, 0.297 mmol) and DIPEA (348 mg, 2.7 mmol) in DMF (1 mL). The mixture was stirred for 1 h, then diluted with EtOAc (20 mL) and H_2O (20 mL). The aqueous and organic layers were separated, and the organic layer was washed with H_2O (3×10 mL), brine (10 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by prep-TLC to give (2S)-2-(1-{1-[(2E)-4-(dimethylamino)but-2-enoyl]azetidin-3-yl}-N-methylformamido)-N-[(8S,14S)-22-ethyl-4-hydroxy-21-[2-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.$1^{2,6}$.$1^{10,14}$.$0^{23,27}$]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methylbutanamide (4.8 mg, 2% yield over 2 steps) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{52}H_{68}N_8O_8$ 932.5; found 933.5; ^{1}H NMR (400 MHz, CD_3OD) δ 8.71 (d, J=3.2 Hz, 1H), 8.50 (s, 1.5H), 8.08-7.85 (m, 2H), 7.65-7.44 (m, 3H), 7.32-7.14 (m, 1H), 7.07-6.95 (m, 1H), 6.80 (dt, J=22.1, 6.8 Hz, 1H), 6.55 (d, J=35.8 Hz, 1H), 6.30 (d, J=15.4 Hz, 1H), 5.56 (dd, J=13.8, 6.7 Hz, 1H), 4.76 (dd, J=19.8, 10.5 Hz, 1H), 4.54 (dd, J=15.9, 7.5 Hz, 2H), 4.48-4.38 (m, 2H), 4.36-4.23 (m, 3H), 4.22-4.14 (m, 1H), 3.96 (qd, J=15.6, 7.9 Hz, 3H), 3.77 (ddd, J=25.8, 23.4, 11.9 Hz, 2H), 3.58 (dd, J=17.2, 8.3 Hz, 2H), 3.38 (s, 2H), 3.25-3.11 (m, 3H), 3.05-2.94 (m, 1H), 2.94-2.81 (m, 4H), 2.73 (dd, J=20.9, 11.0 Hz, 1H), 2.45 (d, J=6.9 Hz, 5H), 2.32-2.07 (m, 3H), 1.92 (d, J=13.2 Hz, 1H), 1.72 (s, 1H), 1.64-1.51 (m, 1H), 1.18 (t, J=7.0 Hz, 2H), 1.00 (ddd, J=14.6, 11.8, 8.5 Hz, 6H), 0.92-0.81 (m, 4H), 0.55-0.41 (m, 3H).

Example A67. Synthesis of (2E)-4-(dimethylamino)-N-(6-{[(1S)-1-{[(8S,14S)-22-ethyl-4-hydroxy-21-[2-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.$1^{2,6}$.$1^{10,14}$.$0^{23,27}$]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]carbamoyl}-2-methylpropyl](methyl)carbamoyl}pyridin-3-yl)but-2-enamide



Step 1.

To a mixture of (6^3S,4S)-4-amino-1^1-ethyl-2^5-hydroxy-1^2-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione TFA salt (225 mg, 0.28 mmol) and (E)-N-(5-(4-(dimethylamino)but-2-enamido)picolinoyl)-N-methyl-L-valine TFA salt (260 mg crude, 0.56 mmol) in DMF (5 mL) at 0° C. were added DIPEA (0.46 mL, 2.8 mmol) followed by HATU (140 mg, 0.36 mmol). The mixture was stirred at 0-10° C. for 1 h, then concentrated under reduced pressure and the residue was purified by prep-HPLC to give (2E)-4-(dimethylamino)-N-(6-{[(1S)-1-{[(8S,14S)-22-ethyl-4-hydroxy-21-[2-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.$1^{2,6}$.$1^{10,14}$.$0^{23,27}$]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]carbamoyl}-2-methylpropyl](methyl)carbamoyl}pyridin-3-

yl)but-2-enamide TFA salt (23.3 mg, 8% yield over 2 steps) as a solid. LCMS (ESI): m/z [M+Na] calc'd for $C_{54}H_{67}N_9O_8Na$ 992.5; found 992.4; ^1H NMR (400 MHz, CD_3OD) δ 9.05 (d, J=2.5 Hz, 1H), 8.85-8.71 (m, 1H), 8.43 (ddd, J=33.3, 18.0, 2.6 Hz, 2H), 8.01-7.87 (m, 2H), 7.83-7.70 (m, 1H), 7.60-7.47 (m, 2H), 7.31-7.19 (m, 1H), 7.07-6.90 (m, 2H), 6.70-6.36 (m, 3H), 5.81-5.61 (m, 1H), 4.50-4.20 (m, 4H), 4.01-3.68 (m, 3H), 3.64-3.35 (m, 5H), 3.27-3.08 (m, 3H), 3.04-2.44 (m, 11H), 2.36-2.10 (m, 3H), 1.93 (d, J=13.0 Hz, 1H), 1.61 (dd, J=34.3, 21.6 Hz, 3H), 1.39-1.16 (m, 3H), 1.12-0.81 (m, 6H), 0.78-0.45 (m, 6H).

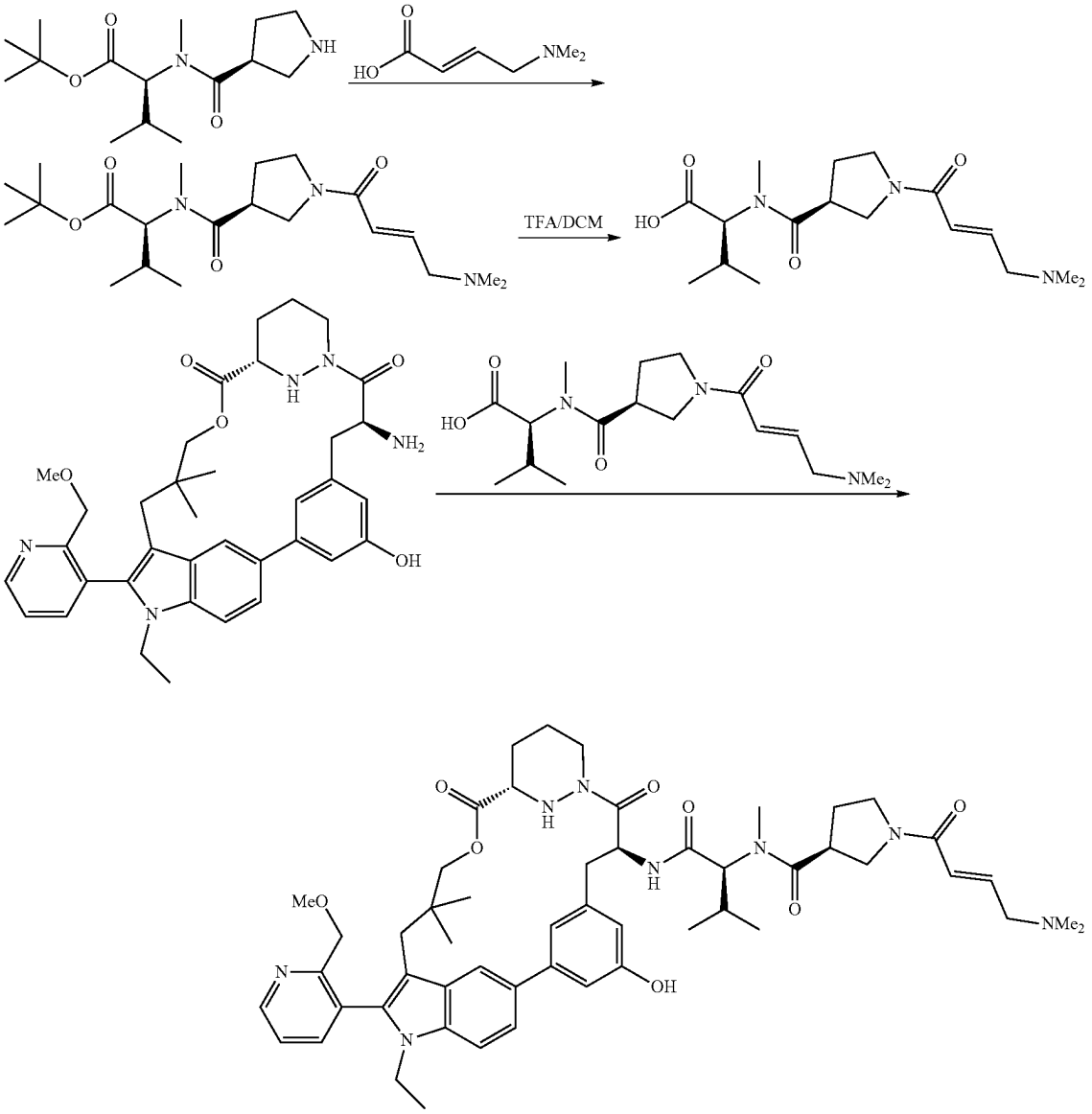
Example A54. Synthesis of (2S)-2-{1-[(3S)-1-[(2E)-4-(dimethylamino)but-2-enoyl]pyrrolidin-3-yl]-N-methylformamido}-N-[(8S,14S)-22-ethyl-4-hydroxy-21-[2-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methylbutanamide

Step 1.

To a mixture of tert-butyl N-methyl-N—((S)-pyrrolidine-3-carbonyl)-L-valinate (210 mg, 0.73 mmol) in DMF (4 mL) at rt were added 4-(dimethylamino)-4-methylpent-2-ynoic acid (450 mg, 2.9 mmol), DIPEA (1.2 mL, 7.3 mmol), and HATU (332 mg, 0.88 mmol). The mixture was stirred at rt for 1 h then diluted with EtOAc, and the mixture washed with H_2O, brine, dried over Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give tert-butyl N—((S)-1-(4-(dimethylamino)-4-methylpent-2-ynoyl)pyrrolidine-3-carbonyl)-N-methyl-L-valinate (140 mg, 45% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{23}H_{39}N_3O_4$ 421.3; found 422.3.

Step 2.

A mixture of tert-butyl N—((S)-1-(4-(dimethylamino)-4-methylpent-2-ynoyl)pyrrolidine-3-carbonyl)-N-methyl-L-valinate (130 mg, 0.31 mmol) in DCM (2 mL) and TFA (1 mL) was stirred at rt for 90 min. The mixture was concen-

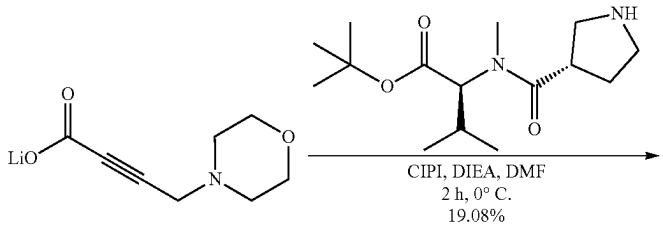


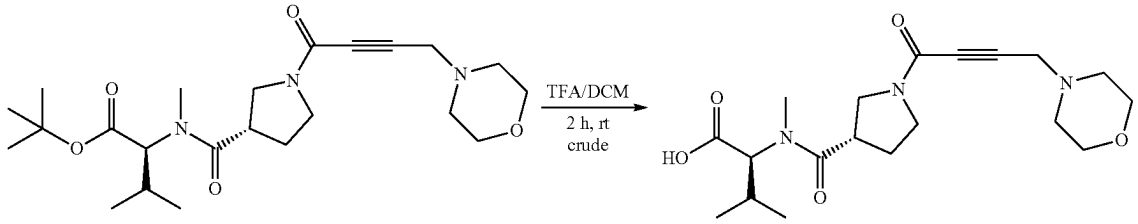
trated under reduced pressure to give N—((S)-1-(4-(dimethylamino)-4-methylpent-2-ynoyl)pyrrolidine-3-carbonyl)-N-methyl-L-valine TFA salt (150 mg) as an oil, which was used directly in the next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{19}H_{31}N_3O_4$ 365.2; found 366.2.

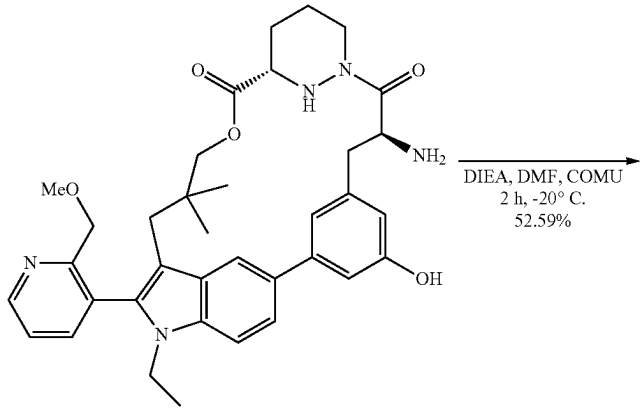
Step 3.

(3S)-1-(4-(dimethylamino)-4-methylpent-2-ynoyl)-N-((2S)-1-(((6[3]S,4S)-1[1]-ethyl-2[5]-hydroxy-1[2]-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6[1],6[2],6[3],6[4],6[5],6[6]-hexahydro-1[1]H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-N-methylpyrrolidine-3-carboxamide TFA salt was synthesized in a manner similar to 1-acryloyl-N-((2S)-1-(((6[3]S,4S)-1[1]-ethyl-2[5]-hydroxy-1[2]-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6[1],6[2],6[3],6[4],6[5],6[6]-hexahydro-1[1]H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-N-methylazetidine-3-carboxamide except (2S)-2-{1-[(3S)-1-[(2E)-4-(dimethylamino)but-2-enoyl]pyrrolidin-3-yl]-N-methylformamido}-N-[(8S,14S)-22-ethyl-4-hydroxy-21-[2-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1[2],[6].1[10],[14].0[23],[27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methylbutanamide TFA salt. (120 mg, 54% yield over 2 steps) as a solid. ^{1}H-NMR (400 MHz, CD_3OD) δ 8.76-8.68 (m, 1H), 8.44 (s, 1H), 8.02-7.94 (m, 1H), 7.94-7.84 (m, 1H), 7.65-7.43 (m, 3H), 7.27-7.14 (m, 1H), 7.06-6.96 (m, 1H), 6.65-6.48 (m, 1H), 5.62-5.46 (m, 1H), 4.81-4.57 (m, 1H), 4.46-4.22 (m, 3H), 4.10-3.35 (m, 11H), 3.26-2.93 (m, 6H), 2.91-2.51 (m, 4H), 2.42-2.09 (m, 9H), 1.95-1.87 (m, 1H), 1.85-1.40 (m, 6H), 1.38-1.10 (m, 6H), 1.07-0.81 (m, 9H), 0.56-0.38 (m, 3H). LCMS (ESI): m/z [M+H] $C_{52}H_{68}N_8O_8$ found 947.7.

Example A95. Synthesis of (2S)—N-[(8S,14S)-22-ethyl-4-hydroxy-21-[2-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1[2],[6].1[10],[14].0[23],[27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methyl-2-{N-methyl-1-[(3S)-1-[4-(morpholin-4-yl)but-2-ynoyl]pyrrolidin-3-yl]formamido}butanamide







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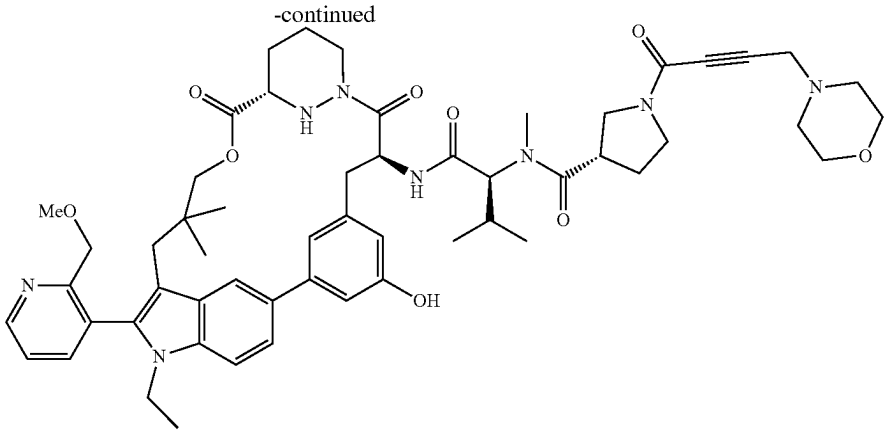


Step 1.

A mixture of tert-butyl (2S)-3-methyl-2-[N-methyl-1-(3S)-pyrrolidin-3-ylformamido]butanoate (500 mg, 1.8 mmol), 4-(morpholin-4-yl)but-2-ynoic acid (1.49 g, 8.8 mmol), DIPEA (682 mg, 5.3 mmol) and CIP (635 mg, 2.3 mmol) in DMF (5 mL) was stirred at 0° C. for 2 h. The mixture was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give tert-butyl N-methyl-N—((S)-1-(4-morpholinobut-2-ynoyl)pyrrolidine-3-carbonyl)-L-valinate (150 mg, 19% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{23}H_{37}N_3O_5$ 435.3; found 436.5.

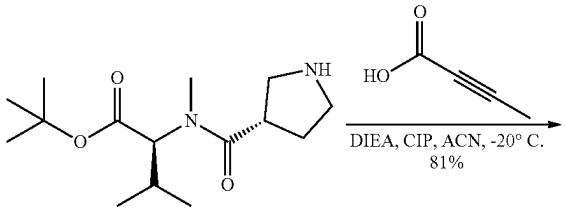
Step 2.

A mixture of tert-butyl N-methyl-N—((S)-1-(4-morpholinobut-2-ynoyl)pyrrolidine-3-carbonyl)-L-valinate (250 mg, 0.57 mmol) in DCM (5 mL) and TFA (2.5 mL) was stirred at rt for 2 h. The mixture was concentrated under reduced pressure to give (2S)-3-methyl-2-[N-methyl-1-[(3S)-1-[4-(morpholin-4-yl)but-2-ynoyl]pyrrolidin-3-yl]formamido]butanoic acid (310 mg, crude) as an oil, which was used directly in the next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{19}H_{29}N_3O_5$ 379.2; found 380.2.

Step 3.

A mixture of (6^3S,4S)-4-amino-1^1-ethyl-2^5-hydroxy-1^2-(2-(methoxymethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (250 mg, 0.4 mmol), DIPEA (516 mg, 4.0 mmol), (2S)-3-methyl-2-[N-methyl-1-[(3S)-1-[4-(morpholin-4-yl)but-2-ynoyl]pyrrolidin-3-yl]formamido]butanoic acid (182 mg, 0.48 mmol), and COMU (205 mg, 0.48 mmol) in DMF (3 mL) was stirred at −20° C. for 2 h. The mixture was diluted with H_2O (10 mL), then extracted with EtOAc (3×10 mL) and the combined organic layers were washed with brine (3×10 mL), dried over anhydrous Na_2SO_4, and filtered. The mixture was concentrated under reduced pressure and the residue was purified by reverse-phase silica gel column chromatography to give (2S)—N-[(8S,14S)-22-ethyl-4-hydroxy-21-[2-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.$1^{2,6}$.$1^{10,14}$.$0^{23,27}$]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methyl-2-{N-methyl-1-[(3S)-1-[4-(morpholin-4-yl)but-2-ynoyl]pyrrolidin-3-yl]formamido}butanamide (207 mg, 53% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{55}H_{70}N_8O_9$ 986.5; found 987.8; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.39-9.28 (m, 1H), 8.74 (t, J=4.8, 1H), 8.70-8.04 (m, 1H), 7.98-7.90 (m, 1.5H), 7.82 (d, J=7.7 Hz, 0.5H), 7.63-7.46 (m, 3H), 7.26-7.10 (m, 1H), 7.03 (s, 1H), 6.58-6.43 (m, 1H), 5.44-5.30 (m, 1H), 5.06 (q, 0.5H), 4.72 (t, J=11.0, 0.5H), 4.39-4.20 (m, 3H), 4.15 (d, J=11.1 Hz, 1H), 4.09-3.85 (m, 4H), 3.66 (s, 2H), 3.65-3.58 (m, 4H), 3.58-3.55 (m, 2H), 3.55-3.48 (m, 3H), 3.47-3.41 (m, 3H), 3.31 (s, 2H), 3.10 (s, 2H), 2.92 (s, 1H), 2.89-2.65 (m, 5H), 2.68 (s, 1H), 2.45-2.38 (m, 1H), 2.29-2.24 (m, 1H), 2.23-1.99 (m, 3H), 1.82 (d, J=12.1 Hz, 1H), 1.76-1.62 (m, 1H), 1.61-1.45 (m, 1H), 1.14-1.04 (m, 2H), 1.02-0.92 (m, 3H), 0.91-0.86 (m, 3H), 0.83-0.77 (m, 3H), 0.77-0.70 (m, 2H), 0.50-0.35 (m, 3H).

Example A145. Synthesis of two atropisomers of (2S)-2-{1-[(3S)-1-(but-2-ynoyl)pyrrolidin-3-yl]-N-methylformamido}-N-[(8S,14S,20M)-22-ethyl-4-hydroxy-21-{2-[(1S)-1-methoxyethyl]pyridin-3-yl}-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.$1^{2,6}$.$1^{10,14}$.$0^{23,27}$]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methylbutanamide



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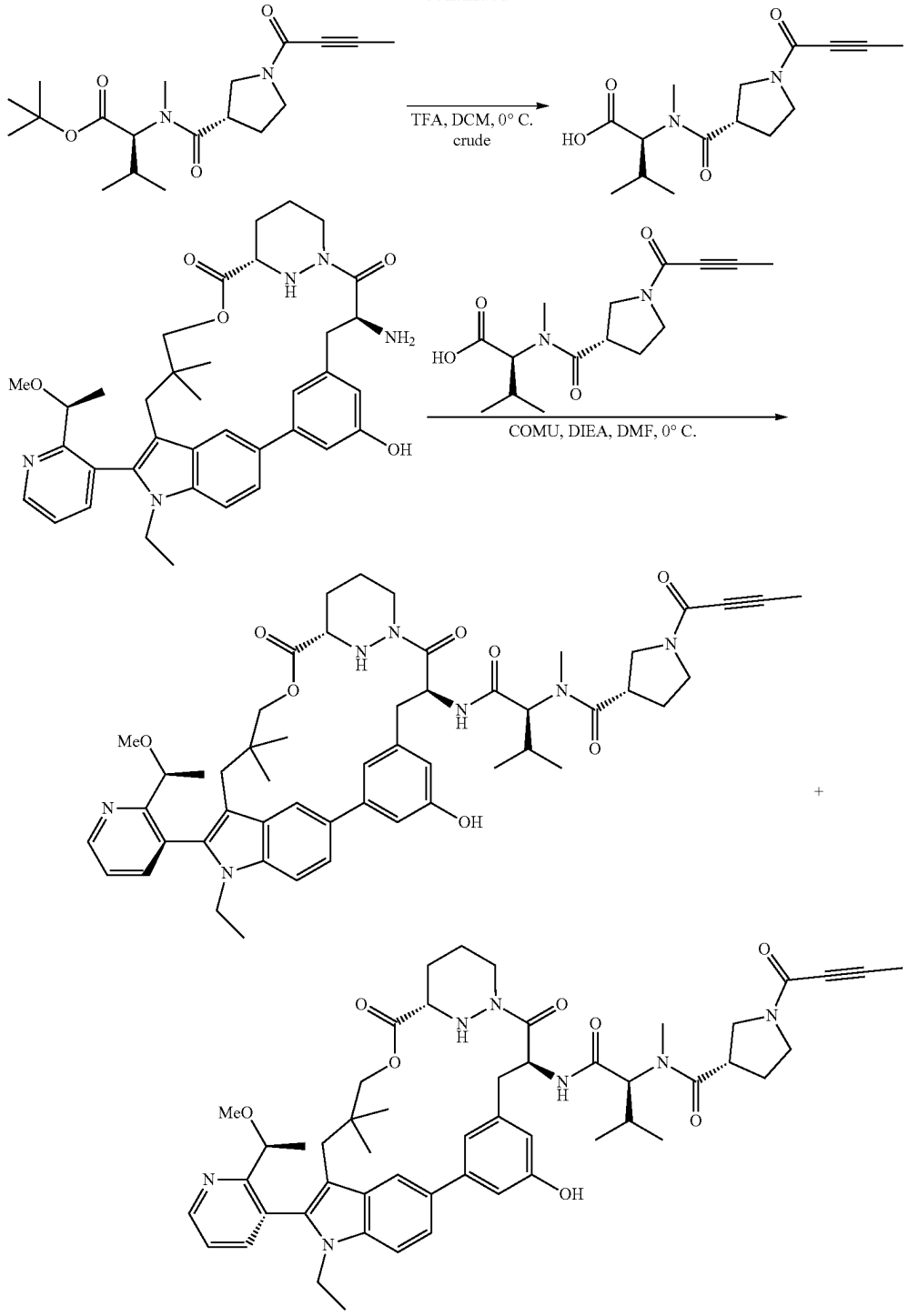


Step 1.

To a mixture of but-2-ynoic acid (222 mg) and CIP (588 mg) in ACN (8 mL) at 0° C. under PGR-1230,C_3 an atmosphere of Ar was added DIPEA (681 mg). The mixture was stirred at 0° C. then tert-butyl N-methyl-N—((S)-pyrrolidine-3-carbonyl)-L-valinate (500 mg) in ACN (3 mL) was added dropwise and the mixture stirred at 0° C. for 2 h. EtOAc was added and the mixture was washed with brine (3×20 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give tert-butyl N—((S)-1-(but-2-ynoyl)pyrrolidine-3-carbonyl)-N-methyl-L-valinate as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{19}H_{30}N_2O_4$ 350.2; found 352.1.

Step 2.

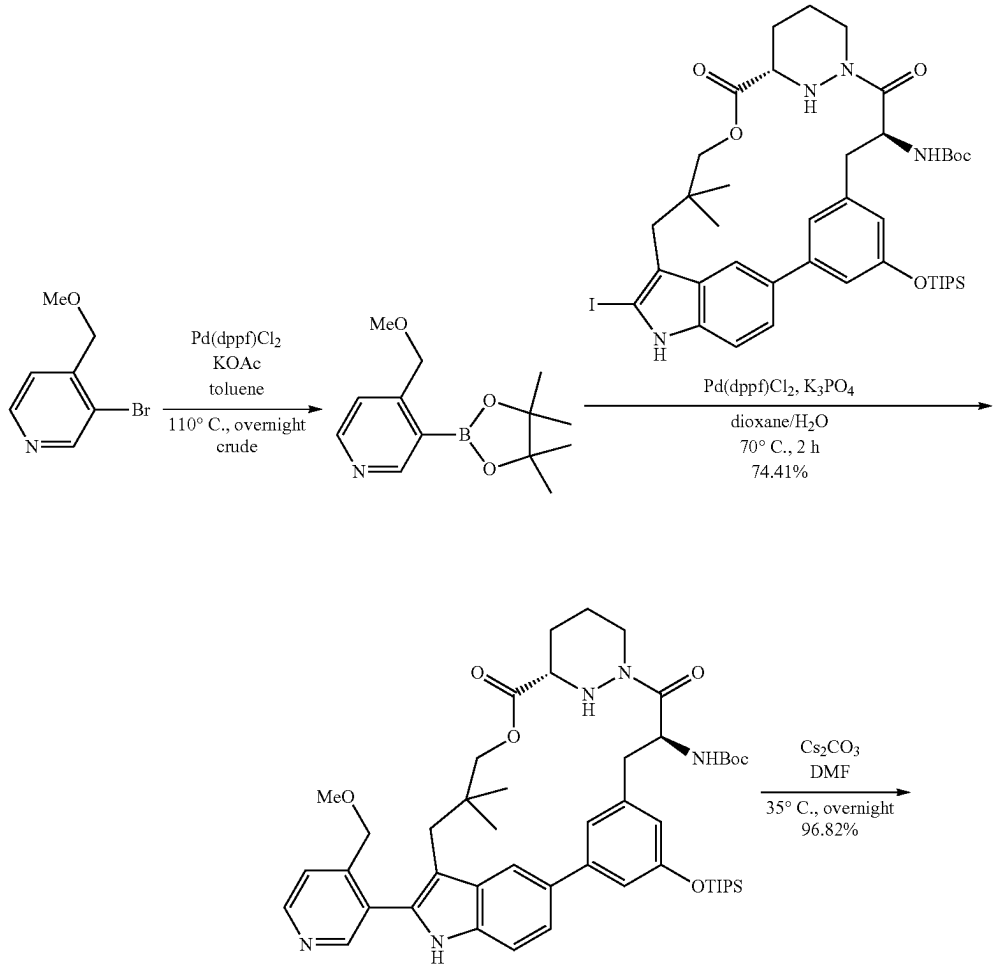
A mixture of tert-butyl N—((S)-1-(but-2-ynoyl)pyrrolidine-3-carbonyl)-N-methyl-L-valinate (200 mg) in DCM (4 mL) and TFA (2 mL) was stirred at 0° C. for 2 h. The mixture

was concentrated under reduced pressure with azeotropic removal of H_2O using toluene (4 mL×2) to give N—((S)-1-(but-2-ynoyl)pyrrolidine-3-carbonyl)-N-methyl-L-valinate as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{15}H_{22}N_2O_4$ 294.2; found 295.2.

Step 3.

Two atropisomers of (2S)-2-{1-[(3S)-1-(but-2-ynoyl)pyrrolidin-3-yl]-N-methylformamido}-N-[(8S,14S,20M)-22-ethyl-4-hydroxy-21-{2-[(1 S)-1-methoxyethyl]pyridin-3-yl}-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methylbutanamide was synthesized in a manner similar to (2S)—N-[(8S,14S)-22-ethyl-4-hydroxy-21-[2-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methyl-2-{N-methyl-1-[(3S)-1-[4-(morpholin-4-yl)but-2-ynoyl]pyrrolidin-3-yl]formamido}butanamide except (2S)-3-methyl-2-[N-methyl-1-[(3S)-1-[4-(morpholin-4-yl)but-2-ynoyl]pyrrolidin-3-yl]formamido]butanoic acid was substituted with N—((S)-1-(but-2-ynoyl)pyrrolidine-3-carbonyl)-N-methyl-L-valinate. (43.3 mg, 12% yield) and (33 mg, 9% yield) both as solids. LCMS (ESI): m/z [M+H] calc'd for $C_{52}H_{65}N_7O_8$ 915.5; found 916.7; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.34-9.27 (m, 1H), 8.78 (t, J=2.5 Hz, 1H), 8.68 (t, J=8.5 Hz, 0.5H), 8.20-8.11 (m, 0.6H), 7.95 (ddt, J=5.4, 3.5, 1.7 Hz, 2H), 7.63-7.60 (m, 1H), 7.61-7.49 (m, 2H), 7.13 (s, 1H), 7.03 (d, J=6.2 Hz, 1H), 6.60-6.49 (d, J=35.5 Hz, 1H), 5.43-5.39 (m, 1H), 5.12-5.00 (m, 0.7H), 4.74 (d, J=10.6 Hz, 0.4H), 4.32-4.25 (m, 1H), 4.18-3.85 (m, 5H), 3.81-3.45 (m, 8H), 3.18-3.02 (m, 5H), 2.93-2.80 (m, 4H), 2.80-2.70 (m, 2H), 2.42-2.36 (m, 1H), 2.31-2.20 (m, 1H), 2.18-1.96 (m, 6H), 1.85-1.74 (m, 1H), 1.74-1.63 (m, 1H), 1.62-1.42 (m, 1H), 1.32-1.16 (m, 4H), 1.15-1.05 (t, J=6.3 Hz, 4H), 1.04-0.95 (m, 2H), 0.95-0.85 (m, 5H), 0.68-0.52 (m, 4H), 0.52-0.37 (m, 4H). and LCMS (ESI): m/z [M+H] calc'd for $C_{52}H_{65}N_7O_8$ 915.5; found 916.7; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.36-9.28 (m, 1H), 8.77 (dd, J=4.7, 1.8 Hz, 1H), 8.62-8.57 (m, 0.5H), 8.15-8.07 (m, 0.5H), 7.95 (s, 1H), 7.87-7.81 (m, 1H), 7.65-7.51 (m, 3H), 7.37-7.25 (m, 1H), 7.10-7.03 (m, 1H), 6.54 (d, J=35.5 Hz, 1H), 5.52-5.21 (m, 2H), 4.78-4.66 (m, 0.5H), 4.34-4.20 (m, 3H), 4.15-3.85 (m, 4H), 3.85-3.42 (m, 7H), 3.22-3.11 (m, 3H), 2.97-2.72 (m, 7H), 2.62-2.54 (m, 1H), 2.28-1.96 (m, 7H), 1.95-1.74 (m, 2H), 1.73-1.44 (m, 2H), 1.42-1.37 (m, 3H), 1.28-1.14 (m, 1H), 1.03-0.85 (m, 6H), 0.83-0.72 (m, 7H), 0.71-0.55 (m, 3H).

Example A28. Synthesis of (2S)—N-[(8S,14S)-22-ethyl-4-hydroxy-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methyl-2-[N-methyl-1-[(3S)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido]butanamide



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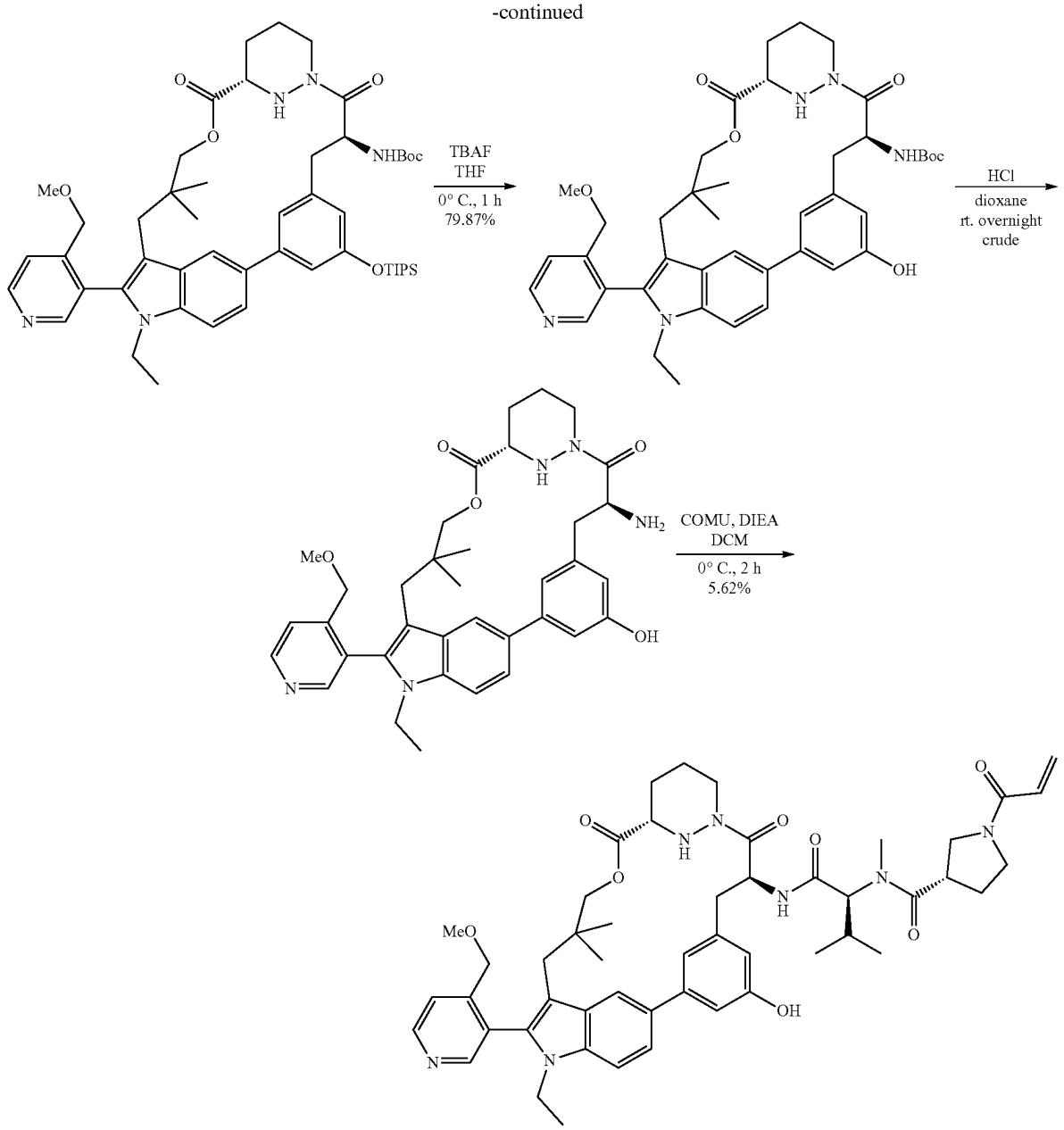


Step 1.

To a mixture of 3-bromo-4-(methoxymethyl)pyridine (1.00 g, 5.0 mmol), 4,4,5,5-tetramethyl-2-(tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,2-dioxaborolane (1.51 g, 5.9 mmol) and KOAc (1.21 g, 12.3 mmol) in toluene (10 mL) at rt under an atmosphere of Ar was added $Pd(dppf)Cl_2$ (362 mg, 0.5 mmol). The mixture was heated to 110° C. and stirred overnight, then concentrated under reduced pressure to give 4-(methoxymethyl)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine, which was used directly in the next step directly without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{13}H_{20}BNO_3$ 249.2; found 250.3.

Step 2.

To a mixture of 4-(methoxymethyl)-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine (290 mg, 1.16 mmol), K_3PO_4 (371 mg, 1.75 mmol) and tert-butyl N-[(8S,14S)-21-iodo-18,18-dimethyl-9,15-dioxo-4-[(triisopropylsilyl)oxy]-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]carbamate (500 mg, 0.58 mmol) in 1,4-dioxane (5 mL) and H_2O (1 mL) at rt under an atmosphere of Ar was added $Pd(dppf)Cl_2$ (43 mg, 0.06 mmol). The mixture was heated to 70° C. and stirred for 2 h, then H_2O added and the mixture extracted with EtOAc (2×10 mL). The combined organic layers were washed with brine (10 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give tert-butyl N-[(8S,14S)-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-4-[(triisopropylsilyl)oxy]-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]carbamate (370 mg, 74% yield) as a foam. LCMS (ESI): m/z [M+H] calc'd for $C_{48}H_{67}N_5O_7Si$ 853.6; found 854.6.

Step 3.

A mixture of tert-butyl N-[(8S,14S)-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-4-[(triisopropylsilyl)oxy]-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]carbamate (350 mg, 0.41 mmol), Cs_2CO_3 (267 mg, 0.82 mmol) and EtI (128 mg, 0.82 mmol) in DMF (4 mL) was stirred at 35° C. overnight. H_2O was added and the mixture was extracted with EtOAc (2×15 mL). The combined organic layers were washed with brine (15 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give tert-butyl N-[(8S,14S)-22-ethyl-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-4-[(triisopropylsilyl)oxy]-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]carbamate (350 mg, 97% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{50}H_{71}N_5O_7Si$ 881.5; found 882.6.

Step 4.

A mixture of tert-butyl N-[(8S,14S)-22-ethyl-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-4-[(triisopropylsilyl)oxy]-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]carbamate (350 mg, 0.4 mmol) and 1M TBAF in THF (0.48 mL, 0.480 mmol) in THF (3 mL) at 0° C. under an atmosphere of Ar was stirred for 1 h. The mixture was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give tert-butyl N-[(8S,14S)-22-ethyl-4-hydroxy-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]carbamate (230 mg, 80% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{41}H_{51}N_5O_7$ 725.4; found 726.6.

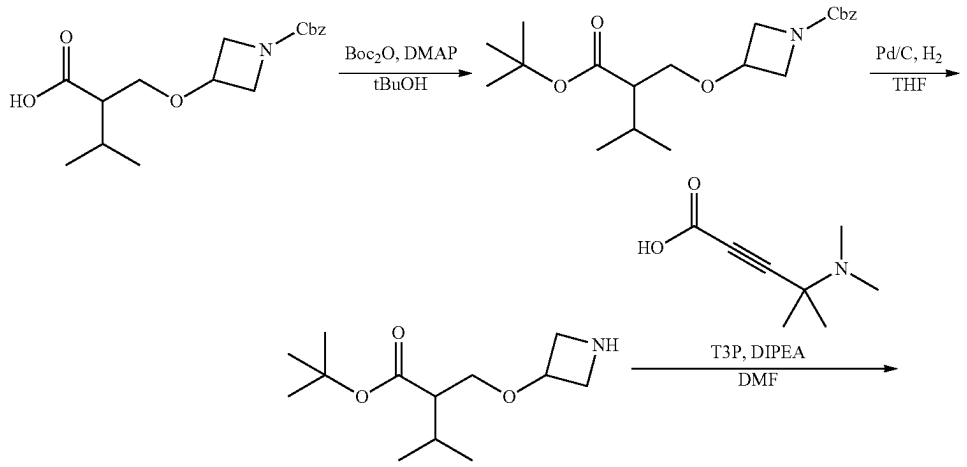
Step 5.

To a mixture of tert-butyl N-[(8S,14S)-22-ethyl-4-hydroxy-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]carbamate (200 mg, 0.28 mmol) in 1,4-dioxane (2 mL) at 0° C. under an atmosphere of Ar was added 4M HCl in 1,4-dioxane (2 mL, 8 mmol). The mixture was allowed to warm to rt and was stirred overnight, then concentrated under reduced pressure to give (8S,14S)-8-amino-22-ethyl-4-hydroxy-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaene-9,15-dione (200 mg). LCMS (ESI): m/z [M+H] calc'd for $C_{36}H_{43}N_5O_5$ 625.3; found 626.5.

Step 6.

To a mixture of (2S)-3-methyl-2-[N-methyl-1-[(3S)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido]butanoic acid (108 mg, 0.38 mmol) and (8S,14S)-8-amino-22-ethyl-4-hydroxy-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaene-9,15-dione (200 mg, 0.32 mmol) in DCM (3 mL) at 0° C. was added DIPEA (165 mg, 1.3 mmol) and COMU (274 mg, 0.64 mmol) in portions. The mixture was stirred at 0° C. for 2 h, H_2O added and extracted with EtOAc (2×10 mL). The combined organic layers were washed with brine (10 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography then prep-HPLC to give (2S)—N-[(8S,14S)-22-ethyl-4-hydroxy-21-[4-(methoxymethyl)pyridin-3-yl]-18,18-dimethyl-9,15-dioxo-16-oxa-10,22,28-triazapentacyclo[18.5.2.1^[2,6].1^[10,14].0^[23,27]]nonacosa-1(26),2,4,6(29),20,23(27),24-heptaen-8-yl]-3-methyl-2-[N-methyl-1-[(3S)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido]butanamide (16 mg, 5.6% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{50}H_{63}N_7O_8$ 889.5; found 890.6; ^1H NMR (400 MHz, DMSO-d_6) δ 9.33 (dd, J=9.1, 6.9 Hz, 1H), 8.79-8.46 (m, 2H), 7.93 (s, 1H), 7.68-7.58 (m, 2H), 7.53 (t, J=8.5 Hz, 1H), 7.26-6.98 (m, 2H), 6.71-6.47 (m, 2H), 6.24-6.07 (m, 1H), 5.80-5.60 (m, 1H), 5.49-5.18 (m, 1H), 4.45-4.07 (m, 4H), 4.08-3.87 (m, 3H), 3.87-3.64 (m, 4H), 3.64-3.40 (m, 5H), 3.34 (s, 2H), 3.30 (s, 2H), 3.23 (d, J=1.8 Hz, 1H), 2.94-2.74 (m, 6H), 2.16-2.01 (m, 3H), 1.82-1.47 (m, 3H), 1.08 (q, J=8.9, 8.0 Hz, 1H), 1.00-0.88 (m, 6H), 0.82 (d, J=10.8 Hz, 4H), 0.76-0.66 (m, 2H), 0.44 (d, J=14.2 Hz, 3H).

Example A316. Synthesis of (2R)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-N-((6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1, 6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methylbutanamide



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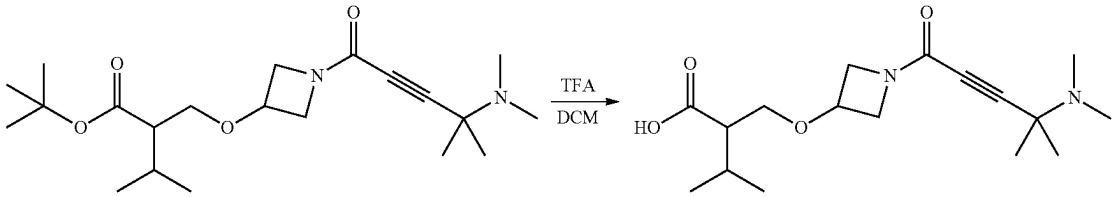
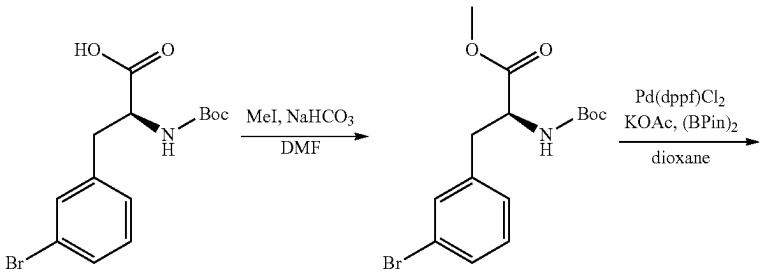

TFA
DCM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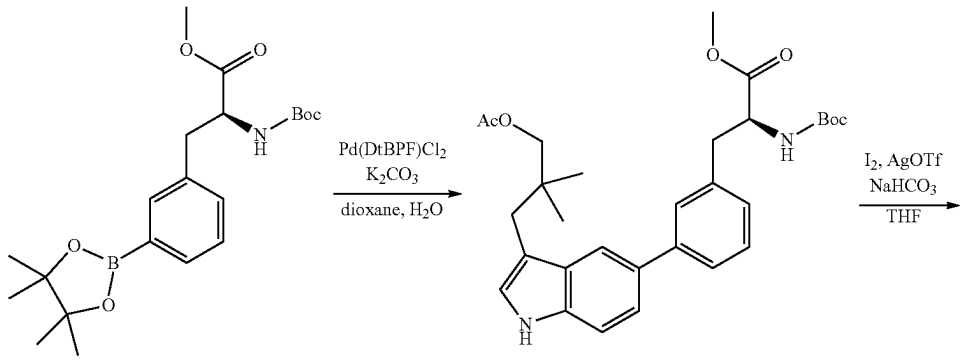
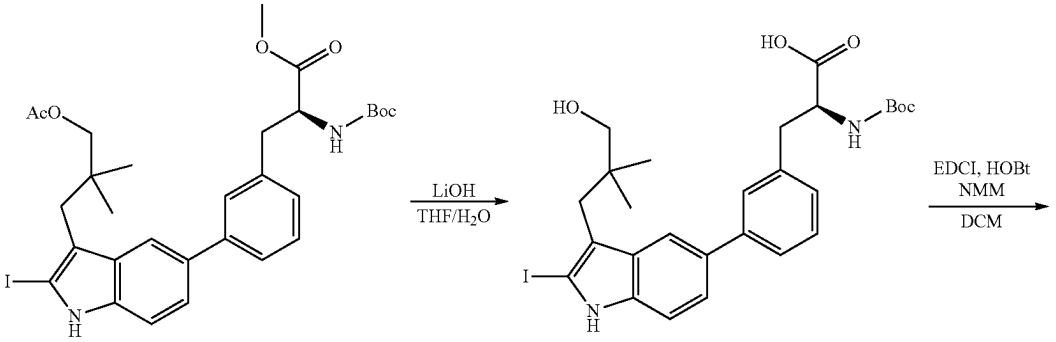
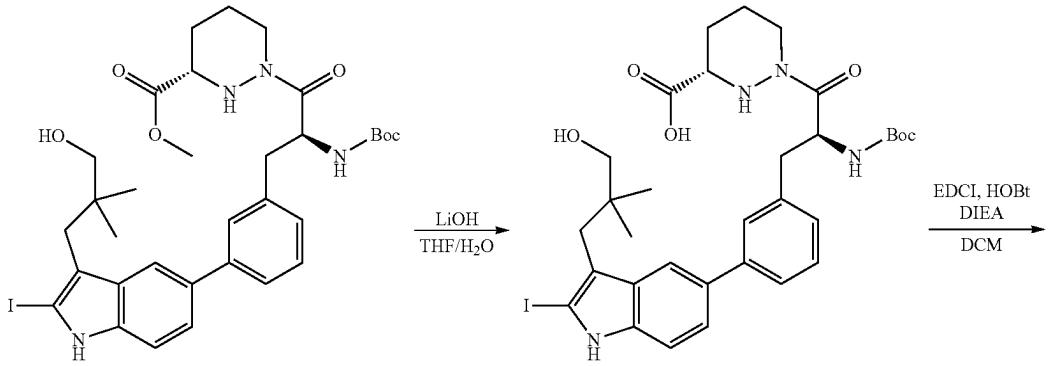
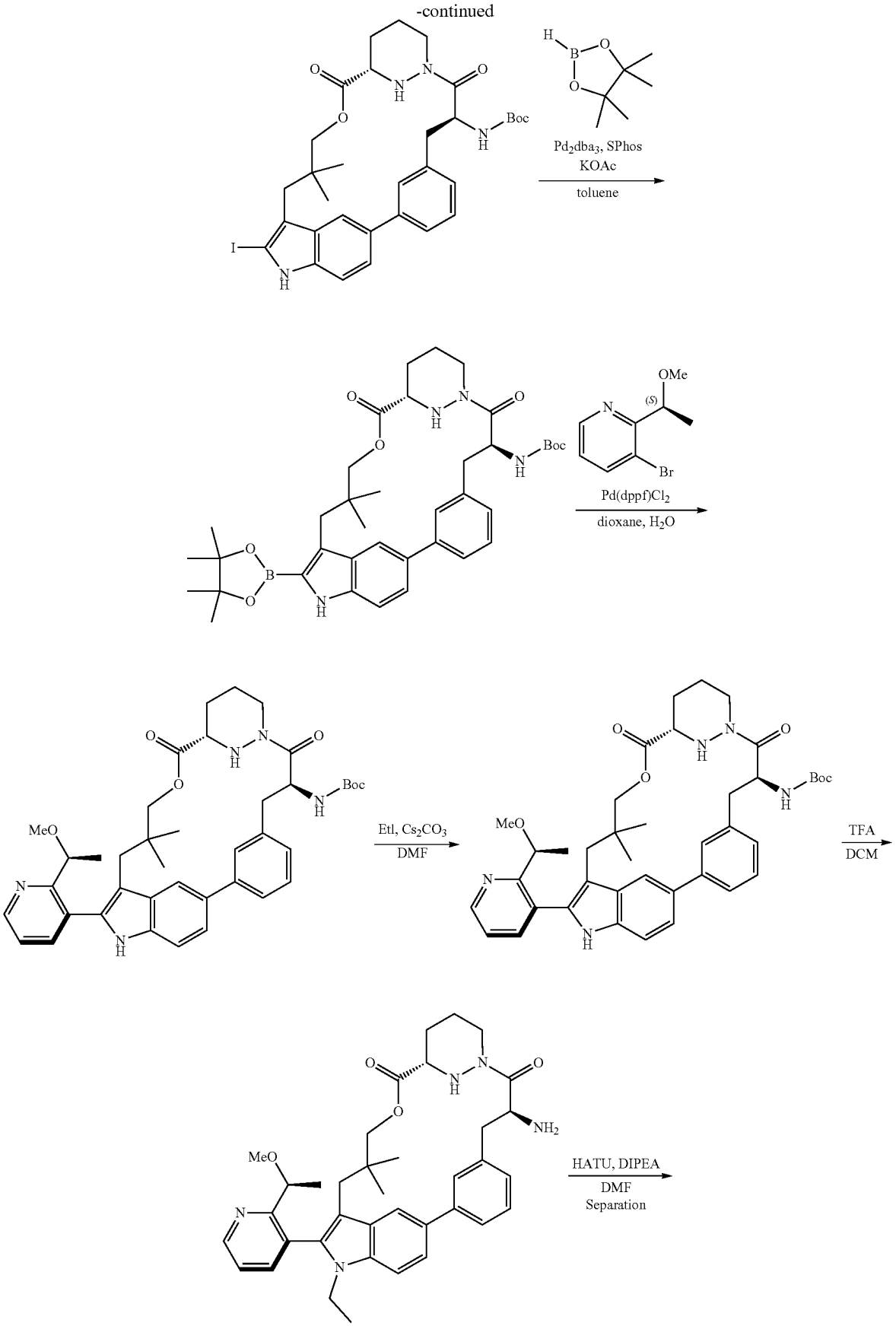
DMF
Pd(dppf)Cl2
KOAc, (BPin)2
dioxane


Pd(DtBPF)Cl2
K2CO3
dioxane, H2O
I2, AgOTf
NaHCO3
THF


LiOH
THF/H2O
EDCI, HOBt
NMM
DCM


LiOH
THF/H2O
EDCI, HOBt
DIEA
DCM

-continued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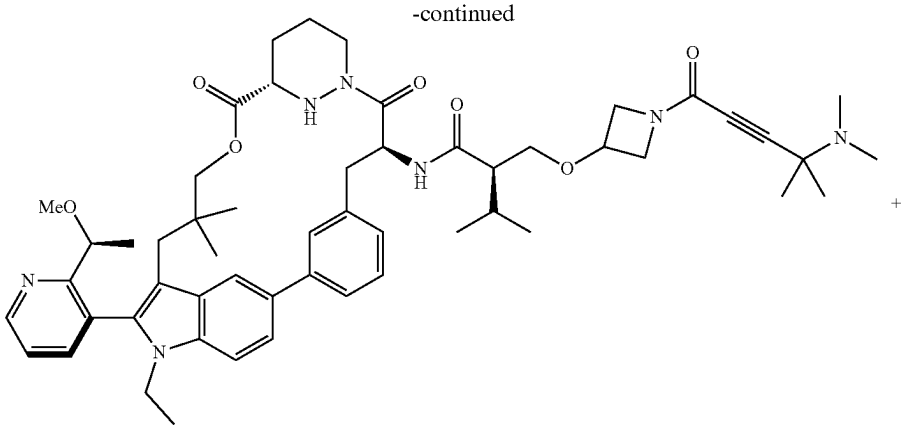
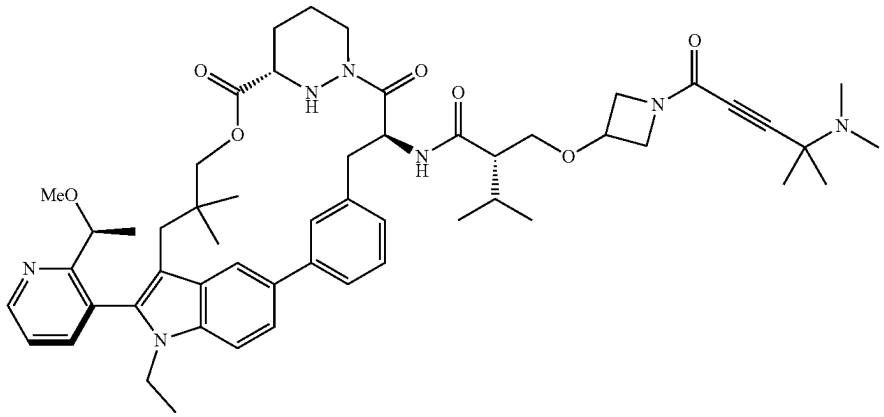
KOAc
toluene
Pd(dppf)Cl2
dioxane, H2O
EtI, Cs2CO3
DMF
TFA
DCM
HATU, DIPEA
DMF
Separation

-continued





Step 1.

To a mixture of 2-(((1-((benzyloxy)carbonyl)azetidin-3-yl)oxy)methyl)-3-methylbutanoic acid (650 mg, 2 mmol) and di-tert-butyl dicarbonate (883 mg, 4 mmol) in ^{t}BuOH (10 mL) was added 4-dimethylaminopyridine (124 mg, 1 mmol). The mixture was heated to 30° C. and stirred for 1 h, then diluted with H_2O (50 mL) and extracted with EtOAc (50 mL×3). The combined organic layers were concentrated under reduced pressure and the residue was purified by silica gel column chromatography to afford benzyl 3-(2-(tert-butoxycarbonyl)-3-methylbutoxy)azetidine-1-carboxylate (450 mg, 56% yield) as an oil. LCMS (ESI): m/z [M+Na] calc'd for $C_{21}H_{31}NO_5Na$ 400.2; found 400.2.

Step 2.

A mixture of benzyl 3-(2-(tert-butoxycarbonyl)-3-methylbutoxy)azetidine-1-carboxylate (450 mg, 1.19 mmol) and Pd/C (50 mg) in THF (30 mL) was stirred for 2 h under an atmosphere of H_2 (15 psi). The mixture was filtered and the filtrate was concentrated under reduced pressure to give tert-butyl 2-((azetidin-3-yloxy)methyl)-3-methylbutanoate (300 mg, 100% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{13}H_{26}NO_3$243.2; found 244.2; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.35-4.25 (m, 1H), 3.71-3.63 (m, 2H), 3.63-3.56 (m, 2H), 3.50 (t, J=8.0 Hz, 1H), 3.43 (dd, J=9.0, 4.0 Hz, 1H), 2.37-2.26 (m, 1H), 2.21 (br. s, 1H), 1.92-1.81 (m, 1H), 1.47 (s, 9H), 0.93 (d, J=6.8 Hz, 6H).

Step 3.

To a mixture of tert-butyl 2-((azetidin-3-yloxy)methyl)-3-methylbutanoate (270 mg, 1.11 mmol), 4-(dimethylamino)-4-methylpent-2-ynoic acid (860 mg, 5.55 mmol) and DIPEA (1.56 g, 11.1 mmol) in DMF (20 mL) at 0° C. was added T3P (2.12 g, 6.7 mmol). The mixture was stirred at 0° C. for 1 h, diluted with EtOAc (200 mL), then washed with H_2O (30 mL×5), brine (30 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give tert-butyl 2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-3-methylbutanoate (200 mg, 47% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{21}H_{36}N_2O_4$ 380.3; found 381.3.

Step 4.

To a mixture of tert-butyl 2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-3-methylbutanoate (190 mg, 0.5 mmol) in DCM (4 mL) was added TFA (2 mL). The mixture was stirred for 1 h, then concentrated under reduced pressure to give 2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-3-methylbutanoic acid (162 mg, 100% yield) as an oil, which was used directly in the next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{17}H_{28}N_2O_4$ 324.2; found 325.3.

Step 5.

To a solution of (2S)-3-(3-bromophenyl)-2-[(tert-butoxycarbonyl)amino]propanoic acid (100 g, 290 mmol) in DMF (1 L) at room temperature was added $NaHCO_3$ (48.8 g, 581.1 mmol) and MeI (61.9 g, 435.8 mmol). The reaction mixture was stirred for 16 h and was then quenched with H_2O (1 L) and extracted with EtOAc (3×1 L). The combined organic layers were washed with brine (3×500 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (13% EtOAc/pet. ether) to give methyl (S)-3-(3-bromophe-

nyl)-2-((tert-butoxycarbonyl)amino)propanoate (109 g, crude). LCMS (ESI): m/z [M+Na] calc'd for $C_{15}H_{20}BrNO_4$ 380.05; found 380.0.

Step 6.

To a stirred solution of methyl (2S)-3-(3-bromophenyl)-2-[(tert-butoxycarbonyl)amino]propanoate (108 g, 301.5 mmol) and bis(pinacolato)diboron (99.53 g, 391.93 mmol) in 1,4-dioxane (3.2 L) was added KOAc (73.97 g, 753.70 mmol) and Pd(dppf)Cl_2 (22.06 g, 30.15 mmol). The reaction mixture was heated to 90° C. for 3 h and was then cooled to room temperature and extracted with EtOAc (2×3 L). The combined organic layers were washed with brine (3×800 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (5% EtOAc/pet. ether) to give methyl (S)-2-((tert-butoxycarbonyl)amino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)propanoate (96 g, 78.6% yield). LCMS (ESI): m/z [M+Na] calc'd for $C_{21}H_{32}BNO_6$ 428.22; found 428.1.

Step 7.

To a mixture of methyl (2S)-2-[(tert-butoxycarbonyl)amino]-3-[3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl]propanoate (94 g, 231.9 mmol) and 3-(5-bromo-1H-indol-3-yl)-2,2-dimethylpropyl acetate (75.19 g, 231.93 mmol) in 1,4-dioxane (1.5 L) and H_2O (300 mL) was added K_2CO_3 (64.11 g, 463.85 mmol) and Pd(DtBPF)Cl_2 (15.12 g, 23.19 mmol). The reaction mixture was heated to 70° C. and stirred for 4 h. The reaction mixture was extracted with EtOAc (2×2 L) and the combined organic layers were washed with brine (3×600 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (20% EtOAc/pet. ether) to give methyl (S)-3-(3-(3-(3-acetoxy-2,2-dimethylpropyl)-1H-indol-5-yl)phenyl)-2-((tert-butoxycarbonyl)amino)propanoate (130 g, crude). LCMS (ESI): m/z [M+H] calc'd for $C_{30}H_{38}N_2O_6$ 523.28; found 523.1.

Step 8.

To a solution of methyl (2S)-3-(3-[3-[3-(acetyloxy)-2,2-dimethylpropyl]-1H-indol-5-yl]phenyl)-2-[(tert-butoxycarbonyl)amino]propanoate (95.0 g, 181.8 mmol) and iodine (36.91 g, 145.41 mmol) in THF (1 L) at −10° C. was added AgOTf (70.0 g, 272.7 mmol) and $NaHCO_3$ (22.9 g, 272.65 mmol). The reaction mixture was stirred for 30 min and was then quenched by the addition of sat. $Na_2S_2O_3$ (100 mL) at 0° C. The resulting mixture was extracted with EtOAc (3×1 L) and the combined organic layers were washed with brine (3×500 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (50% EtOAc/pet. ether) to give methyl (S)-3-(3-(3-(3-acetoxy-2,2-dimethylpropyl)-2-iodo-1H-indol-5-yl)phenyl)-2-((tert-butoxycarbonyl)amino)propanoate (49.3 g, 41.8% yield). LCMS (ESI) m/z: [M+H] calcd for $C_{30}H_{37}IN_2O_6$: 649.18; found 649.1.

Step 9.

To a solution of methyl (2S)-3-(3-[3-[3-(acetyloxy)-2,2-dimethylpropyl]-2-iodo-1H-indol-5-yl]phenyl)-2-[(tert-butoxycarbonyl)amino]propanoate (60 g, 92.5 mmol) in THF (600 mL) was added a solution of LiOH·H_2O (19.41 g, 462.5 mmol) in H_2O (460 mL). The resulting solution was stirred overnight and then the pH was adjusted to 6 with HCl (1 M). The resulting solution was extracted with EtOAc (2×500 mL) and the combined organic layers was washed with sat. brine (2×500 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give (S)-2-((tert-butoxycarbonyl)amino)-3-(3-(3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1H-indol-5-yl)phenyl)propanoic acid (45 g, 82.1% yield). LCMS (ESI): m/z [M+Na] calc'd for $C_{27}H_{33}IN_2O_6$ 615.13; found 615.1.

Step 10.

To a solution of (2S)-2-[(tert-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1H-indol-5-yl]phenyl]propanoic acid (30 g, 50.6 mmol) and methyl (3S)-1,2-diazinane-3-carboxylate (10.9 g, 75.9 mmol) in DCM (400 mL) was added NMM (40.97 g, 405.08 mmol), HOBT (2.05 g, 15.19 mmol), and EDCI (19.41 g, 101.27 mmol). The reaction mixture was stirred overnight and then the mixture was washed with sat. NH_4Cl (2×200 mL) and sat. brine (2×200 mL), and the mixture was dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give methyl (S)-1-((S)-2-((tert-butoxycarbonyl)amino)-3-(3-(3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1H-indol-5-yl)phenyl)propanoyl)hexahydropyridazine-3-carboxylate (14 g, 38.5% yield). LCMS (ESI): m/z [M+H] calc'd for $C_{33}H_{43}IN_4O_6$ 718.23; found 719.4.

Step 11.

To a solution of methyl (S)-1-((S)-2-((tert-butoxycarbonyl)amino)-3-(3-(3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1H-indol-5-yl)phenyl)propanoyl)hexahydropyridazine-3-carboxylate (92 g, 128.0 mmol) in THF (920 mL) at 0° C. was added a solution of LiOH·H_2O (26.86 g, 640.10 mmol) in H_2O (640 mL). The reaction mixture was stirred for 2 h and was then concentrated under reduced pressure to give (S)-1-((S)-2-((tert-butoxycarbonyl)amino)-3-(3-(3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1H-indol-5-yl)phenyl)propanoyl)hexahydropyridazine-3-carboxylic acid (90 g, crude). LCMS (ESI): m/z [M+H] calc'd for $C_{32}H_{41}IN_4O_6$ 705.22; found 705.1.

Step 12.

To a solution of (3S)-1-[(2S)-2-[(tert-butoxycarbonyl)amino]-3-[3-[3-(3-hydroxy-2,2-dimethylpropyl)-2-iodo-1H-indol-5-yl]phenyl]propanoyl]-1,2-diazinane-3-carboxylic acid (90 g, 127.73 mmol) in DCM (10 L) at 0° C. was added HOBt (34.52 g, 255.46 mmol), DIPEA (330.17 g, 2554.62 mmol) and EDCI (367.29 g, 1915.96 mmol). The reaction mixture was stirred for 16 h and was then concentrated under reduced pressure. The mixture was extracted with DCM (2×2 L) and the combined organic layers were washed with brine (3×1 L), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (50% EtOAc/pet. ether) to give tert-butyl ((6^{3}S,4S)-1^2-iodo-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1 ^{1}H-8-oxa-1 (5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (70 g, 79.8% yield). LCMS (ESI): m/z [M+H] calc'd for $C_{32}H_{39}IN_4O_5$ 687.21; found 687.1.

Step 13.

A1 L round-bottom flask was charged with tert-butyl ((6^{3}S,4S)-1^2-iodo-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (22.0 g, 32.042 mmol), toluene (300.0 mL), $Pd_2(dba)_3$ (3.52 g, 3.845 mmol), S-Phos (3.95 g, 9.613 mmol), and KOAc (9.43 g, 96.127 mmol) at room temperature. To the mixture was added 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (26.66 g, 208.275 mmol) dropwise with stirring at room temperature. The resulting solution was stirred for 3 h at 60° C. The resulting mixture was filtered, and the filter cake was washed with EtOAc. The filtrate was concentrated under reduced pressure and the remaining residue was purified by silica gel column chromatography to afford tert-butyl ((6^{3}S,4S)-10,10-dimethyl-5,7-dioxo-12-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,

3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (22 g, 90% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{38}H_{51}BN_4O_7$ 687.3; found 687.4.

Step 14.

A mixture of tert-butyl ((6^{3}S,4S)-10,10-dimethyl-5,7-dioxo-1^2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl) carbamate (2.0 g, 2.8 mmol), 3-bromo-2-[(1S)-1-methoxyethyl]pyridine (0.60 g, 2.8 mmol), Pd(dppf)Cl_2 (0.39 g, 0.5 mmol), and K_3PO_4 (1.2 g, 6.0 mmol) in 1,4-dioxane (50 mL) and H_2O (10 mL) under an atmosphere of N_2 was heated to 70° C. and stirred for 2 h. The mixture was diluted with H_2O (50 mL) and extracted with EtOAc (3×50 mL). The combined organic layers were washed with brine (3×50 mL), dried over anhydrous Na_2SO_4, and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give tert-butyl ((6^{3}S,4S)-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (1.5 g, 74% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{40}H_{49}N_5O_6$ 695.4; found 696.5.

Step 15.

To a solution of tert-butyl ((6^{3}S,4S)-1^2-(2-((S)-1-methoxyethyl) pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl) carbamate (20 g, 28.7 mmol) and Cs_2CO_3 (18.7 g, 57.5 mmol) in DMF (150 mL) at 0° C. was added a solution of ethyl iodide (13.45 g, 86.22 mmol) in DMF (50 mL). The resulting mixture was stirred overnight at 35° C. and was then diluted with H_2O (500 mL). The mixture was extracted with EtOAc (2×300 mL) and the combined organic layers were washed with brine (3×100 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel chromatography to give tert-butyl ((6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl) pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)- benzenacycloundecaphane-4-yl)carbamate (4.23 g, 18.8% yield) and the atropisomer (5.78 g, 25.7% yield) as solids. LCMS (ESI): m/z [M+H] calc'd for $C_{42}H_{53}N_5O_6$ 724.4; found 724.6.

Step 16.

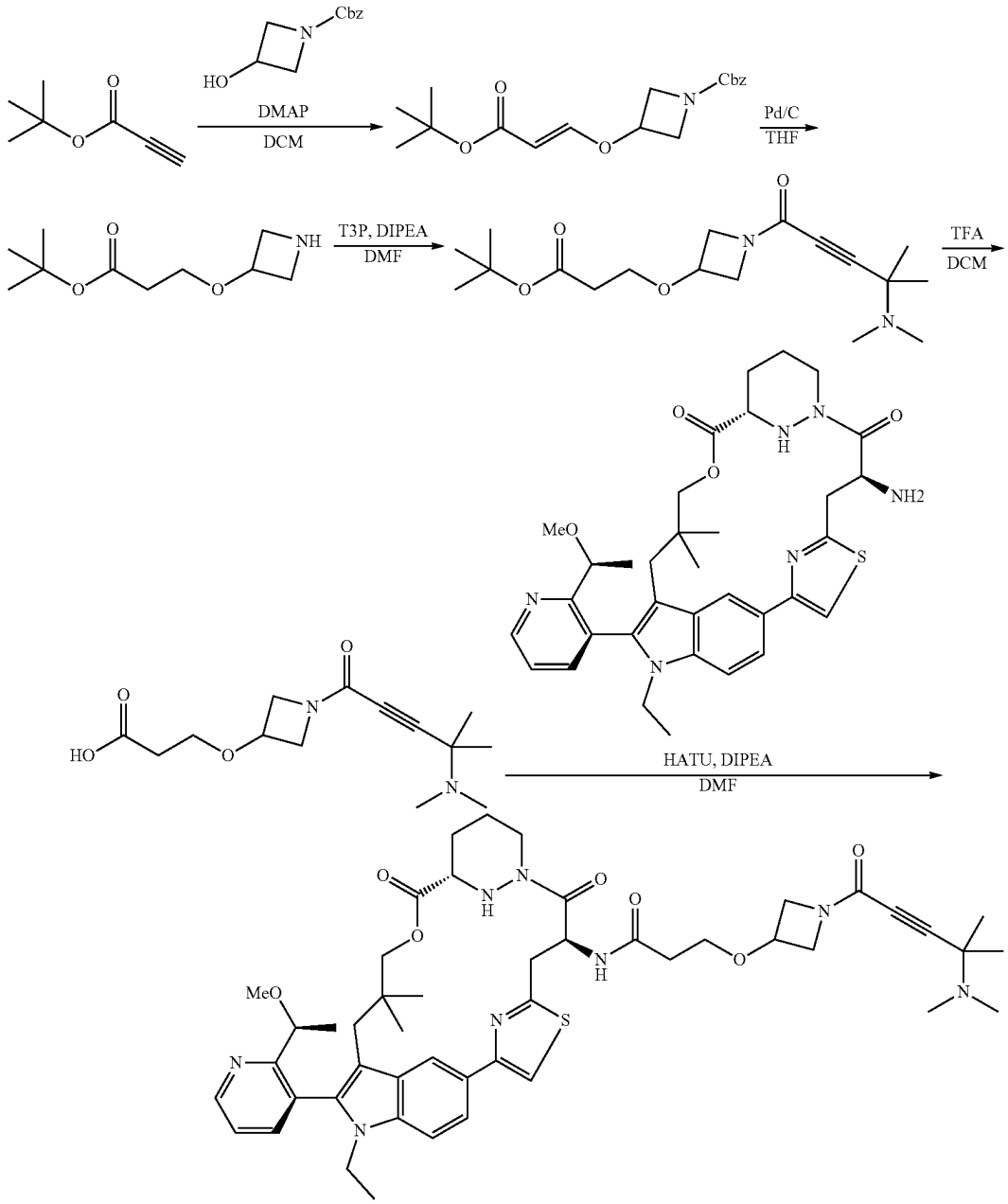
A mixture of tert-butyl ((6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1 ^{1}H-8-oxa-1 (5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl) carbamate (1.3 g, 1.7 mmol) in TFA (10 mL) and DCM (20 mL) was stirred at 0° C. for 2 h. The mixture was concentrated under reduced pressure to afford (6^{3}S,4S)-4-amino-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (1.30 g, crude) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{37}H_{45}N_5O_4$ 623.3; found 624.4.

Step 17.

To a mixture of (6^{3}S,4S)-4-amino-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (258 mg, 0.41 mmol) and 2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-3-methylbutanoic acid (162 mg, 0.5 mmol) in DMF (4 mL) at 0° C. was added a mixture of HATU (188 mg, 0.5 mmol) and DIPEA (534 mg, 4.14 mmol) in DMF (2 mL). The mixture was stirred at 0° C. for 1 h, then diluted with H_2O (30 mL) and extracted with EtOAc (30 mL×3). The combined organic layers were concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl) oxy)methyl)-N-((6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methylbutanamide (250 mg, 64% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{54}H_{71}N_7O_7$ 929.5; found 930.5; ^{1}H NMR (400 MHz, CD_3OD) δ 8.90-8.79 (m, 1H), 8.54-8.21 (m, 1H), 8.15-7.91 (m, 2H), 7.88-7.67 (m, 2H), 7.65-7.52 (m, 2H), 7.47-7.15 (m, 2H), 5.80-5.52 (m, 1H), 4.53-4.23 (m, 5H), 4.23-3.93 (m, 3H), 3.90-3.76 (m, 2H), 3.75-3.58 (m, 3H), 3.57-3.44 (m, 1H), 3.38 (s, 1H), 3.29-3.26 (m, 2H), 3.21-2.85 (m, 8H), 2.82-2.65 (m, 3H), 2.51-2.30 (m, 1H), 2.24-2.03 (m, 1H), 1.99-1.87 (m, 1H), 1.86-1.69 (m, 6H), 1.67-1.57 (m, 2H), 1.57-1.39 (m, 4H), 1.45-1.05 (m, 2H), 1.04-0.96 (m, 3H), 0.96-0.88 (m, 3H), 0.88-0.79 (m, 3H), 0.79-0.63 (m, 3H), 0.56 (s, 1H).

Step 18. 2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-N-((6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methylbutanamide (180 mg, 0.194 mmol) was purified by prep-HPLC to afford (2R)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-N-((6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methylbutanamide (41.8 mg, 23.2% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{54}H_{71}N_7O_7$ 930.5; found 930.5; ^{1}H NMR (400 MHz, MeOD) δ 8.74 (d, J=4.0 Hz, 1H), 8.53-8.30 (m, 1H), 8.10-7.95 (m, 1H), 7.94-7.80 (m, 2H), 7.68 (t, J=8.0 Hz, 1H), 7.65-7.58 (m, 1H), 7.58-7.46 (m, 2H), 7.38-7.17 (m, 2H), 5.73-5.60 (m, 1H), 4.52-4.40 (m, 1H), 4.35-4.15 (m, 4H), 4.14-3.95 (m, 2H), 3.90-3.72 (m, 3H), 3.71-3.45 (m, 4H), 3.30-3.20 (m, 3H), 3.06-2.72 (m, 5H), 2.49-2.28 (m, 4H), 2.28-2.20 (m, 3H), 2.18-2.06 (m, 1H), 2.00-1.90 (m, 1H), 1.90-1.52 (m, 4H), 1.52-1.40 (m, 5H), 1.40-1.22 (m, 4H), 1.09-0.92 (m, 8H), 0.90-0.75 (m, 3H), 0.71-0.52 (m, 3H) and (2S)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-N-((6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1 (5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methylbutanamide (51.2 mg, 28.4% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{54}H_{71}N_7O_7$ 930.5; found 930.3; ^{1}H NMR (400 MHz, MeOD) δ 8.74 (d, J=4.0 Hz, 1H), 8.28-8.20 (m, 0.6H), 8.11-7.98 (m, 1H), 7.97-7.80 (m, 2H), 7.73-7.48 (m, 4H), 7.46-7.36 (m, 0.4H), 7.33-7.26 (m, 1H), 7.25-7.13 (m, 1H), 5.79-5.66 (m, 1H), 4.54-4.43 (m, 1H), 4.42-4.01 (m, 7H), 3.90-3.75 (m, 2H), 3.73-3.48 (m, 4H), 3.27-3.12 (m, 3H), 3.08-2.99 (m, 1H), 2.96-2.85 (m, 2H), 2.84-2.69 (m, 2H), 2.69-2.49 (m, 6H), 2.41-2.29 (m, 1H), 2.15-2.05 (m, 1H), 1.95-1.85 (m, 1H), 1.84-1.71 (m, 1H), 1.71-1.38 (m, 11H), 1.14-1.00 (m, 3H), 1.00-0.71 (m, 9H), 0.70-0.56 (m, 3H).

Example A427. Synthesis of 3-((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)-N-((6^{3}S,4S,Z)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)propanamide



Step 1.

To a mixture of tert-butyl prop-2-ynoate (5 g, 40 mmol) and [3-(3-hydroxyazetidin-1-yl)phenyl]methyl formate (4.1 g, 20 mmol) in DCM (150 mL) was added DMAP (9.8 g, 80 mmol). The mixture was stirred for 2 h, then diluted with H_2O and washed with H_2O (60 mL×3). The organic layer was dried over Na_2SO_4, filtered, the filtrate was concentrated under reduced pressure and the residue purified by silica gel column chromatography to give benzyl (E)-3-((3-(tert-butoxy)-3-oxoprop-1-en-1-yl)oxy)azetidine-1-carboxylate (6.6 g, 90% yield) as an oil. LCMS (ESI): m/z [M+Na] calc'd for $C_{18}H_{23}NO_5Na$ 356.2; found 356.2.

Step 2.

A mixture of benzyl (E)-3-((3-(tert-butoxy)-3-oxoprop-1-en-1-yl)oxy)azetidine-1-carboxylate (1.4 g, 4 mmol) and Pd/C (200 mg) in THF (10 mL) was stirred under an atmosphere of H_2 (1 atmosphere) for 16 h. The mixture was filtered and the filtrate and was concentrated under reduced pressure to give tert-butyl 3-(azetidin-3-yloxy)propanoate, which was used directly in the next step. LCMS (ESI): m/z [M+H] calc'd for $C_{10}H_{19}NO_3$ 201.1; found 202.2.

Step 3.

To a mixture of tert-butyl 3-(azetidin-3-yloxy)propanoate (300 mg, 1.5 mmol) and 4-(dimethylamino)-4-methylpent-2-ynoic acid (2.3 g, 15 mmol) in DMF (15 mL) at 5° C. was added DIPEA (1.9 g, 15 mmol) and T3P (4.77 g, 7.5 mmol) dropwise. The mixture was stirred at 5° C. for 2 h, then H_2O and EtOAc (80 mL) were added. The organic and aqueous layers were separated and the organic layer was washed with H_2O (20 mL×3), brine (30 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-HPLC to afford tert-butyl 3-((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)propanoate (60 mg, 12% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{18}H_{30}N_2O_4$ 338.2; found 339.2.

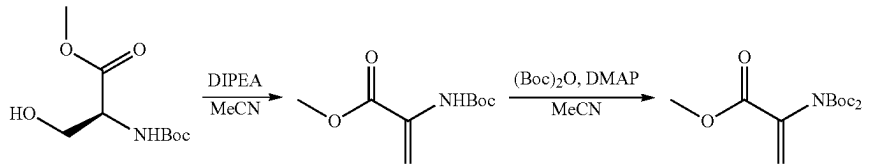
Step 4.

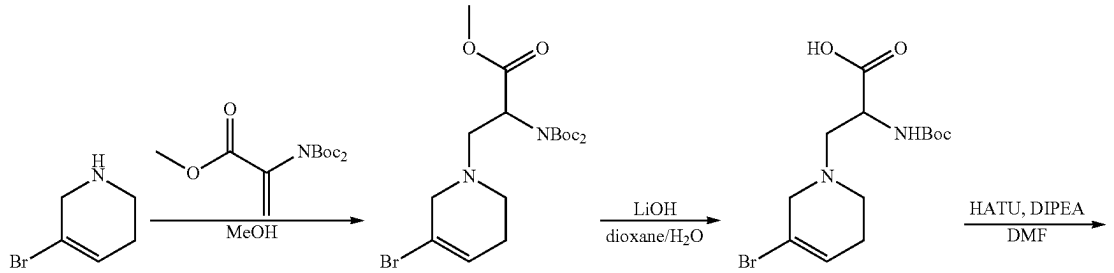
A mixture tert-butyl 3-((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)propanoate (70 mg, 0.21 mmol) in TFA/DCM (1:3, 2 mL) was stirred at 0-5° C. for 1 h, then concentrated under reduced pressure to give 3-({1-[4-(dimethylamino)-4-methylpent-2-ynoyl]azetidin-3-yl}oxy)propanoic acid (56 mg, 95% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{14}H_{22}N_2O_4$ 282.2; found 283.3.

Step 5.

To a mixture of 3-((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)propanoic acid (56 mg, 0.19 mmol), (6^3S,4S,Z)-4-amino-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane- 5,7-dione (90 mg, 0.14 mmol) and DIPEA (200 mg, 1.9 mmol) in DMF (1 mL) at 0° C. was added HATU (110 mg, 0.38 mmol) portion-wise. The mixture was stirred at 0° C. for 1 h, then H_2O added and the mixture extracted with EtOAx (150 mL×2). The combined organic layers were washed with H_2O (150 mL) and brine (150 mL), then dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-HPLC to give 3-((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)-N-((6^3S,4S,Z)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl) propanamide (12.6 mg, 7.5% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{48}H_{62}N_8O_7S$ 894.5; found 895.3; ^{1}H NMR (400 MHz, CD_3OD) δ 8.73 (dd, J=4.8, 1.6 Hz, 1H), 8.57 (s, 1H), 8.28 (s, 0.3H), 7.84 (m, 1H), 7.71 (m, 1H), 7.52 (m, 3H), 5.76 (dd, J=30.2, 7.8 Hz, 1H), 4.40 (m, 4H), 4.32-4.12 (m, 4H), 4.06 (dd, J=12.4, 6.0 Hz, 1H), 3.97-3.86 (m, 1H), 3.79-3.66 (m, 4H), 3.46 (dd, J=14.8, 4.8 Hz, 1H), 3.41-3.33 (m, 3H), 3.29-3.19 (m, 1H), 3.17-3.05 (m, 1H), 2.79 (m, 1H), 2.73-2.50 (m, 3H), 2.49-2.43 (m, 3H), 2.38 (s, 3H), 2.21 (dd, J=12.6, 9.6 Hz, 1H), 1.95 (d, J=12.8 Hz, 1H), 1.86-1.73 (m, 1H), 1.61 (dd, J=12.6, 3.6 Hz, 1H), 1.51 (s, 2H), 1.46-1.43 (m, 4H), 1.38-1.27 (m, 3H), 1.01-0.86 (m, 6H), 0.44 (d, J=11.6 Hz, 3H).

Example A716. Synthesis of (3S)-1-acryloyl-N-((2S)-1-(((6^3S,4S)-1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^1,2^2,2^3,2^6,6^1,6^2,6^3,6^4,6^5,6^6-decahydro-1^1H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,1)-pyridinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-N-methylpyrrolidine-3-carboxamide





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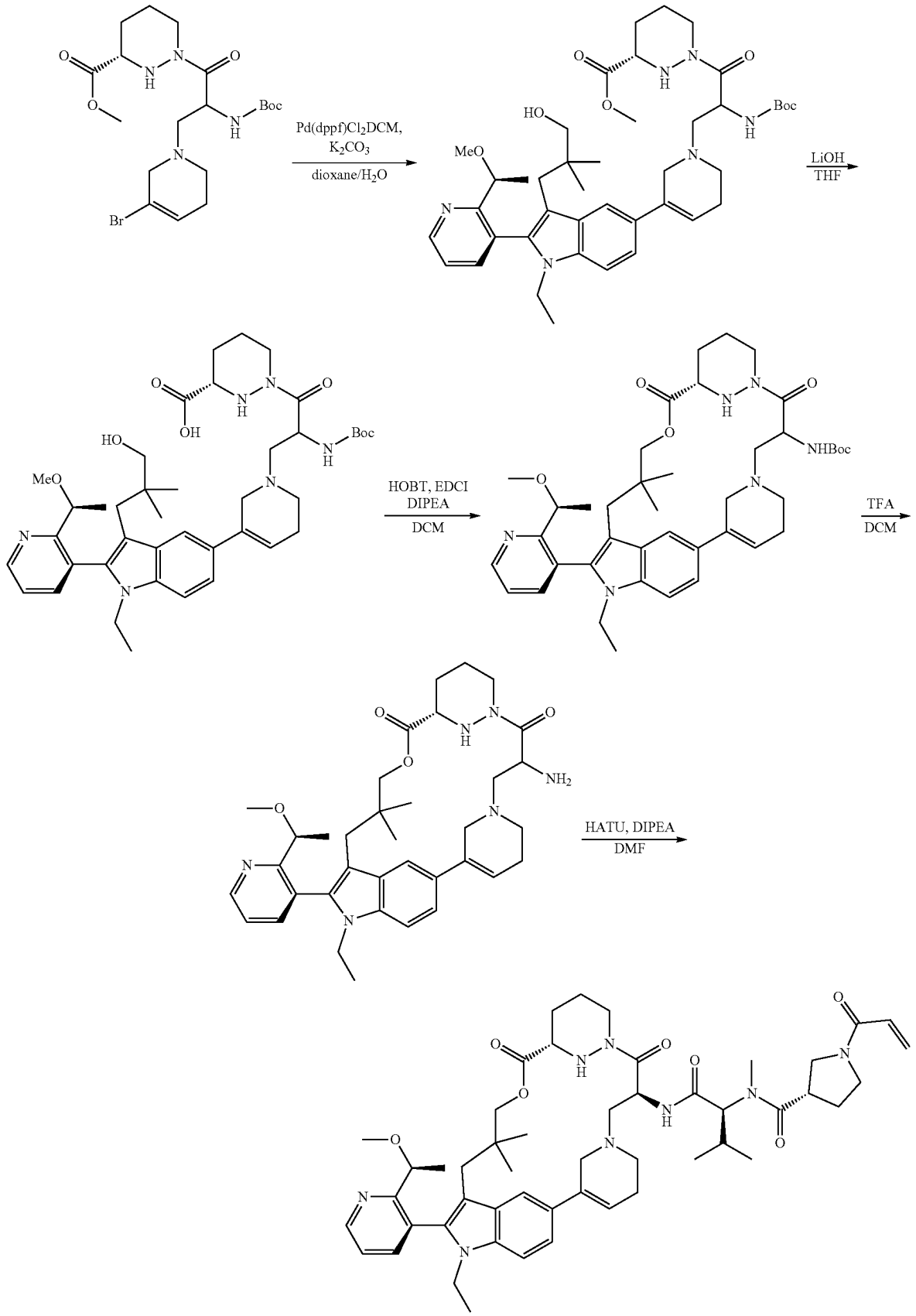

Pd(dppf)Cl2DCM,
K2CO3
dioxane/H2O
LiOH
THF
HOBT, EDCI
DIPEA
DCM
TFA
DCM
HATU, DIPEA
DMF

Step 1.

To a solution of methyl (tert-butoxycarbonyl)-L-serinate (10 g, 45 mmol) in anhydrous MeCN (150 mL), was added DIPEA (17 g, 137 mmol). The reaction mixture was stirred at 45° C. for 2 h to give methyl 2-((tert-butoxycarbonyl) amino)acrylate in solution. LCMS (ESI): m/z [M+Na] calc'd for $C_9H_{15}NO_4$ 201.1; found 224.1.

Step 2.

To a solution of methyl 2-((tert-butoxycarbonyl)amino) acrylate (12 g, 60 mmol) in anhydrous MeCN (150 mL) at 0° C., was added 4-DMAP (13 g, 90 mmol) and $(BoC)_2O$ (26 g, 120 mmol). The reaction was stirred for 6 h, then quenched with H_2O (100 mL) and extracted with DCM (200 mL×3). The combined organic layers were washed with brine (150 mL), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give methyl 2-(bis(tert-butoxycarbonyl)amino)acrylate (12.5 g, 65% yield) as solid. LCMS (ESI): m/z [M+Na] calc'd for $C_{14}H_{23}NO_6$ 301.2; found 324.1.

Step 3.

To a mixture of 5-bromo-1,2,3,6-tetrahydropyridine (8.0 g, 49 mmol) in MeOH (120 mL) under an atmosphere of Ar was added methyl 2-{bis[(tert-butoxy)carbonyl] amino}prop-2-enoate (22 g, 74 mmol). The mixture was stirred for 16 h, then concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl 2-(bis(tert-butoxycarbonyl)amino)-3-(5-bromo-3,6-dihydropyridin-1(2H)-yl)propanoate (12 g, 47% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{19}H_{31}BrN_2O_6$ 462.1; found 463.1.

Step 4.

To a mixture of methyl 2-(bis(tert-butoxycarbonyl) amino)-3-(5-bromo-3,6-dihydropyridin-1(2H)-yl)propanoate (14 g, 30 mmol) in 1,4-dioxane (30 mL) and H_2O (12 mL) was added LiOH (3.6 g, 151 mmol). The mixture was heated to 35° C. and stirred for 12 h, then 1M HCl was added and the pH adjusted to ~3-4. The mixture was extracted with DCM (300 mL×2) and the combined organic layers were dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to give 3-(5-bromo-3,6-dihydropyridin-1(2H)-yl)-2-((tert-butoxycarbonyl)amino) propanoic acid (10 g, 85% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{13}H_{21}BrN_2O_4$ 348.1; found 349.0.

Step 5.

To a mixture of 3-(5-bromo-3,6-dihydropyridin-1(2H)-yl)-2-((tert-butoxycarbonyl)amino)propanoic acid (10 g, 30 mmol), DIPEA (12 g, 93 mmol) and methyl (3S)-1,2-diazinane-3-carboxylate (5.4 g, 37 mmol) in DMF (100 mL) at 0° C. under an atmosphere of Ar was added HATU (13 g, 34 mmol). The mixture was stirred at 0° C. for 2 h, then H_2O added and the mixture extracted with EtOAc (300 mL×2). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, the filtrate was concentrated under reduced pressure and the residue was purified by preparative-HPLC to give methyl (3S)-1-(3-(5-bromo-3,6-dihydropyridin-1 (2H)-yl)-2-((tert-butoxycarbonyl)amino)propanoyl)hexahydropyridazine-3-carboxylate (9.0 g, 55% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{19}H_{31}BrN_4O_5$ 474.1; found 475.1.

Step 6.

A mixture of methyl (3S)-1-(3-(5-bromo-3,6-dihydropyridin-1(2H)-yl)-2-((tert-butoxycarbonyl)amino)propanoyl) hexahydropyridazine-3-carboxylate (9.0 g, 18 mmol), K_2CO_3 (4.5 g, 32 mmol), Pd(dppf)Cl_2.DCM (1.4 g, 2 mmol), 3-(1-ethyl-2-{2-[(1S)-1-methoxyethyl]pyridin-3-yl}-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)indol-3-yl)-2,2-dimethylpropan-1-ol (9.8 g, 20 mmol) in 1,4-dioxane (90 mL) and H_2O (10 mL) under an atmosphere of Ar was heated to 75° C. and stirred for 2 h. H_2O was added and the mixture was extracted with EtOAc (200 mL×3). The combined organic layers were dried over Na_2SO_4, filtered, the filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl (3S)-1-(2-((tert-butoxycarbonyl)amino)-3-(5-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1H-indol-5-yl)-3,6-dihydropyridin-1(2H)-yl)propanoyl)hexahydropyridazine-3-carboxylate (4.0 g, 25% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{42}H_{60}N_6O_7$ 760.5; found 761.4.

Step 7.

To a mixture of methyl (3S)-1-(2-((tert-butoxycarbonyl) amino)-3-(5-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1H-indol-5-yl)-3,6-dihydropyridin-1(2H)-yl)propanoyl)hexahydropyridazine-3-carboxylate (4.1 g, 5.0 mmol) in THF (35 mL) at 0° C. was added LiOH (0.60 g, 27 mmol). The mixture was stirred at 0° C. for 1.5 h, then 1M HCl added to adjust pH to ~6-7 and the mixture extracted with EtOAc (200 mL×3). The combined organic layers were dried over Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure to give (3S)-1-(2-((tert-butoxycarbonyl)amino)-3-(5-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl) pyridin-3-yl)-1H-indol-5-yl)-3,6-dihydropyridin-1 (2H)-yl) propanoyl)hexahydropyridazine-3-carboxylic acid (3.6 g, 80% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{41}H_{58}N_6O_7$ 746.4; found 747.4.

Step 8.

To a mixture of (3S)-1-(2-((tert-butoxycarbonyl)amino)-3-(5-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1H-indol-5-yl)-3,6-dihydropyridin-1(2H)-yl)propanoyl)hexahydropyridazine-3-carboxylic acid (3.6 g, 5.0 mmol) and DIPEA (24 g, 190 mmol) in DCM (700 mL) under an atmosphere of Ar was added EDCl·HCl (28 g, 140 mmol) and HOBT (6.5 g, 50 mmol). The mixture was heated to 30° C. and stirred for 16 h at 30° C., then concentrated under reduced pressure. The residue was diluted with EtOAc (200 mL) and washed with H_2O (200 mL×2), brine (200 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give tert-butyl ((6^{3}S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^1 2^2,2^3,2^6,6^1,6^2,6^3,6^4,6^5,6^6-decahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,1)-pyridinacycloundecaphane-4-yl)carbamate (1.45 g, 40% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{41}H_{56}N_6O_6$ 728.4; found 729.4.

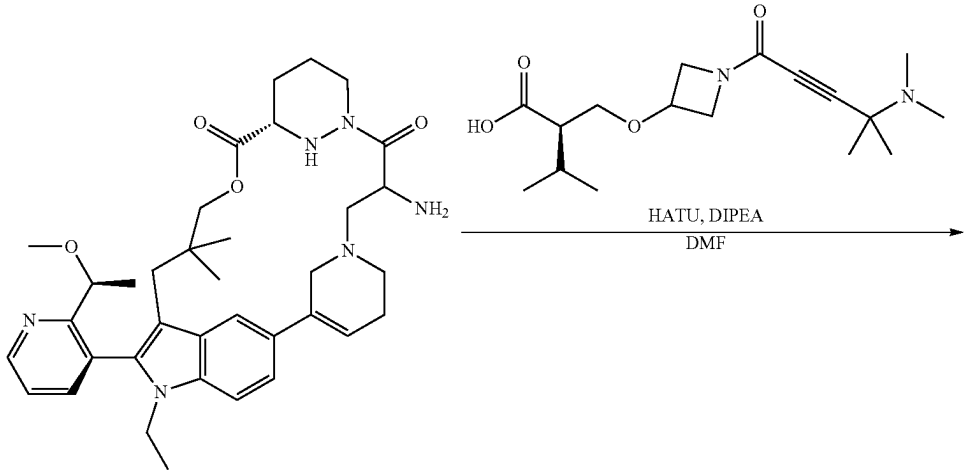
Step 9.

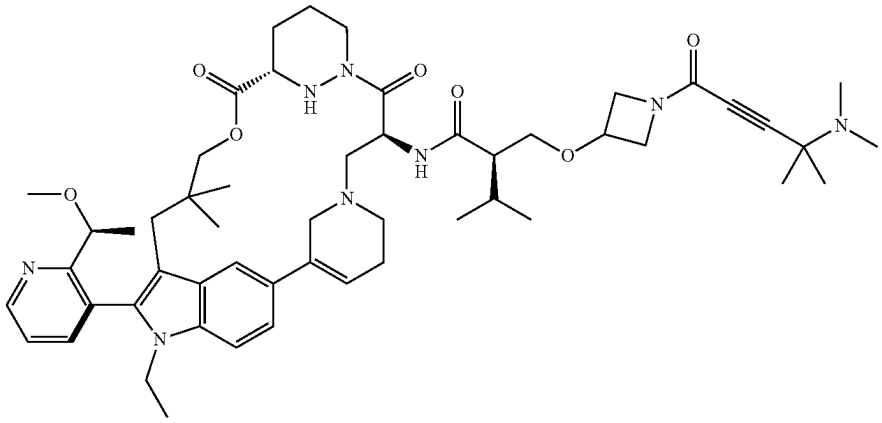
To a mixture of tert-butyl ((6^{3}S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^1 2^2,2^3,2^6,6^1,6^2,6^3,6^4,6^5,6^6-decahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,1)-pyridinacycloundecaphane-4-yl)carbamate (130 mg, 0.20 mmol) in DCM (1.0 mL) at 0° C. was added TFA (0.3 mL). The mixture was warmed to room temperature and stirred for 2 h, then concentrated under reduced pressure to give (6^{3}S)-4-amino-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-2^1 2^2,2^3,2^6,6^1,6^2,6^3,6^4,6^5, 6^6-decahydro-1 ^{1}H-8-oxa-1 (5,3)-indola-6(1,3)-pyridazina-2(5,1)-pyridinacycloundecaphane-5,7-dione, which was used directly in the next step directly without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{36}H_{48}N_6O_4$ 628.4; found 629.4.

Step 10.

To a mixture of ((6^{3}S)-4-amino-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-2^1 2^2,2^3,2^6,6^1,6^2,6^3,6^4,6^5,6^6-decahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,1)- pyridinacycloundecaphane-5,7-dione (130 mg, 0.2 mmol), DIPEA (270 mg, 2.0 mmol) and (2S)-3-methyl-2-{N-methyl-1-[(3S)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido}butanoic acid (118 mg, 0.40 mmol) in DMF (3.0 mL) at 0° C. under an atmosphere of Ar was added HATU (87 mg, 0.30 mmol) in portions. The mixture was stirred at 0° C. for 1 h, then diluted with H_2O extracted with EtOAc (30 mL×2). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, the filtrate was concentrated under reduced pressure and the residue was purified by preparative-HPLC to give (3S)-1-acryloyl-N-((2S)-1-(((6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl) pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^1 2^2,2^3,2^6,6^1,6^2,6^3,6^4,6^5,6^6-decahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,1)-pyridinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-N-methylpyrrolidine-3-carboxamide (17.2 mg, 10% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{50}H_{68}N_6O_4$ 892.5; found 893.5; ^{1}H NMR (400 MHz, CD_3OD) δ 8.74 (d, J=4.4 Hz, 1H), 7.93-7.90 (m, 1H), 7.56-7.51 (m, 3H), 7.43 (d, J=4.4 Hz, 1H), 6.63-6.53 (m, 2H), 6.33-6.23 (m, 2H), 5.83-5.70 (m, 1H), 4.73-4.70 (d, J=11.0 Hz, 1H), 4.48-4.45 (d, J=13.0 Hz, 1H), 4.12-4.10 (m, 3H), 3.86-3.81 (m, 4H), 3.79-3.75 (m, 1H), 3.72-3.69 (m, 3H), 3.57-3.47 (m, 2H), 3.21-3.09 (m, 1H), 3.07-3.04 (q, 4H), 3.02-2.95 (m, 3H), 2.86-2.82 (m, 3H), 2.66-2.48 (m, 2H), 2.29-2.17 (m, 4H), 2.11-1.98 (m, 2H), 1.95-1.91 (m, 1H), 1.45 (d, J=6.2 Hz, 3H), 1.23-1.16 (m, 2H), 1.09-1.04 (m, 1H), 0.97-0.93 (m, 3H), 0.92-0.81 (m, 5H), 0.67-0.63 (m, 3H).

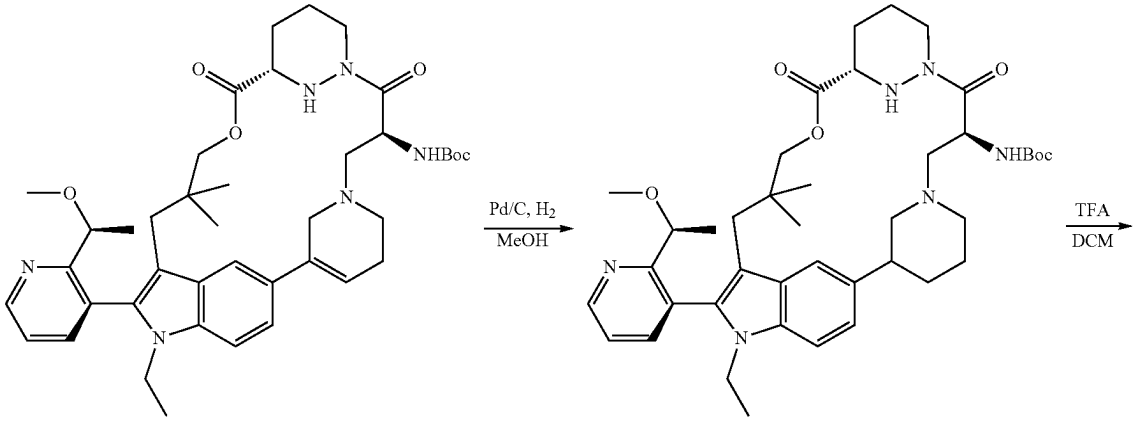
Example A663. The synthesis of (2R)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)405yridine405-3-yl)oxy)methyl)-N-((6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)405yridine-3-yl)-10,10-dimethyl-5,7-dioxo-2^1,2^2,2^3,2^6,6^1,6^2,6^3,6^4,6^5,6^6-decahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,1)-pyridinacycloundecaphane-4-yl)-3-methylbutanamide

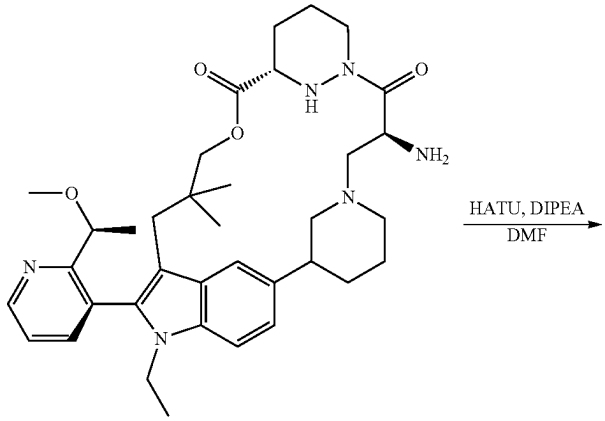




To a mixture of (6^3S,4S)-4-amino-1-ethyl-1^2-(2-((S)-1-methoxyethyl)405yridine-3-yl)-10,10-dimethyl-2^1 2^2,2^3,2^6,6^1,6^2 6^3 6^4 6^5 6^6-decahydro-1^1H-8-oxa-1 (5,3)-indola-6(1,3)-pyridazina-2(5,1)- pyridinacycloundecaphane-5,7-dione (100 mg, 0.16 mmol),®-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)405yridine405-3-yl)oxy)methyl)-3-methylbutanoic acid (80 mg, 0.24 mmol) and DIPEA (825 mg, 6.4 mmol) in DMF (2 mL) at 0° C., was added HATU (95 mg, 0.24 mmol). The reaction mixture was stirred at 0° C. for 1 h, then poured into H_2, (60 mL), extracted with EtOAc (80 mL×2). The combined organic layers were washed with H_2S (80 mL) and brine (80 mL, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography to afford (2R)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)405yridine405-3-yl)oxy)methyl)-N-((6^3S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)405yridine-3-yl)-10,10-dimethyl-5,7-dioxo-2^1 2^2,2^3,2^6,6^1,$6^2$$6^3$ 6^4,6^5,6^6-decahydro-1^1H-8-oxa-1 (5,3)-indola-6(1,3)-pyridazina-2(5,1)-pyridinacycloundecaphane-4-yl)-3-methylbutanamide (55 mg, 36% yield) as solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.76-8.70 (m, 1H), 8.49 (dd, J=4.3, 1.4 Hz, 0.1H), 7.93-7.87 (m, 1H), 7.58-7.50 (m, 3H), 7.41 (dd, J=8.8, 3.2 Hz, 1H), 6.26 (d, J=16.8 Hz, 1H), 5.96 (t, J=9.6 Hz, 1H), 4.47 (d, J=12.8 Hz, 1H), 4.39-4.28 (m, 2H), 4.21-3.97 (m, 5H), 3.96-3.70 (m, 5H), 3.68-3.54 (m, 3H), 3.51-3.35 (m, 1H), 3.11 (d, J=22.7 Hz, 3H), 3.00-2.67 (m, 5H), 2.46-2.30 (m, 7H), 2.24 (s, 3H), 2.11 (d, J=12.4 Hz, 1H), 1.92 (d, J=13.2 Hz, 1H), 1.85-1.60 (m, 3H), 1.45 (d, J=7.8 Hz, 6H), 1.32 (d, J=16.0 Hz, 3H), 1.12 (dt, J=24.5, 6.8 Hz, 3H), 0.95 (m, 6H), 0.76 (in, 6H). LCMS (ESI): m/z [M+H] calc'd for $C_{53}H_{74}N_8O_7$ 934.6; found 935.5.

Example A646. The synthesis of (2R)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-N-((6^3S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(3,1)-piperidinacycloundecaphane-4-yl)-3-methylbutanamide





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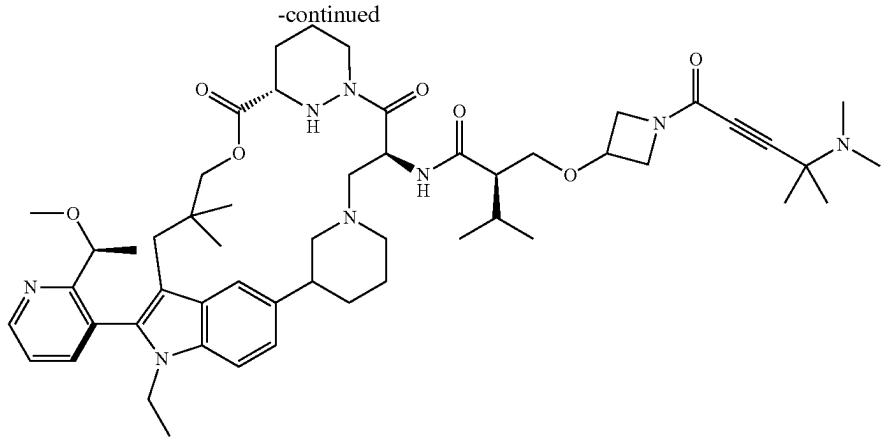


Step 1.

A mixture of tert-butyl ((6^{3}S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-2^1,2^2,2^3,2^6,6^1,6^2,6^3,6^4,6^5,6^6-decahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,1)-pyridinacycloundecaphane-4-yl)carbamate (0.2 g, 0.28 mmol) and Pd/C (0.2 g, 2 mmol) in MeOH (10 mL) was stirred at 25° C. for 16 h under an H_2 atmosphere. The reaction mixture was filtered through Celite, concentrated under reduced pressure to afford tert-butyl ((6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(3,1)-piperidinacycloundecaphane-4-yl)carbamate as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{41}H_{58}N_6O_6$ 730.4; found 731.4.

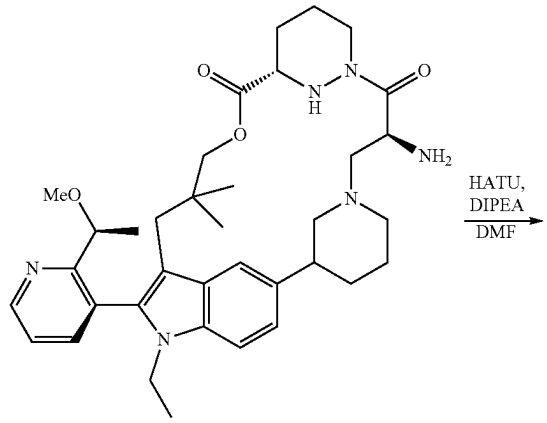
Step 2.

To a solution of tert-butyl ((6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(3,1)-piperidinacycloundecaphane-4-yl) carbamate (150 mg, 0.2 mmol) in DCM (1.5 mL) at 0° C. was added TFA (0.5 mL). The reaction mixture was stirred at 20° C. for 1 h, then concentrated under reduced pressure to afford (6^{3}S,4S)-4-amino-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(3,1)-piperidinacycloundecaphane-5,7-dione as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{36}H_{50}N_6O_4$ 630.4; found 631.4.

Step 3.

To a mixture of (6^{3}S,4S)-4-amino-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(3,1)-piperidinacycloundecaphane-5,7-dione (240 mg, 0.4 mmol), DIPEA (982 mg, 2 mmol) and (R)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-3-methylbutanoic acid (148 mg, 0.45 mmol) in DMF (4 mL) at 0° C. under argon atmosphere, was added HATU (173 mg, 0.46 mmol) in portions. The reaction mixture was stirred at 0° C. under an argon atmosphere for 1 h, then quenched with H_2O at 0° C. The resulting mixture was extracted with EtOAc (30 mL×2). The combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by reverse phase chromatography to afford (2R)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-N-((6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(3,1)-piperidinacycloundecaphane-4-yl)-3-methylbutanamide (150 mg, 38% yield) as solid. ^{1}H NMR (400 MHz, CD3OD) δ 8.72 (d, J=4.8 Hz, 1H), 7.88 (d, J=8.0 Hz, 1H), 7.53-7.49 (m, 2H), 7.42 (d, J=8.4 Hz, 1H), 7.18 (d, J=8.4 Hz, 1H), 5.95-5.91 (m, 1H), 4.52-4.49 (m, 1H), 4.37-4.25 (m, 3H), 4.18-4.15 (m, 2H), 3.99-3.98 (m, 2H), 3.90-3.86 (m, 1H), 3.76-3.68 (m, 2H), 3.55-3.50 (m, 2H), 3.39-3.36 (m, 2H), 3.20 (s, 3H), 3.02 (s, 3H), 2.89-2.79 (m, 3H), 2.62-2.50 (m, 2H), 2.36 (s, 3H), 2.35-2.30 (m, 1H), 2.26 (s, 3H), 2.20-1.15 (m, 1H), 1.97-1.93 (m, 3H), 1.81-1.76 (m, 4H), 1.64-1.61 (m, 2H), 1.46-1.43 (m, 6H), 1.36 (d, J=14.8 Hz, 3H), 1.02 (s, 3H), 0.94 (m, 6H), 0.81 (s, 3H), 0.65 (s, 3H). LCMS (ESI): m/z [M+H] calc'd for $C_{53}H_{76}N_8O_7$ 936.6; found 937.5.

Example A740. Synthesis of (3S)-1-acryloyl-N-((2S)-1-(((2^{3}S,6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(3,1)-piperidinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-N-methylpyrrolidine-3-carboxamide



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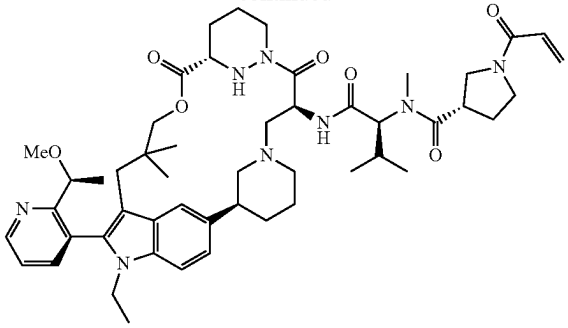
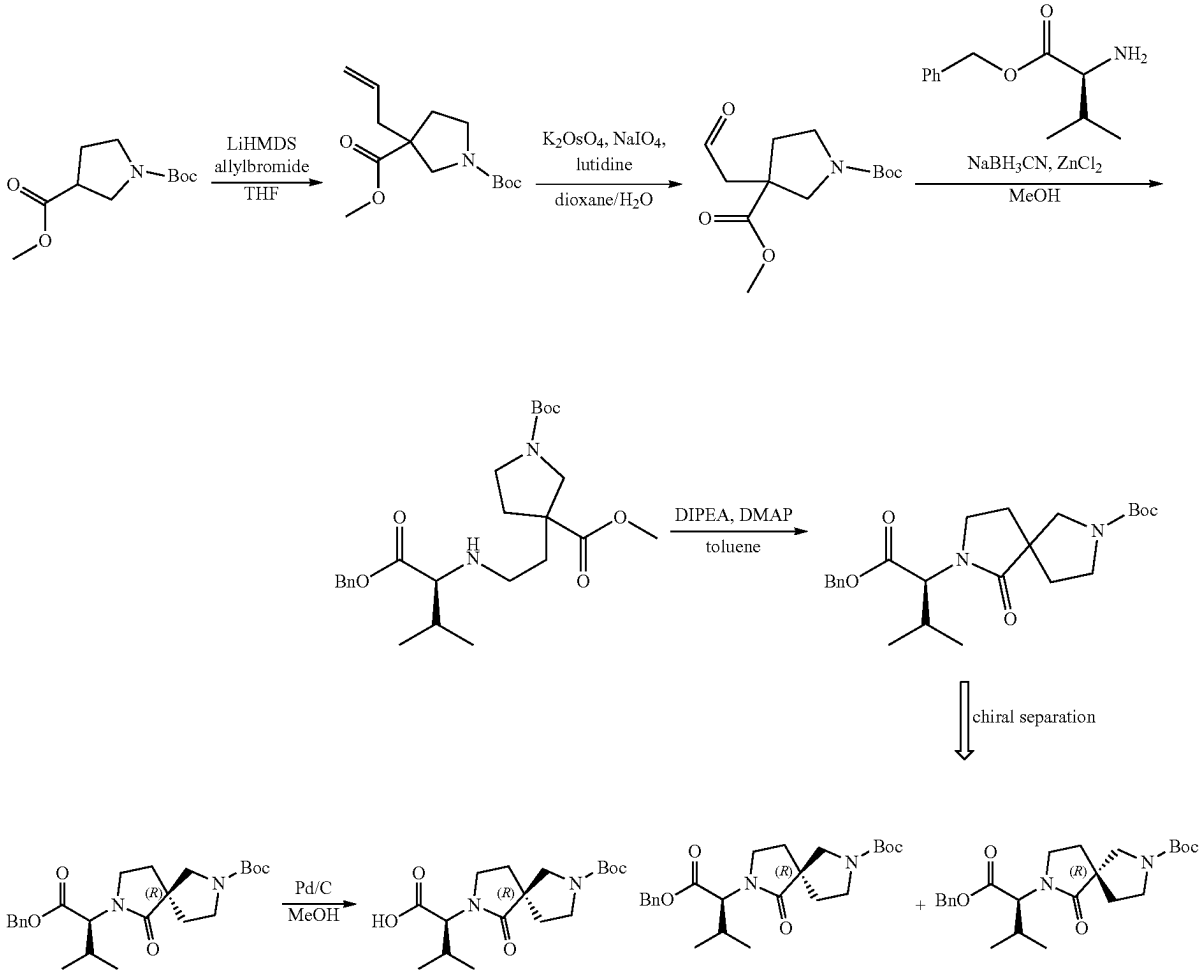


To a mixture of (6^3S,4S)-4-amino-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(3,1)-piperidinacycloundecaphane-5,7-dione (140 mg, 0.20 mmol), DIPEA (570 mg, 4.4 mmol) and (2S)-3-methyl-2-{N-methyl-1-[(3S)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido}butanoic acid (124 mg, 0.40 mmol) in DMF (3.0 mL) at 0° C. under an atmosphere of Ar was added HATU (100 mg, 0.30 mmol) in portions. The mixture was stirred at 0° C. for 1 h, then H_2O was added and the mixture extracted with EtOAc (2×30 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-HPLC to give (3S)-1-acryloyl-N-((2S)-1-(((2^{3}S,6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(3,1)-piperidinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-N-methylpyrrolidine-3-carboxamide (41 mg, 20% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{50}H_{70}N_8O_7$ 894.5; found 895.5; ^{1}H NMR (400 MHz, CD_3OD) δ 8.72 (d, J=4.8 Hz, 1H), 7.87 (d, J=7.6 Hz, 1H), 7.51-7.49 (m, 2H), 7.42-7.37 (m, 1H), 7.18-7.14 (m, 1H), 6.64-6.54 (m, 1H), 6.30-6.23 (m, 1H), 5.77-5.70 (m, 2H), 4.65-4.60 (m, 1H), 4.50-4.40 (m, 1H), 4.27-4.16 (m, 2H), 4.00-3.95 (m, 2H), 3.83-3.78 (m, 2H), 3.73-3.60 (m, 4H), 3.51-3.36 (m, 3H), 3.22-3.19 (m, 4H), 3.07 (d, J=6.8 Hz, 2H), 2.99 (d, J=12.0 Hz, 3H), 2.90-2.78 (m, 2H), 2.75-2.64 (m, 3H), 2.20-2.10 (m, 4H), 2.02-1.93 (m, 3H), 1.87-1.64 (m, 4H), 1.45 (d, J=4.8 Hz, 3H), 1.06-1.00 (m, 4H), 0.97-0.89 (m, 3H), 0.83-0.79 (m, 3H), 0.66 (s, 3H).

Example A534. (2S)-2-((S)-7-(4-(dimethylamino)-4-methylpent-2-ynoyl)-1-oxo-2,7-diazaspiro[4.4]nonan-2-yl)-N-((6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methylbutanamide



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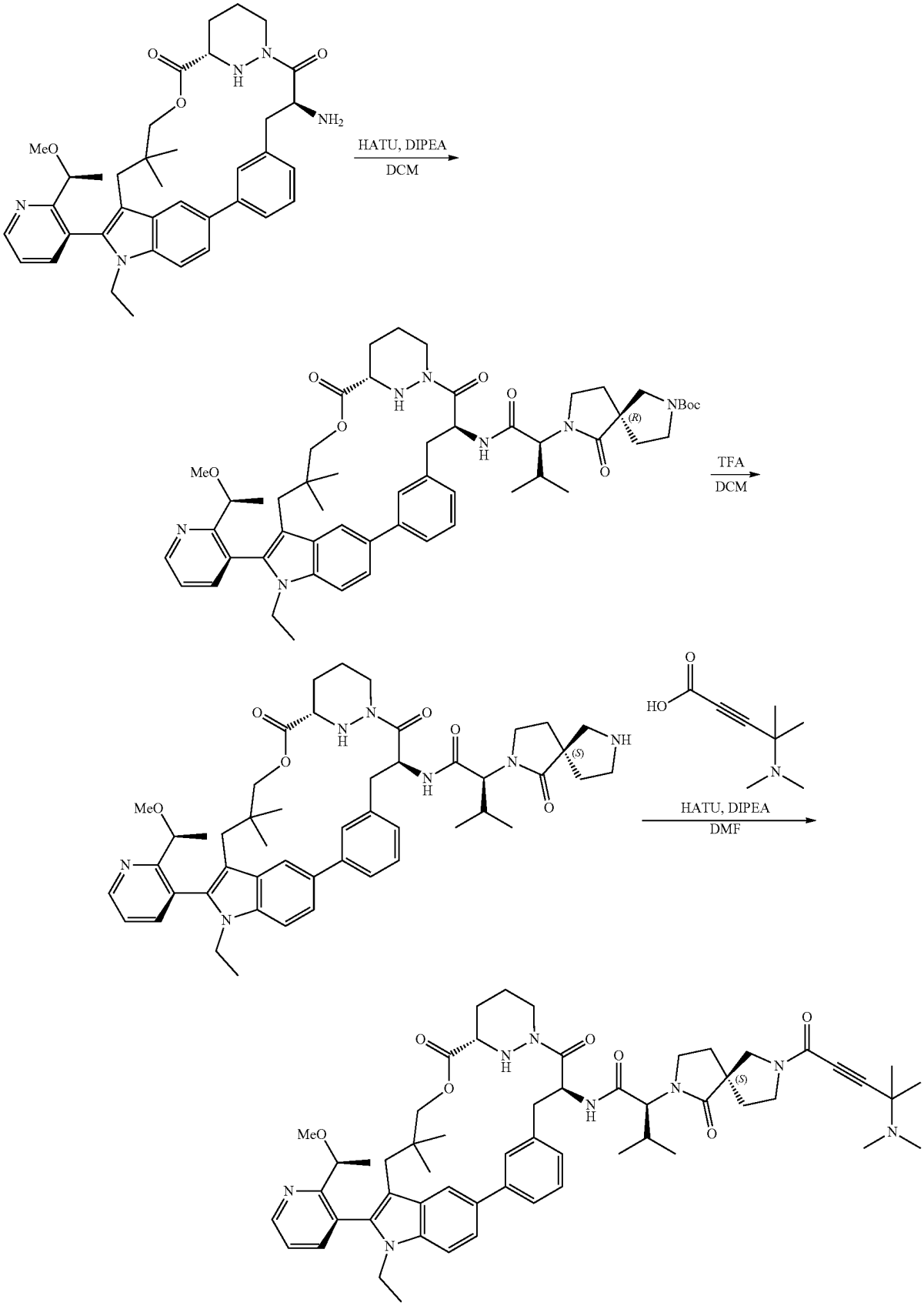


Step 1.

To a mixture of 1-tert-butyl 3-methyl pyrrolidine-1,3-dicarboxylate (20.0 g, 87.2 mmol) in THF (150 mL) at −78° C. under an atmosphere of nitrogen was added 1M LiHMDS in THF (113.4 mL, 113.4 mmol). After stirring at −78° C. for 40 min, allyl bromide (13.72 g, 113.4 mmol) was added and the mixture was allowed to warm to room temperature and stirred for 4 h. The mixture was cooled to 0° C., saturated NaCl (30 mL) was added and the mixture extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 1-(tert-butyl) 3-methyl 3-allylpyrrolidine-1,3-dicarboxylate (17 g, 72% yield) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.80-5.60 (m, 1H), 5.16-5.02 (m, 2H), 3.71 (s, 4H), 3.42 (d, J=9.3 Hz, 2H), 3.27 (t, J=11.2 Hz, 1H), 2.42 (d, J=7.6 Hz, 2H), 2.38-2.24 (m, 1H), 2.05 (s, 1H), 1.85 (dt, J=14.3, 7.5 Hz, 1H), 1.46 (s, 10H), 1.27 (t, J=7.1 Hz, 1H).

Step 2.

To a mixture of 1-(tert-butyl) 3-methyl 3-allylpyrrolidine-1,3-dicarboxylate (4.0 g, 14.9 mmol) and 2,6-dimethylpyridine (3.18 g, 29.7 mmol) in 1,4-dioxane (200 mL) and H_2O (100 mL) at 0° C. was added K_2OsO_4 $2H_2O$ (0.11 g, 0.3 mmol) in portions. The mixture was stirred for 15 min at 0° C., then $NaIO_4$ (6.35 g, 29.7 mmol) was added in portions. The mixture was stirred at room temperature for 3 h at room temperature, then cooled to 0° C. and saturated aqueous $Na_2S_2O_3$ (50 mL) added. The mixture was extracted with EtOAc (3×100 mL) and the combined organic layers were washed with 2 M HCl, then dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to give 1-(tert-butyl) 3-methyl 3-(2-oxoethyl)pyrrolidine-1,3-dicarboxylate (4 g, 52% yield) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.80-5.60 (m, 1H), 5.16-5.04 (m, 2H), 3.72 (s, 3H), 3.41 (s, 3H), 3.28 (d, J=11.0 Hz, 1H), 2.44 (s, 2H), 2.31 (d, J=9.1 Hz, 1H), 1.85 (dt, J=12.7, 7.5 Hz, 1H), 1.69 (s, 1H), 1.47 (s, 10H).

Step 3.

To a mixture of 1-(tert-butyl) 3-methyl 3-(2-oxoethyl) pyrrolidine-1,3-dicarboxylate (6.30 g, 23.2 mmol), in MeOH (70 mL) at 0° C. was added benzyl (2S)-2-amino-3-methylbutanoate (7.22 g, 34.8 mmol) and $ZnCl_2$ (4.75 g, 34.8 mmol). The mixture was warmed to room temperature and stirred for 30 min, then cooled to 0° C. and $NaCNBH_3$ (2.92 g, 46.4 mmol) was added in portions. The mixture was warmed to room temperature and stirred for 2 h, then cooled to 0° C. and saturated aqueous NH_4Cl added. The mixture was extracted with EtOAc (3×200 mL) and the combined organic layers were washed with brine (150 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 1-(tert-butyl) 3-methyl 3-(2-(((S)-1-(benzyloxy)-3-methyl-1-oxobutan-2-yl)amino)ethyl)pyrrolidine-1,3-dicarboxylate (6.4 g, 54% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{25}H_{38}N_2O_6$ 462.3; found 463.4.

Step 4.

To a mixture of 1-(tert-butyl) 3-methyl 3-(2-(((S)-1-(benzyloxy)-3-methyl-1-oxobutan-2-yl)amino)ethyl)pyrrolidine-1,3-dicarboxylate (4.50 g, 9.7 mmol) in toluene (50 mL) was added DIPEA (12.57 g, 97.3 mmol) and DMAP (1.19 g, 9.7 mmol). The resulting mixture was heated to 80° C. and stirred for 24 h, then concentrated under reduced pressure and the residue was purified by preparative-HPLC, then by chiral-HPLC to give tert-butyl (R)-7-((S)-1-(benzyloxy)-3-methyl-1-oxobutan-2-yl)-6-oxo-2,7-diazaspiro[4.4]nonane-2-carboxylate (1.0 g, 32% yield) and tert-butyl (S)-7-((S)-1-(benzyloxy)-3-methyl-1-oxobutan-2-yl)-6-oxo-2,7-diazaspiro[4.4]nonane-2-carboxylate (1.0 g, 32% yield) and as a an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{24}H_{34}N_2O_5$ 430.5; found 431.2 and LCMS (ESI): m/z [M+H] calc'd for $C_{24}H_{34}N_2O_5$ 430.3; found 431.2.

Step 5.

A mixture of tert-butyl (R)-7-((S)-1-(benzyloxy)-3-methyl-1-oxobutan-2-yl)-6-oxo-2,7-diazaspiro[4.4]nonane-2-carboxylate (4.0 g) and 10% Pd/C (1 g) in MeOH (40 mL) was stirred at room temperature under an atmosphere of H_2. The mixture was filtered through a pad of Celite pad and the filtrate was concentrated under reduced pressure to give (S)-2-((R)-7-(tert-butoxycarbonyl)-1-oxo-2,7-diazaspiro[4.4]nonan-2-yl)-3-methylbutanoic acid (4.9 g) as a solid. LCMS (ESI): m/z [M−H] calc'd for $C_{17}H_{28}N_2O_5$ 340.2; found 339.3.

Step 6.

To a mixture of (6^3S,4S)-4-amino-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (500 mg, 0.8 mmol) in DCM at 0° C. were added DIPEA (829 mg, 6.4 mmol), ((S)-2-((R)-7-(tert-butoxycarbonyl)-1-oxo-2,7-diazaspiro[4.4]nonan-2-yl)-3-methylbutanoic acid (273 mg, 0.8 mmol) and HATU (396 mg, 1.0 mmol) in portions over 1 min. The mixture was allowed to warm to room temperature and stirred 2 h, then concentrated under reduced pressure and the residue was purified by preparative-TLC to give tert-butyl (5R)-7-((2S)-1-((((6^3S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-6-oxo-2,7-diazaspiro[4.4]nonane-2-carboxylate (500 mg, 64% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{54}H_{71}N_7O_8$ 945.5; found 946.5.

Step 7.

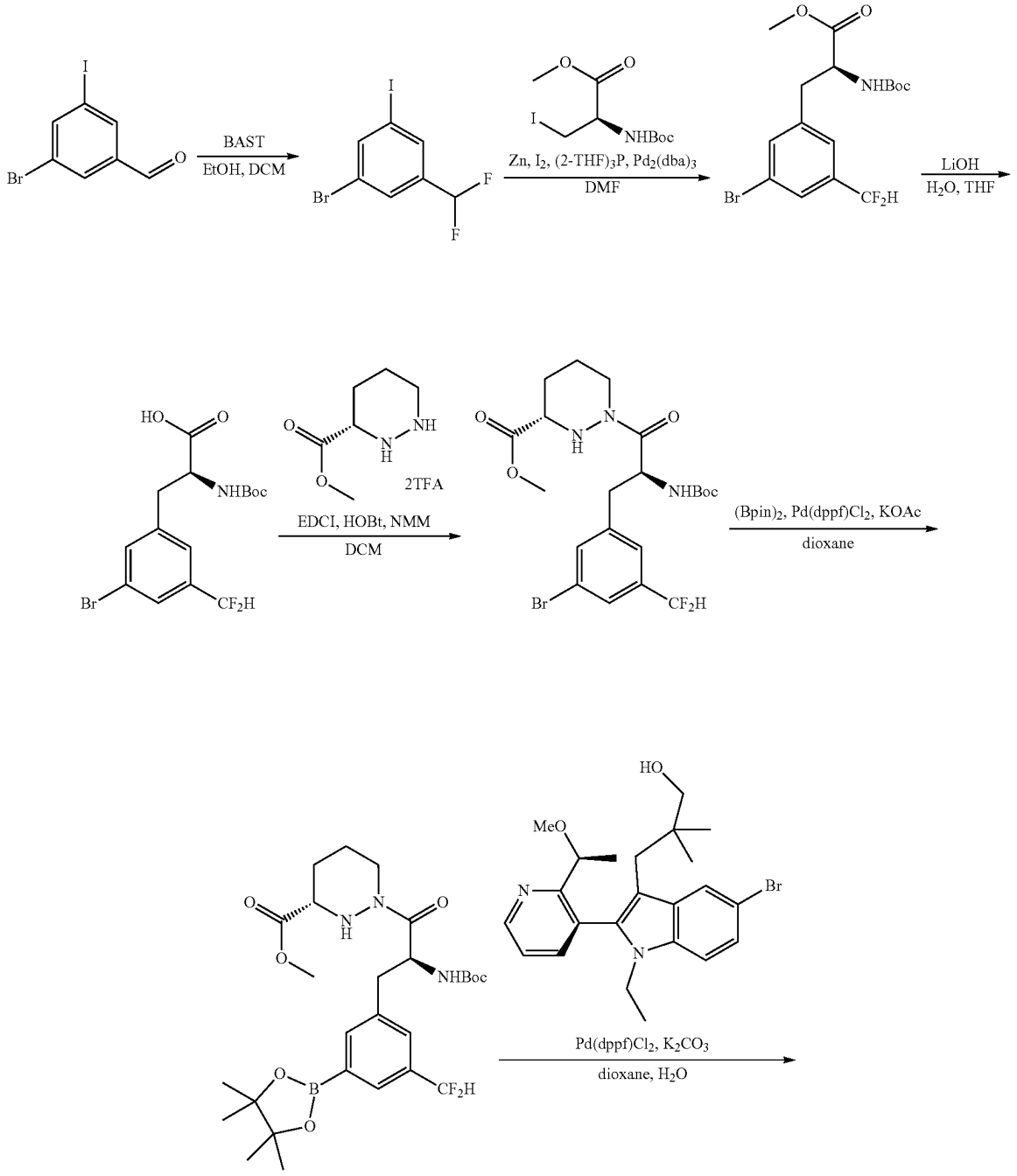
To a mixture of tert-butyl (5R)-7-((2S)-1-((((6^3S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-6-oxo-2,7-diazaspiro[4.4]nonane-2-carboxylate (1.0 g, 1.06 mmol) in DCM (10 mL) at 0° C. was added TFA (3 mL) dropwise. The mixture was warmed to room temperature and stirred for 1 h, then concentrated under reduced pressure to give (2S)—N-((6^3S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methyl-2-((S)-1-oxo-2,7-diazaspiro[4.4]nonan-2-yl)butanamide (1.3 g). LCMS (ESI): m/z [M−H] calc'd for $C_{49}H_{63}N_7O_6$ 846.1; found 845.5.

Step 8.

To a mixture of (2S)—N-((6^3S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methyl-2-((S)-1-oxo-2,7-diazaspiro[4.4]nonan-2-yl)butanamide (500 mg, 0.59 mmol) and DIPEA (764 mg, 5.9 mmol) in DMF (5 mL) at 0° C. were added 4-(dimethylamino)-4-methylpent-2-ynoic acid (110 mg, 0.71 mmol) and HATU (292 mg, 0.77 mmol) in portions. The mixture was warmed to room temperature and stirred for 1 h, then H_2O (10 mL) was added and the mixture extracted with EtOAc (10 mL×3). The combined organic layers were washed with brine (10 mL), dried over anhydrous Na_2SO_4 and filtered.

The filtrate was concentrated under reduced pressure and the residue was purified by preparative-HPLC to give (2S)-2-((S)-7-(4-(dimethylamino)-4-methylpent-2-ynoyl)-1-oxo-2,7-diazaspiro[4.4]nonan-2-yl)-N-((6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1 ^{1}H-8-oxa-1 (5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methylbutanamide (177 mg, 28.94% yield) as a white solid. LCMS (ESI): m/z [M+H] calc'd for $C_{57}H_{74}N_8O_7$ 982.6; found 983.8; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 8.76 (dd, J=4.7, 1.7 Hz, 1H), 8.52 (d, J=7.9 Hz, 1H), 7.99 (d, J=1.7 Hz, 1H), 7.83 (d, J=10.2 Hz, 2H), 7.74-7.58 (m, 3H), 7.53 (dd, J=7.7, 4.8 Hz, 1H), 7.24 (t, J=7.6 Hz, 1H), 7.12 (d, J=7.6 Hz, 1H), 5.32 (d, J=9.7 Hz, 2H), 4.33-4.20 (m, 4H), 4.03 (dd, J=15.0, 8.6 Hz, 2H), 3.88-3.82 (m, 1H), 3.63 (dq, J=20.5, 10.3 Hz, 4H), 3.42-3.34 (m, 2H), 3.21 (s, 1H), 3.13 (d, J=2.8 Hz, 3H), 2.87 (s, 2H), 2.83-2.72 (m, 2H), 2.69-2.62 (m, 1H), 2.21 (d, J=22.6 Hz, 6H), 2.12-1.76 (m, 7H), 1.75-1.47 (m, 2H), 1.46-1.28 (m, 9H), 0.99-0.89 (m, 6H), 0.79-0.71 (m, 6H), 0.52 (s, 3H).

Example A341. Synthesis of (3S)-1-acryloyl-N-((2S)-1-(((6^{3}S,4S)-2^5-(difluoromethyl)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-N-methylpyrrolidine-3-carboxamide



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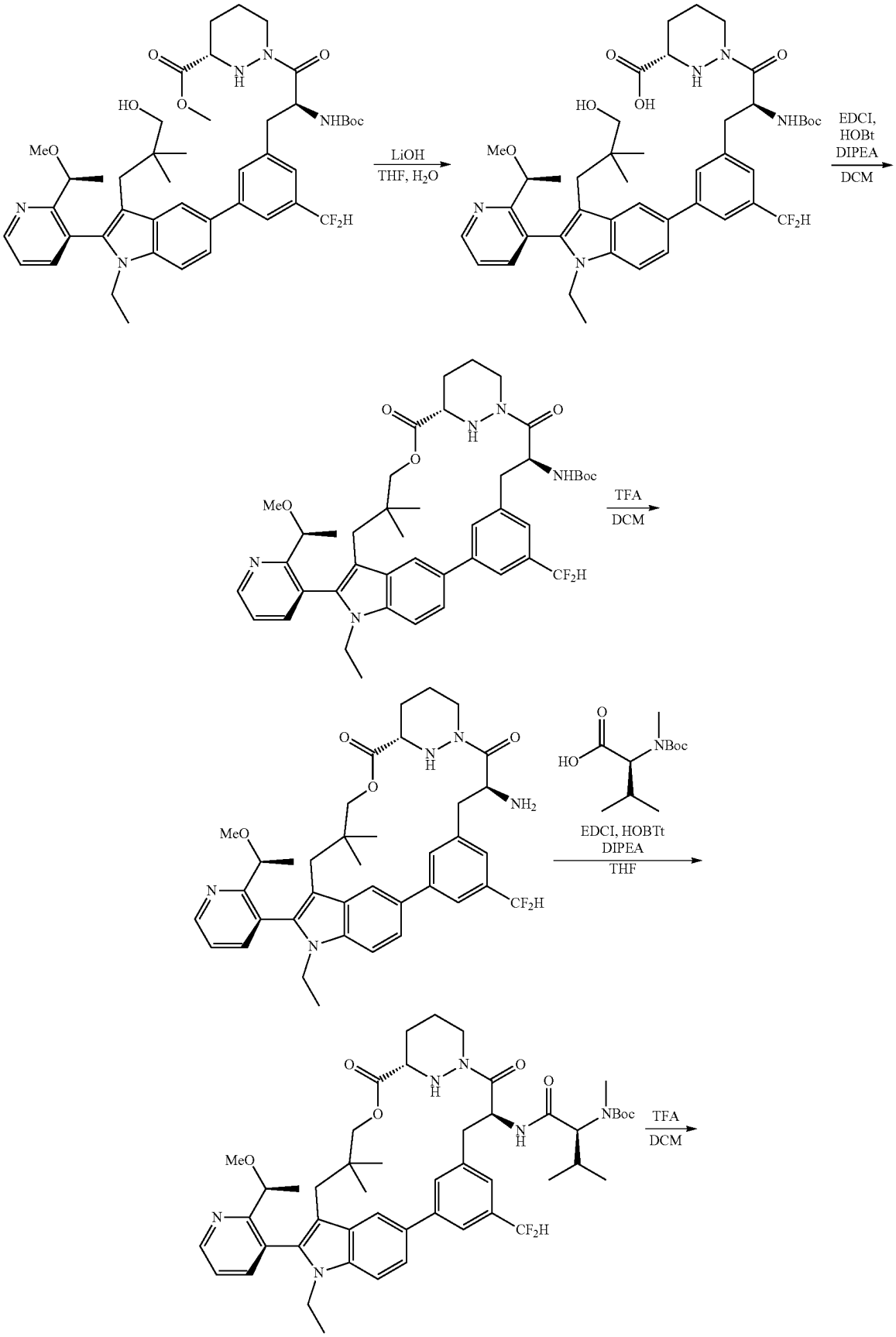
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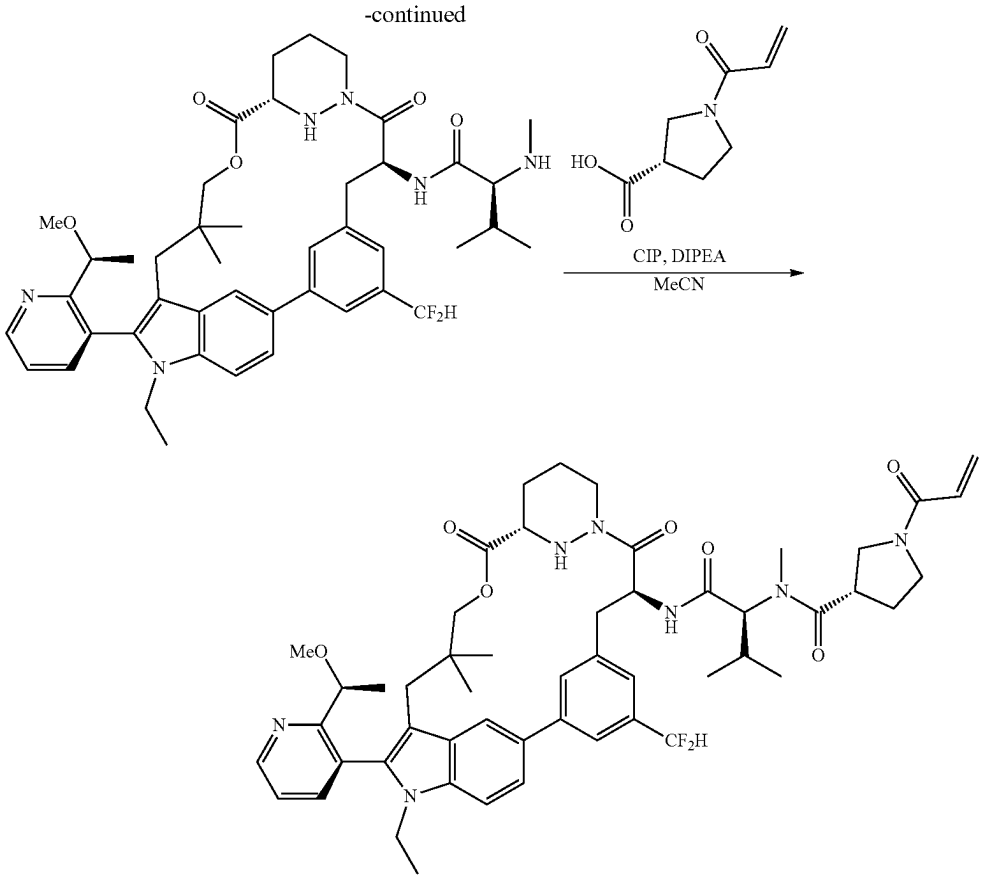


Step 1.

To a mixture of 3-bromo-5-iodobenzaldehyde (4.34 g, 14.0 mmol) in DCM at 0° C. under an atmosphere of N_2 was added BAST (6.8 g, 30.7 mmol) and EtOH (129 mg, 2.8 mmol) dropwise. The mixture was heated with microwave heating at 27° C. for 14 h. H_2O (500 mL) was added and the mixture was extracted with DCM (200 mL×3), the combined organic layers were concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give 1-bromo-3-(difluoromethyl)-5-iodobenzene (3.2 g, 65% yield) as a solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.16 (p, J=1.2 Hz, 1H), 7.94 (p, J=1.3 Hz, 1H), 7.81 (p, J=1.3 Hz, 1H), 7.00 (t, J=55.3 Hz, 1H).

Step 2.

A mixture of Zn (2.28 g, 34.8 mmol) and 12 (442 mg, 1.74 mmol) in DMF (20 mL) under an atmosphere of Ar was stirred at 50° C. for 0.5 h. To this mixture was added a solution of methyl (methyl (R)-2-((tert-butoxycarbonyl) amino)-3-iodopropanoate (2.39 g, 7.25 mmol) in DMF (20 mL) and the mixture was stirred at 50° C. for 2 h. After cooling, the mixture was added to 1-bromo-3-(difluoromethyl)-5-iodobenzene (2.90 g, 8.7 mmol), $Pd_2(dba)_3$ (239 mg, 0.26 mmol) and tri-2-furylphosphine (162 mg, 0.7 mmol) in DMF (20 mL). The mixture was heated to 70° C. and stirred for 2 h, then H_2O (200 mL) was added and the mixture extracted with EtOAc (200 mL×3). The combined organic layers were concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl (S)-3-(3-bromo-5-(difluoromethyl) phenyl)-2-((tert-butoxycarbonyl)amino)propanoate (560 mg, 19% yield) as a solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.65 (d, J=10.0 Hz, 2H), 7.47 (s, 1H), 7.36 (d, J=8.4 Hz, 1H), 7.00 (t, J=55.6 Hz, 1H), 4.25 (td, J=9.6, 4.7 Hz, 1H), 3.64 (s, 3H), 3.11 (dd, J=13.6, 4.9 Hz, 1H), 3.00-2.80 (m, 1H), 1.32 (s, 9H).

Step 3.

To a mixture of methyl (S)-3-(3-bromo-5-(difluoromethyl)phenyl)-2-((tert-butoxycarbonyl)amino)propanoate (650 mg, 1.6 mmol) in THF (1.5 mL) at 0° C. under an atmosphere of N_2 was added LiOH (114 mg, 4.8 mmol) in H_2O (1.50 mL). The mixture was stirred at 0° C. for 1 h, then acidified to pH 5 with 1M HCl. The mixture was extracted with DCM/MeOH (10/1) (100 mL×3) and the combined organic layers were dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to give (S)-3-(3-bromo-5-(difluoromethyl)phenyl)-2-((tert-butoxycarbonyl)amino)propanoic acid (500 mg), which was used directly in the next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{15}H_{18}BrF_2NO_4$ 393.0; found 392.1.

Step 4.

To a mixture of methyl (3S)-1,2-diazinane-3-carboxylate (475 mg, 3.3 mmol) in DCM (10 mL) at 0° C. under an atmosphere of N_2 were added N-methylmorpholine (3.34 g, 33.0 mmol) and (S)-3-(3-bromo-5-(difluoromethyl)phenyl)-2-((tert-butoxycarbonyl)amino)propanoic acid (650 mg, 1.7 mmol) and HOBt (45 mg, 0.33 mmol) and EDCI (632 mg, 3.3 mmol). The mixture was warmed to room temperature and stirred for 16 h, then diluted with DCM (100 mL) and H_2O. The organic and aqueous layer was separated and the aqueous layer was extracted with DCM (100 mL×3). The combined organic layers were dried over anhydrous Na_2SO_4

and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl (S)-1-((S)-3-(3-bromo-5-(difluoromethyl)phenyl)-2-((tert-butoxycarbonyl)amino) propanoyl)hexahydropyridazine-3-carboxylate (510 mg, 56% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{21}H_{28}BrF_2N_3O_5$ 519.1; found 520.3.

Step 5.

To a mixture of 4,4,5,5-tetramethyl-2-(tetramethyl-1,3,2-dioxaborolan-2-yl)-1,3,2-dioxaborolane (488 mg, 1.92 mmol) and methyl (S)-1-((S)-3-(3-bromo-5-(difluoromethyl)phenyl)-2-((tert-butoxycarbonyl)amino)propanoyl) hexahydropyridazine-3-carboxylate (500 mg, 0.96 mmol) in 1,4-dioxane (5 mL) was added Pd(dppf)Cl_2 (70 mg, 0.07 mmol) and KOAc (236 mg, 2.4 mmol) in portions. The mixture was heated to 90° C. and stirred for 4 h then diluted with H_2O (100 mL). The mixture was extracted with DCM (100 mL×3) and the combined organic layers were dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl (S)-1-((S)-2-((tert-butoxycarbonyl)amino)-3-(3-(difluoromethyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)propanoyl)hexahydropyridazine-3-carboxylate (423 mg, 73% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{27}H_{40}BF_2N_3O_7$ 567.3; found 568.2.

Step 6.

To a mixture of methyl (S)-1-((S)-2-((tert-butoxycarbonyl)amino)-3-(3-(difluoromethyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)propanoyl)hexahydropyridazine-3-carboxylate (260 mg, 0.47 mmol), (S)-3-(5-bromo-1-ethyl-2-(2-(1-methoxyethyl)pyridin-3-yl)-1H-indol-3-yl)-2,2-dimethylpropan-1-ol and Pd(dppf)Cl_2 (34 mg, 0.05 mmol) in 1,4-dioxane (3 mL) and H_2O (0.6 mL) was added K_2CO_3 (163 mg, 1.12 mmol). The mixture was heated to 60° C. and stirred for 16 h, then diluted with H_2O (100 mL) and extracted with DCM (100 mL×3). The combined organic layers were dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl (S)-1-((S)-2-((tert-butoxycarbonyl)amino)-3-(3-(difluoromethyl)-5-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1H-indol-5-yl)phenyl)propanoyl) hexahydropyridazine-3-carboxylate (350 mg, 78% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{44}H_{57}F_2N_5O_7$ 805.4; found 806.6.

Step 7.

To a mixture of methyl (S)-1-((S)-2-((tert-butoxycarbonyl)amino)-3-(3-(difluoromethyl)-5-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1H-indol-5-yl)phenyl)propanoyl)hexahydropyridazine-3-carboxylate (350 mg, 0.43 mmol) in THF (2.8 mL) at 0° C. was added LiOH H_2O (54 mg, 1.3 mmol) in H_2O (0.7 mL). The mixture was warmed to room temperature and stirred for 2 h, then acidified to pH 5 with 1M HCl and extracted with EtOAc (50 mL×3). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure to give (S)-1-((S)-2-((tert-butoxycarbonyl)amino)-3-(3-(difluoromethyl)-5-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1H-indol-5-yl)phenyl) propanoyl)hexahydropyridazine-3-carboxylic acid (356 mg) was used directly in the next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{43}H_{55}F_2N_5O_7$ 791.4; found 792.6.

Step 8.

To a mixture of S)-1-((S)-2-((tert-butoxycarbonyl)amino)-3-(3-(difluoromethyl)-5-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1H-indol-5-yl)phenyl)propanoyl)hexahydropyridazine-3-carboxylic acid (356 mg, 0.45 mmol) and DIPEA (1.74 g, 13.5 mmol) in DCM were added EDCI (2.41 g, 12.6 mmol) and HOBt (304 mg, 2.3 mmol). The mixture was stirred for 16 h then H_2O was added and the mixture extracted with EtOAc (200 mL×3). The combined organic layers were washed with brine (50 mL×4), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give tert-butyl ((6^{3}S,4S)-2^5-(difluoromethyl)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (202 mg, 51% yield). LCMS (ESI): m/z [M+H] calc'd for $C_{43}H_{53}F_2N_5O_6$ 773.4; found 774.6.

Step 9.

To a mixture of tert-butyl ((6^{3}S,4S)-2^5-(difluoromethyl)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (202 mg, 0.26 mmol) in DCM (2 mL) at 0° C. was added TFA (1.0 mL) dropwise. The mixture was stirred at 0° C. for 1.5 h, then concentrated under reduced pressure and dried azeotropically with toluene (3 mL×3) to give (6^{3}S,4S)-4-amino-2^5-(difluoromethyl)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione, which was used directly in the next without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{38}H_{45}F_2N_5O_4$ 673.3; found 674.5.

Step 10.

To a mixture of (6^{3}S,4S)-4-amino-2^5-(difluoromethyl)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (202 mg, 0.3 mmol) and (2S)-2-[(tert-butoxycarbonyl)(methyl)amino]-3-methylbutanoic acid (139 mg, 0.6 mmol) in THF under an atmosphere of Ar were added DIPEA (581 mg, 4.5 mmol), EDCI (86 mg, 0.45 mmol) and HOBt (61 mg, 0.45 mmol). The mixture was stirred for 16 h, then H_2O (100 mL) added and the mixture extracted with EtOAc (200 mL×3). The combined organic layers were washed with brine (50 mL×3), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give tert-butyl ((2S)-1-(((6^{3}S,4S)-2^5-(difluoromethyl)-11-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate (135 mg, 46% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{49}H_{64}F_2N_6O_7$ 886.5; found 887.6.

Step 11.

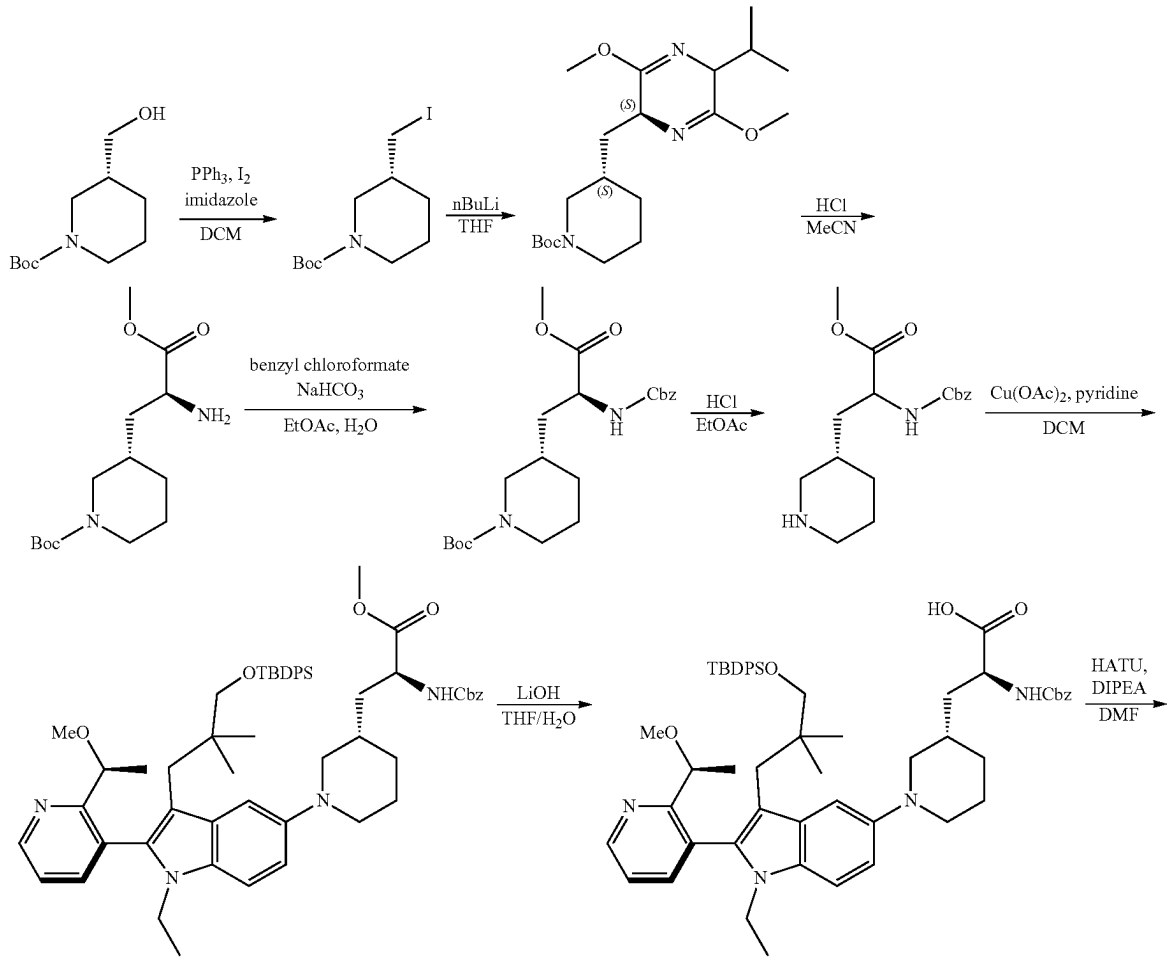
To a mixture of tert-butyl ((2S)-1-(((6^{3}S,4S)-2^5-(difluoromethyl)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate (130 mg, 0.15 mmol) in DCM at 0° C. under an atmosphere of N_2 was added TFA

(1.0 mL) dropwise. The mixture was stirred at 0° C. for 1.5 h, then concentrated under reduced pressure and dried azeotropically with toluene (3 mL×3) to give (2S)—N-((6^{3}S,4S)-2^5-(difluoromethyl)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methyl-2-(methylamino)butanamide (130 mg), which was used directly in the next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{44}H_{56}F_2N_6O_5$ 786.4; found 787.6.

Step 12.

To a mixture of (2S)—N-((6^{3}S,4S)-2^5-(difluoromethyl)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methyl-2-(methylamino)butanamide (130 mg, 0.17 mmol) and (3S)-1-(prop-2-enoyl)pyrrolidine-3-carboxylic acid (56 mg, 0.33 mmol) in MeCN (1.5 mL) at 0° C. under an atmosphere of N_2 were added DIPEA (427 mg, 3.3 mmol) and CIP (69 mg, 0.25 mmol). The mixture was stirred at 0° C. for 1 h, then H_2O (100 mL) was added and the mixture extracted with EtOAc (200 mL×3). The combined organic layers were washed with brine (50 mL×3), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-HPLC to give (3S)-1-acryloyl-N-((2S)-1-(((6^{3}S,4S)-2^5-(difluoromethyl)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-N-methylpyrrolidine-3-carboxamide (58 mg, 36% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{52}H_{65}F_2N_7O_7$ 937.4; found 938.1; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 8.78 (dd, J=4.8, 1.7 Hz, 1H), 8.43-8.21 (m, 1H), 8.02 (s, 2H), 7.93-7.81 (m, 2H), 7.76 (dd, J=9.3, 3.9 Hz, 1H), 7.66 (d, J=8.7 Hz, 1H), 7.56 (dd, J=7.7, 4.8 Hz, 1H), 7.34 (d, J=5.4 Hz, 1H), 7.20-6.86 (m, 1H), 6.80-6.40 (m, 1H), 6.15 (ddt, J=16.8, 4.9, 2.4 Hz, 1H), 5.90-5.60 (m, 1H), 5.59-5.19 (m, 2H), 4.71 (dd, J=10.7, 3.1 Hz, 1H), 4.40-4.17 (m, 3H), 4.12-3.90 (m, 3H), 3.85-3.71 (m, 1H), 3.61 (tdd, J=23.4, 9.9, 4.3 Hz, 6H), 3.40-3.30 (m, 2H), 3.11 (d, J=6.8 Hz, 3H), 3.08-2.90 (m, 2H), 2.87 (s, 2H), 2.84 (s, 3H), 2.69-2.30 (d, J=16.5 Hz, 1H), 2.30-1.79 (m, 5H), 1.75-1.45 (m, 2H), 1.40 (d, J=6.1 Hz, 3H), 1.05-0.85 (m, 6H), 0.85-0.66 (m, 6H), 0.57 (d, J=11.8 Hz, 3H).

Example A741. Synthesis of (3S)-1-acryloyl-N-((2S)-1-(((2^{3}S,6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-piperidinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-N-methylpyrrolidine-3-carboxamide



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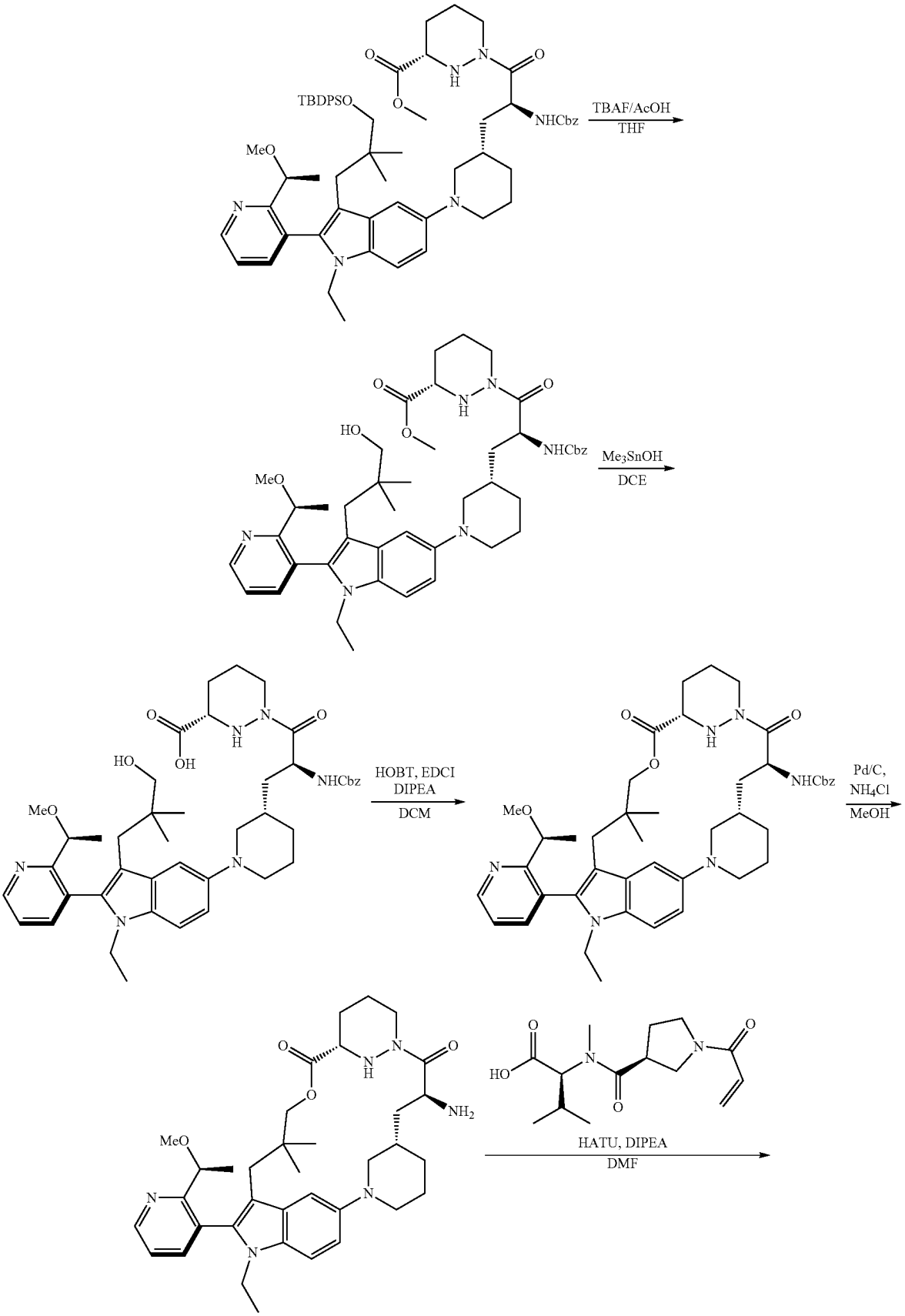
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THF
HO
Me3SnOH
DCE
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NH4Cl
MeOH
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HATU, DIPEA
DMF

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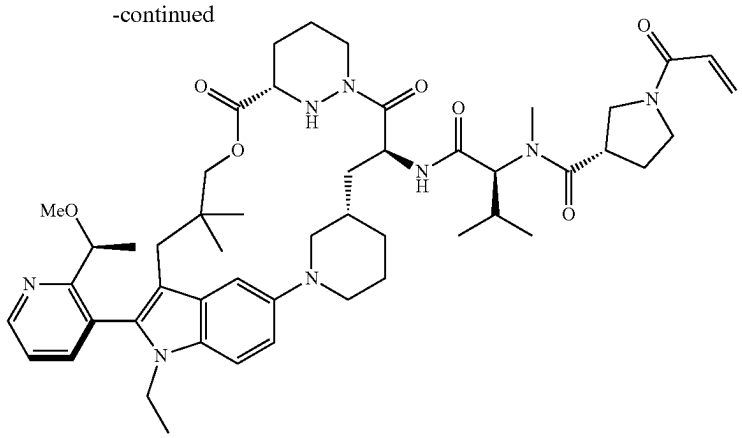


Step 1.

A solution of tert-butyl (3R)-3-(hydroxymethyl) piperidine-1-carboxylate (10 g, 46.45 mmol) in DCM (200 mL) at 0° C., was added PPh_3 (15.8 g, 60.4 mmol), Imidazole (4.7 g, 69.7 mmol) and I_2 (14.1 g, 55.74 mmol). The reaction suspension was stirred at 20° C. for 17 h, then concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford tert-butyl (3R)-3-(iodomethyl) piperidine-1-carboxylate (10 g, 66% yield) as oil. LCMS (ESI): m/z [M+H] calc'd for $C_{11}H_{20}INO_2$ 325.1; found no mass.

Step 2.

To a mixture of 3-isopropyl-2,5-dimethoxy-3,6-dihydropyrazine (10.8 g, 58.9 mmol) in THF (150 mL) at −60° C. under an atmosphere of N_2 was added n-BuLi (47 mL, 2.5 M in hexane, 117.7 mmol) dropwise. The mixture was warmed to 0° C. and was stirred for 2 h, then re-cooled to −60° C., and a solution of tert-butyl (3R)-3-(iodomethyl) piperidin-1-yl formate (9.60 g, 29.4 mmol) in THF (50 mL) was slowly added dropwise. The mixture was stirred at −60° C. for 2 h then warmed to room temperature and stirred for 2 h. Saturated NH_4Cl (150 mL) was slowly added and the mixture extracted with EtOAc (150 mL×2). The combined organic layers were washed with brine (200 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was reduced under reduced pressure and the residue was purified by silica gel column chromatography to give tert-butyl (3S)-3-{[(2S)-5-isopropyl-3,6-dimethoxy-2,5-dihydropyrazin-2-yl] methyl}piperidin-1-yl formate (5.3 g, 46% yield) as a gum. LCMS (ESI): m/z [M+H] calc'd for $C_{20}H_{35}N_3O_4$ 381.5; found 382.3.

Step 3.

A mixture of tert-butyl (3S)-3-{[(2S)-5-isopropyl-3,6-dimethoxy-2,5-dihydropyrazin-2-yl]methyl}piperidin-1-yl formate (5.30 g, 13.9 mol) in MeCN (4 mL) was added 1M HCl (27.7 mL, 27.7 mmol) dropwise. The mixture was stirred for 2 h, then saturated $NaHCO_3$ until ~pH 7-8, then extracted with DCM (30 mL×2). The combined organic layers was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give methyl (S)-tert-butyl 3-((S)-2-amino-3-methoxy-3-oxopropyl)piperidine-1-carboxylate (4.3 g, 95% yield) as an oil, which was used in next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{14}H_{26}N_2O_4$ 286.2; found 287.3.

Step 4.

To a mixture of methyl (S)-tert-butyl 3-((S)-2-amino-3-methoxy-3-oxopropyl)piperidine-1-carboxylate (4.30 g, 15.0 mmol) in EtOAc (30 mL) and H_2O (20 mL) at −10° C. was added $NaHCO_3$ (3.77 g, 44.88 mmol). The mixture was stirred at −10° C. for 10 min, then a solution of benzyl chloroformate (3.83 g, 22.44 mmol) was added dropwise. The mixture was warmed to 0° C. and stirred for 1 h, then H_2O (50 mL) was added and the mixture extracted with EtOAc (50 mL×2). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, the filtrate was concentrated under reduced pressure to give tert-butyl (3S)-3-[(2S)-2-{[(benzyloxy)carbonyl] amino}-3-methoxy-3-oxopropyl] piperidine-1-carboxylate (4.0 g, 60% yield) as a gum. LCMS (ESI): m/z [M−Boc+H] calc'd for $C_{17}H_{24}N_2O_4$ 320.2; found 321.3.

Step 5.

To a mixture of tert-butyl (3S)-3-[(2S)-2-{[(benzyloxy) carbonyl] amino}-3-methoxy-3-oxopropyl]piperidine-1-carboxylate (1.0 g, 2.38 mmol) in EtOAc (8 mL) was added 2M HCl in EtOAc (11.9 mL, 23.8 mmol). The mixture was stirred for 2 h, then saturated $NaHCO_3$ added until ~pH 8-9, and the mixture extracted with DCM (30 mL×3). The combined organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give (2S)-2-{[(benzyloxy)carbonyl]amino}-3-[(3S)-piperidin-3-yl]propanoate (740 mg, 91% yield) as a gum. LCMS (ESI): m/z [M+H] calc'd for $C_{17}H_{24}N_2O_4$ 320.2; found 321.2.

Step 6.

To a mixture of (3-{3-[(tert-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl}-1-ethyl-2-{2-[(1 S)-1-methoxyethyl]pyridin-3-yl}indol-5-yl)boranediol (5.47 g, 8.43 mmol) and methyl (2S)-2-{[(benzyloxy)carbonyl]amino}-3-[(3S)-piperidin-3-yl]propanoate (2.70 g, 8.43 mmol) in DCM (70 mL) was added $Cu(OAc)_2$ (6.06 g, 16.86 mmol) and pyridine (2.0 g, 25.3 mmol). The mixture was stirred under an atmosphere of 02 for 48 h, then diluted with DCM (200 mL) and washed with H_2O (150 mL×2). The organic layer was dried over anhydrous Na_2SO_4, filtered, concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl 2-{[(benzyloxy) carbonyl]amino}-3-[(3S)-1-(3-{3-[(tert-butyldiphenylsilyl) oxy]-2,2-dimethylpropyl}-1-ethyl-2-{2-[(1 S)-1-methoxyethyl]pyridin-3-yl}indol-5-yl)piperidin-3-yl]propanoate (3.6 g, 42% yield) as a solid. LCMS (ESI): m/z [M/2+H] calc'd for $C_{56}H_{70}N_4O_6Si$ 462.3; found 462.3.

Step 7.

To a mixture of methyl 2-{[(benzyloxy)carbonyl]amino}-3-[(3S)-1-(3-{3-[(tert-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl}-1-ethyl-2-{2-[(1 S)-1-methoxyethyl]pyridin-3-

yl}indol-5-yl)piperidin-3-yl]propanoate (3.60 g, 3.57 mmol) in THF (60 mL) and H_2O (30 mL) was added LiOH (342 mg, 14.28 mmol). The mixture was stirred for 2 h, then diluted with H_2O (150 mL), then 1M HCl was added slowly until ~pH 3-4 and the mixture extracted with EtOAc (200 mL×3). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and the filtrate concentrated under reduced pressure to give 2-{[(benzyloxy)carbonyl]amino}-3-[(3S)-1-(3-{3-[(tert-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl}-1-ethyl-2-{2-[(1 S)-1-methoxyethyl]pyridin-3-yl}indol-5-yl)piperidin-3-yl]propanoic acid (3.3 g, 85% yield) as a solid, which was used directly in the next step without further purification. LCMS (ESI): m/z [M/2+H] calc'd for $C_{55}H_{68}N_4O_6Si$ 455.3; found 455.3.

Step 8.

To a mixture of methyl 2-{[(benzyloxy)carbonyl]amino}-3-[(3S)-1-(3-{3-[(tert-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl}-1-ethyl-2-{2-[(1 S)-1-methoxyethyl]pyridin-3-yl}indol-5-yl)piperidin-3-yl]propanoate (3.30 g, 2.91 mmol) in DMF (40 mL) was added methyl (3S)-1,2-diazinane-3-carboxylate (0.42 g, 2.91 mmol), HATU (2.21 g, 5.82 mmol) and DIPEA (2.26 g, 17.46 mmol). The mixture was stirred for 3 h, then poured into ice-H_2O and extracted with EtOAc (120 mL×2). The combined organic layers were washed with saturated $NaHCO_3$ (150 mL), brine (150 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl (3S)-1-[(2S)-2-{[(benzyloxy)carbonyl]amino}-3-[(3S)-1-(3-{3-[(tert-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl}-1-ethyl-2-{2-[(1 S)-1-methoxyethyl]pyridin-3-yl}indol-5-yl)piperidin-3-yl]propanoyl]-1,2-diazinane-3-carboxylate (2.9 g, 95% yield) as a gum. LCMS (ESI): m/z [M/2+H] calc'd for $C_{61}H_{78}N_6O_7Si$ 518.3; found 518.3.

Step 9.

To a mixture of methyl (3S)-1-[(2S)-2-{[(benzyloxy)carbonyl]amino}-3-[(3S)-1-(3-{3-[(tert-butyldiphenylsilyl)oxy]-2,2-dimethylpropyl}-1-ethyl-2-{2-[(1 S)-1-methoxyethyl]pyridin-3-yl}indol-5-yl)piperidin-3-yl]propanoyl]-1,2-diazinane-3-carboxylate (1.70 g, 1.64 mmol) was added a mixture of 1M TBAF in THF (19.68 mL, 19.68 mmol) and AcOH (1.18 g, 19.68 mmol). The reaction was heated to 60° C. and stirred for 22 h, then diluted with EtOAc (80 mL) and washed with saturated $NaHCO_3$ (80 mL), H_2O (60 mL×2) and brine (60 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, the filtrate was concentrated under reduced pressure and the residue was purified by preparative-HPLC to give methyl (3S)-1-[(2S)-2-{[(benzyloxy)carbonyl]amino}-3-[(3S)-1-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-{2-[(1 S)-1-methoxyethyl]pyridin-3-yl}indol-5-yl]piperidin-3-yl]propanoyl]-1,2-diazinane-3-carboxylate (1.0 g, 73% yield) as a solid. LCMS (ESI): m/z [M/2+H] calc'd for $C_{45}H_{60}N_6O_7$ 399.2; found 399.4.

Step 10.

To a mixture m methyl (3S)-1-[(2S)-2-{[(benzyloxy)carbonyl]amino}-3-[(3S)-1-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-{2-[(1 S)-1-methoxyethyl]pyridin-3-yl}indol-5-yl]piperidin-3-yl]propanoyl]-1,2-diazinane-3-carboxylate (1.0 g, 1.1 mmol) in 1,2-dichloroethane (10 mL) was added Me_3SnOH (1.42 g 7.84 mmol). The mixture was heated to 65° C. and stirred for 10 h, then filtered and the filtrate was concentrated under reduced pressure to give (3S)-1-[(2S)-2-{[(benzyloxy)carbonyl]amino}-3-[(3S)-1-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-{2-[(1 S)-1-methoxyethyl]pyridin-3-yl}indol-5-yl]piperidin-3-yl]propanoyl]-1,2-diazinane-3-carboxylic acid (1.0 g, 99% yield) as a gum. The product was used in the next step without further purification. LCMS (ESI): m/z [M/2+H] calc'd for $C_{44}H_{58}N_6O_7$ 392.2; found 392.3.

Step 11.

To a mixture of (3S)-1-[(2S)-2-{[(benzyloxy)carbonyl]amino}-3-[(3S)-1-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-{2-[(1 S)-1-methoxyethyl]pyridin-3-yl}indol-5-yl]piperidin-3-yl]propanoyl]-1,2-diazinane-3-carboxylic acid (1.0 g, 1.1 mmol) in DCM (30 mL) at 0° C. was added HOBT (1.51 g, 11.2 mmol), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide HCl (6.44 g, 33.6 mmol) and DIPEA (5.79 g, 44.8 mmol). The mixture was warmed to room temperature and stirred for 6 h, then diluted with H_2O and extracted with EtOAc (100 mL×3). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, the filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give benzyl N-[(6S,8S,14S)-22-ethyl-21-{2-[(1S)-1-methoxyethyl]pyridin-3-yl}-18,18-dimethyl-9,15-dioxo-16-oxa-2,10,22,28-tetraazapentacyclo [18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),20,23(27),24-tetraen-8-yl]carbamate (340 mg, 36% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{44}H_{56}N_6O_6$ 383.2; found 383.3.

Step 12.

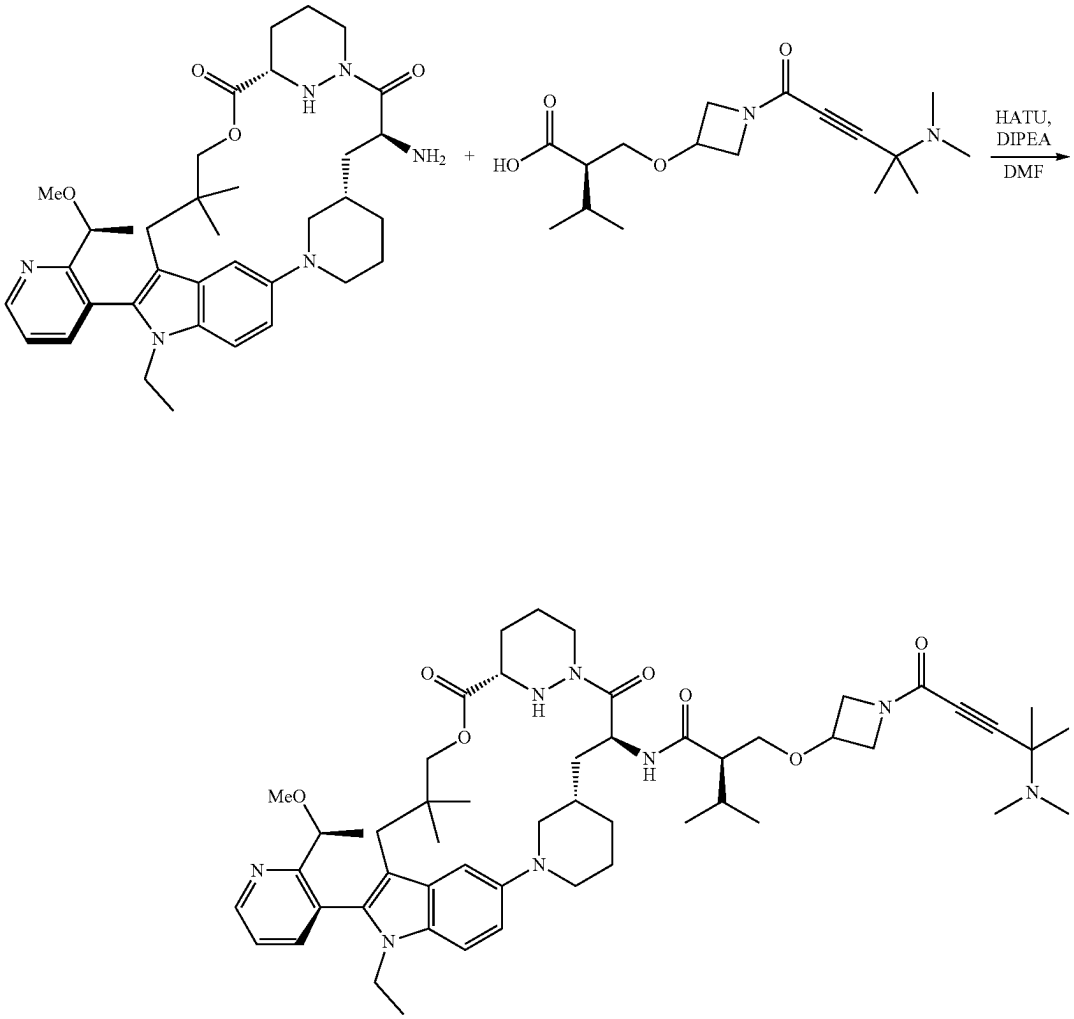
A mixture of benzyl N-[(6S,8S,14S)-22-ethyl-21-{2-[(1 S)-1-methoxyethyl]pyridin-3-yl}-18,18-dimethyl-9,15-dioxo-16-oxa-2,10,22,28-tetraazapentacyclo [18.5.2.1^{2,6}.1^{10, 14}.0^{23,27}]nonacosa-1(26),20,23(27),24-tetraen-8-yl]carbamate (250 mg, 0.33 mmol), Pd/C (100 mg) and NH_4Cl (353 mg, 6.6 mmol) in MeOH (5 mL) was stirred under an atmosphere of H_2 for 4 h. The mixture was filtered through Celite and the filtrate was concentrated under reduced pressure. The residue was dissolved in DCM (30 mL) and washed with saturated $NaHCO_3$ (20 mL), H_2O (20 mL) and brine (20 mL). The organic layer was dried over Na_2SO_4, filtered, and the filtrate concentrated under reduced pressure to give (6S,8S,14S)-8-amino-22-ethyl-21-{2-[(1 S)-1-methoxyethyl]pyridin-3-yl}-18,18-dimethyl-16-oxa-2,10,22,28-tetraazapentacyclo[18.5.2.1{2,6}.1{10,14}.0{23,27}]nonacosa-1(26),20,23(27),24-tetraene-9,15-dione, which was used in next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{36}H_{50}N_6O_4$ 631.4; found 631.4.

Step 13.

To a mixture of (6S,8S,14S)-8-amino-22-ethyl-21-{2-[(1S)-1-methoxyethyl]pyridin-3-yl}-18,18-dimethyl-16-oxa-2,10,22,28-tetraazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),20,23(27),24-tetraene-9,15-dione (300 mg, 0.48 mmol), (2S)-3-methyl-2-{N-methyl-1-[(3S)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido}butanoic acid (136 mg, 0.48 mmol) and DIPEA (620 mg, 4.8 mmol) in DMF (5 mL) at 0° C. was added HATU (183 mg, 0.48 mmol). The mixture was stirred at 0-5° C. for 1 h, then diluted with EtOAc (50 mL), washed with H_2O (50 mL×2), brine (50 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give (2S)—N-[(6S,8S,14S)-22-ethyl-21-{2-[(1 S)-1-methoxyethyl]pyridin-3-yl}-18,18-dimethyl-9,15-dioxo-16-oxa-2,10,22,28-tetraazapentacyclo[18.5.2.1^{2,6}.1^{10,14}.0^{23,27}]nonacosa-1(26),20,23(27),24-tetraen-8-yl]-3-methyl-2-{N-methyl-1-[(3S)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido}butanamide (90 mg, 20% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{50}H_{70}N_8O_7$ 895.5; found 895.4; ^1H NMR (400 MHz, CD_3OD) δ 8.71 (dd, J=4.8, 1.5 Hz, 1H), 7.86 (dd, J=7.7, 1.5 Hz, 1H), 7.51 (dd, J=7.7, 4.8 Hz, 1H), 7.36 (dd, J=8.9, 1.9 Hz, 1H), 7.22 (d, J=12.1 Hz, 1H), 7.09 (dd, J=8.9, 1.9 Hz, 1H), 6.59 (dt,

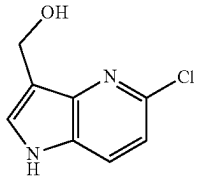
J=16.9, 9.9 Hz, 1H), 6.26 (ddd, J=16.8, 5.0, 1.9 Hz, 1H), 5.80-5.67 (m, 1H), 5.59-5.46 (m, 1H), 4.93 (d, J=12.4 Hz, 1H), 4.66 (dd, J=11.1, 6.4 Hz, 1H), 4.45 (d, J=12.6 Hz, 1H), 4.28-4.19 (m, 1H), 4.13 (dd, J=14.5, 7.2 Hz, 1H), 4.02-3.87 (m, 1H), 3.87-3.36 (m, 11H), 3.16 (s, 2H), 3.10 (d, J=3.4 Hz, 2H), 2.76 (dd, J=26.9, 13.5 Hz, 3H), 2.61 (s, 1H), 2.35-1.97 (m, 5H), 1.78 (dd, J=25.4, 22.1 Hz, 10H), 1.45 (d, J=6.2 Hz, 3H), 1.04 (d, J=6.2 Hz, 3H), 0.95 (dd, J=6.5, 1.8 Hz, 3H), 0.83 (d, J=6.6 Hz, 3H), 0.72 (d, J=31.8 Hz, 6H).

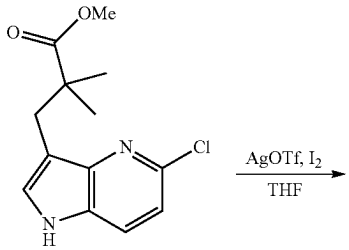
Example A715. Synthesis of benzyl ((2^{3}S,6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-piperidinacycloundecaphane-4-yl)carbamate

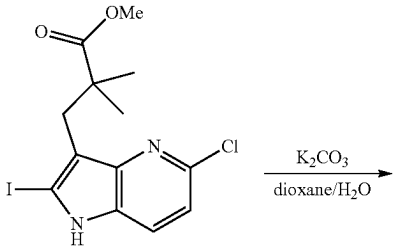


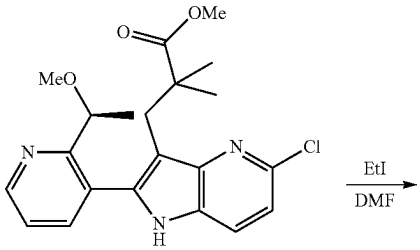
To a solution of ((2^{3}S,6^{3}S,4S)-4-amino-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-piperidinacycloundecaphane-5,7-dione (50 mg, 0.08 mmol), (R)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-3-methylbutanoic acid (26 mg, 0.08 mmol) and DIPEA (31 mg, 0.24 mmol) in DMF (1 mL) at 0° C., was added HATU (30 mg, 0.08 mmol). The reaction mixture was stirred at 0-5° C. for 1 h, then diluted with EtOAc (20 mL), washed with H_2O (20 mL×2) and brine (20 mL). The organic phase was separated and dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give (2R)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-N-((2^{3}S,6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-piperidinacycloundecaphane-4-yl)-3-methylbutanamide as solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.71 (d, J=4.7 Hz, 1H), 8.24 (s, 1H), 8.10 (dd, J=26.7, 7.8 Hz, 1H), 7.86 (d, J=7.7 Hz, 1H), 7.51 (dd, J=7.7, 4.8 Hz, 1H), 7.39 (dd, J=8.9, 3.1 Hz, 1H), 7.26 (d, J=16.6 Hz, 1H), 7.11 (d, J=8.8 Hz, 1H), 5.61 (s, 1H), 4.50-4.28 (m, 3H), 4.27-4.07 (m, 3H), 3.98 (ddd, J=25.6, 13.4, 5.1 Hz, 2H), 3.84-3.72 (m, 2H), 3.62 (dd, J=10.7, 4.8 Hz, 2H), 3.55 (d, J=7.1 Hz, 2H), 3.47 (d, J=6.5 Hz, 2H), 3.16 (s, 3H), 3.03-2.91 (m, 1H), 2.76 (dd, J=28.7, 15.2 Hz, 3H), 2.62 (s, 1H), 2.40 (t, J=7.0 Hz, 3H), 2.33 (dd, J=14.3, 5.0 Hz, 4H), 2.05 (d, J=11.6 Hz, 1H), 1.99-1.64 (m, 10H), 1.64-1.55 (m, 1H), 1.51-1.42 (m, 6H), 1.37 (d, J=12.3 Hz, 3H), 1.05 (s, 3H), 0.94 (ddd, J=9.3, 6.7, 2.0 Hz, 6H), 0.76 (d, J=3.8 Hz, 3H), 0.69 (s, 3H). LCMS (ESI): m/z [M+H] calc'd for $C_{53}H_{76}N_8O_7$ 936.6; found 937.4.

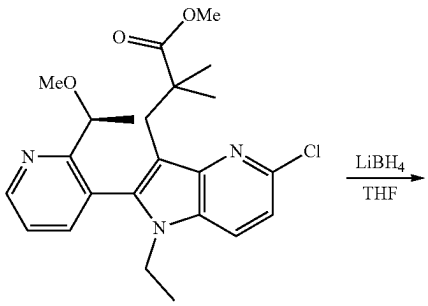
Example A347. Synthesis of (2S)—N-[(7S,13S)-21-ethyl-20-{2-[(1S)-1-methoxyethyl]pyridin-3-yl}-17,17-dimethyl-8,14-dioxo-15-oxa-4-thia-9,21,25,27,28-pentaazapentacyclo[17.5.2.1^{2,5}.1^{9,13}.0^{22,26}]octacosa-1(25),2,5(28),19,22(26),23-hexaen-7-yl]-3-methyl-2-{N-methyl-1-[(3S)-1-(prop-2-enoyl)pyrrolidin-3-yl]formamido}butanamide











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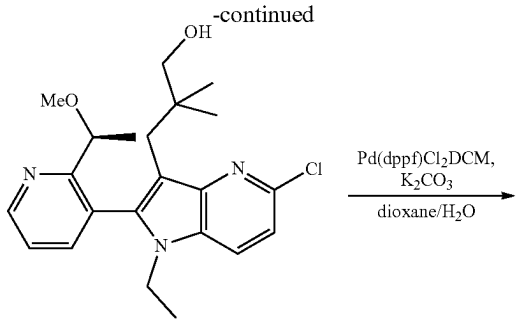

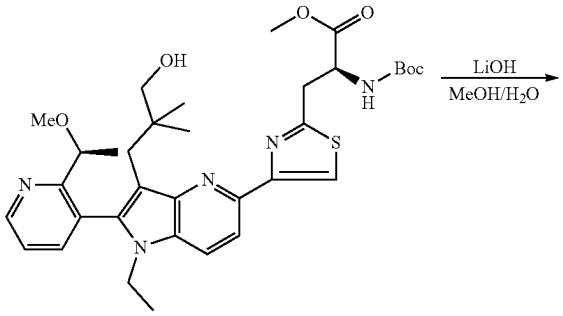

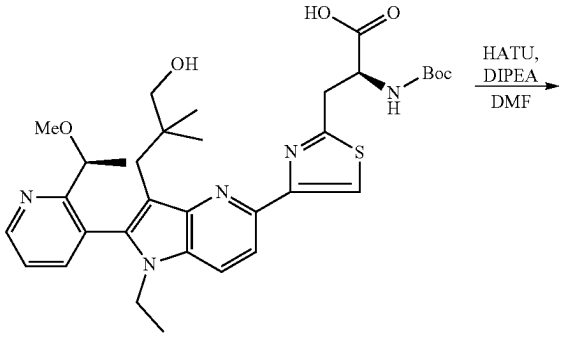

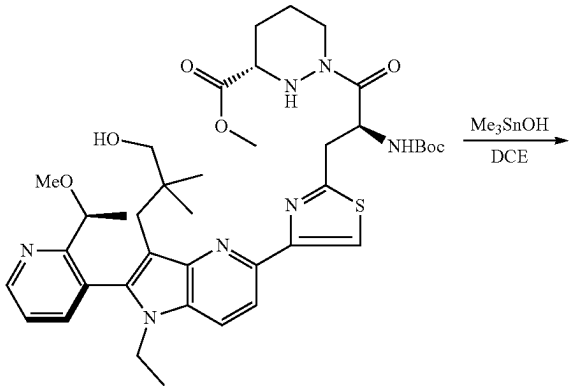

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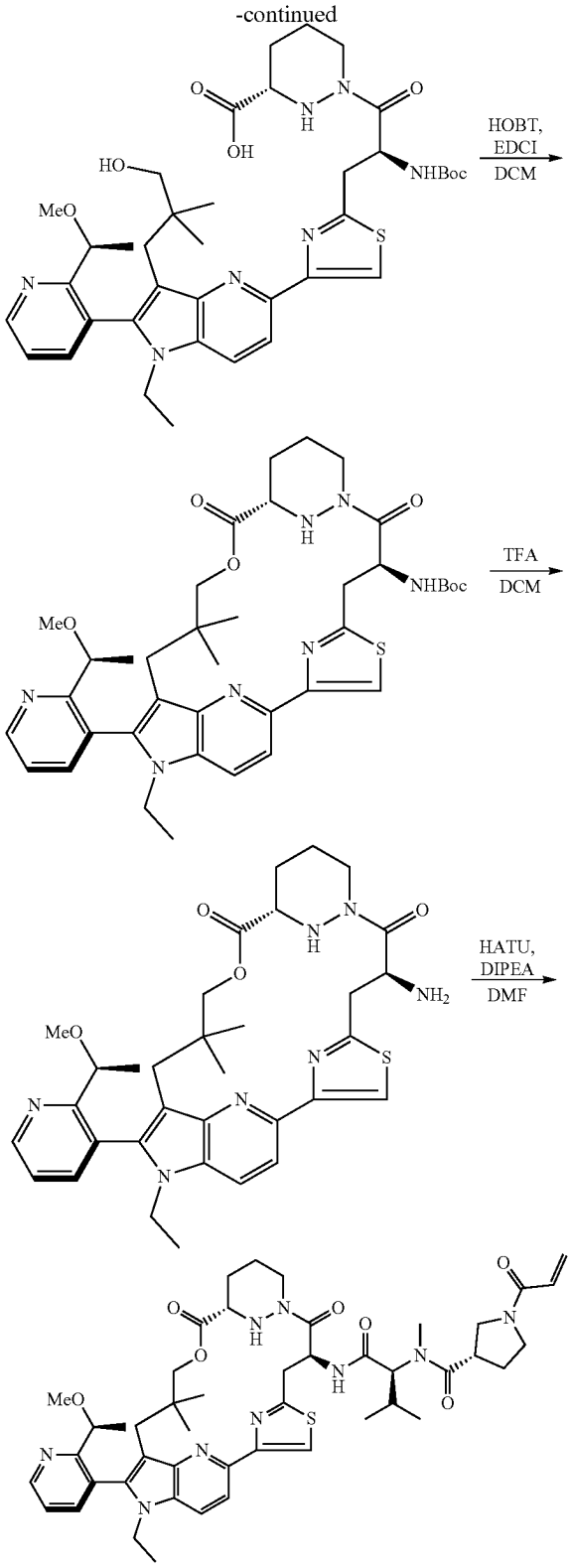


Step 1.

To a mixture of (5-chloro-1H-pyrrolo[3,2-b]pyridin-3-yl) methanol (3.5 g, 19 mmol), and ((1-methoxy-2-methylprop-1-en-1-yl)oxy)trimethylsilane (6.7 g, 38 mmol) in THF (50 ml) at 0° C. was added TMSOTf (3.8 g, 17 mmol) dropwise. The mixture was stirred at 0-5° C. for 2 h, then diluted with EtOAc (100 mL) and washed with saturated $NaHCO_3$ (50 mL) and brine (50 mL×2). The organic layer was dried over Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography to give methyl 3-(5-chloro-1H-pyrrolo[3,2-b]pyridin-3-yl)-2,2-dimethylpropanoate (3.0 g, 59% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{13}H_{15}ClN_2O_2$ 266.1; found 267.1.

Step 2.

To a mixture of methyl 3-(5-chloro-1H-pyrrolo[3,2-b] pyridin-3-yl)-2,2-dimethylpropanoate (3.0 g, 11 mmol) in anhydrous THF (50 mL) at 0° C. was added AgOTf (4.3 g, 17 mmol) and 12 (2.9 g, 11 mmol). The mixture was stirred at 0° C. for 2 h, then saturated Na_2SO_3 (20 mL) and EtOAc (50 mL) added. The mixture was filtered and the filtrate was washed with brine (50 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl 3-(5-chloro-2-iodo-1H-pyrrolo[3,2-b]pyridin-3-yl)-2,2-dimethylpropanoate (2.3 g, 52% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{13}H_{18}ClIN_2O_2$ 392.0; found 393.0.

Step 3.

To a mixture of methyl 3-(5-chloro-2-iodo-1H-pyrrolo[3,2-b]pyridin-3-yl)-2,2-dimethylpropanoate (2.3 g, 5.9 mmol) and 2-(2-(2-methoxyethyl)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (1.6 g, 7.1 mmol) and K_2CO_3 (2.4 g, 18 mol) in 1,4-dioxane (25 mL) and H_2O (5 mL) under an atmosphere of N_2 was added Pd(dppf)Cl_2.DCM (480 mg, 0.59 mmol). The mixture was heated to 70° C. and for 4 h, then diluted with EtOAc (200 mL) and washed with brine (25 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl (S)-3-(5-chloro-2-(2-(1-methoxyethyl)pyridin-3-yl)-1H-pyrrolo[3,2-b]pyridin-3-yl)-2,2-dimethylpropanoate (2.0 g, 84% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{21}H_{24}ClN_3O_3$ 401.2; found 402.2.

Step 4.

A mixture of ethyl 3-(5-chloro-2-(2-((S)-1-methoxyethyl) pyridin-3-yl)-1H-pyrrolo[3,2-b]pyridin-3-yl)-2-methylpropanoate (2.0 g, 5.0 mmol), Cs_2CO_3 (3.3 g, 10 mmol) and EtI (1.6 g, 10 mmol) in DMF (30 mL) was stirred for 10 h. The mixture was diluted with EtOAc (100 mL) and washed with brine (20 mL×4), dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give two diastereomers of methyl (S)-3-(5-chloro-1-ethyl-2-(2-(1-methoxyethyl)pyridin-3-yl)-1H-pyrrolo[3,2-b]pyridin-3-yl)-2,2-dimethylpropanoate (0.7 g, 32% yield; 0.6 g, 28% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{23}H_{28}ClN_3O_3$ 429.2; found 430.2.

Step 5.

To a mixture of methyl (S)-3-(5-chloro-1-ethyl-2-(2-(1-methoxyethyl)pyridin-3-yl)-1H-pyrrolo[3,2-b]pyridin-3-yl)-2,2-dimethylpropanoate (1.9 g, 4.4 mmol) in anhydrous THF (20 mL) at 0° C. was added $LiBH_4$ (200 mg, 8.8 mmol). The mixture was heated to 60° C. and stirred for 4 h, then saturated NH_4Cl (20 mL) and EtOAc (50 mL) added. The aqueous and organic layers were separated and the organic layer was washed with brine (30 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give (S)-3-(5-chloro-1-ethyl-2-(2-(1-methoxyethyl)pyridin-3-yl)-1H-pyrrolo[3,2-b]pyridin-3-

yl)-2,2-dimethylpropan-1-ol (1.5 g, 85% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{22}H_{28}ClN_3O_2$ 401.2; found 402.2.

Step 6.

To a mixture of (S)-3-(5-chloro-1-ethyl-2-(2-(1-methoxyethyl)pyridin-3-yl)-1H-pyrrolo[3,2-b]pyridin-3-yl)-2,2-dimethylpropan-1-ol (550 mg, 1.37 mmol), (S)-(2-(2-((tert-butoxycarbonyl)amino)-3-methoxy-3-oxopropyl)thiazol-4-yl)boronic acid (907.4 mg, 2.74 mmol, 2 eq) and K_2CO_3 (568 mg, 4.11 mmol) in 1,4-dioxane (25 mL) and H_2O (5 mL) under an atmosphere of N_2 was added Pd(dppf)Cl_2.DCM (89 mg, 0.14 mmol). The mixture was heated to 70° C. and stirred for 4 h, then H_2O (50 mL) added and the mixture extracted with EtOAc (100 mL×3). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl (S)-2-((tert-butoxycarbonyl)amino)-3-(4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1H-pyrrolo[3,2-b]pyridin-5-yl)thiazol-2-yl)propanoate (440 mg, 22% yield) as a solid, which was used directly in the next step. LCMS (ESI): m/z [M+H] calc'd for $C_{34}H_{45}N_5O_6S$ 651.3; found 652.3.

Step 7.

To a mixture of (2S)-methyl 2-((tert-butoxycarbonyl)amino)-3-(4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1H-pyrrolo[3,2-b]pyridin-5-yl)thiazol-2-yl)propanoate (280 mg, 0.43 mmol) in MeOH (4 mL) was added a solution of LiOH (51 mg, 2.2 mmol) in H_2O (2 mL). The mixture was stirred for 5 h, then pH adjusted to ~3-4 by addition of 1M HCl. The mixture was diluted with H_2O (30 mL) and extracted with EtOAc (15 mL×3). The combined organic layers were washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered and the filtrate was concentrated under reduced pressure to give (2S)-2-((tert-butoxycarbonyl)amino)-3-(4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1H-pyrrolo[3,2-b]pyridin-5-yl)thiazol-2-yl)propanoic acid (280 mg) as solid, which was used directly in the next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{33}H_{43}N_5O_6S$ 637.3; found 638.3.

Step 8.

To a mixture of (2S)-2-((tert-butoxycarbonyl)amino)-3-(4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1H-pyrrolo[3,2-b]pyridin-5-yl)thiazol-2-yl)propanoic acid (274 mg, 0.43 mmol) and methyl (3S)-1,2-diazinane-3-carboxylate (280 mg, 0.64 mmol) in DMF (3 mL) at 0-5° C. was added a solution of HATU (245 mg, 0.64 mmol) and DIPEA (555 mg, 4.3 mmol) in DMF (2 mL). The mixture was stirred for 1 h, then diluted with EtOAc (20 mL) and H_2O (20 mL). The aqueous and organic layers were partitioned and the organic layer was washed with H_2O (20 mL×3), brine (20 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl (3S)-1-[(2S)-2-{[(tert-butoxy)carbonyl]amino}-3-{4-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-{2-[(1 S)-1-methoxyethyl]pyridin-3-yl}pyrrolo[3,2-b]pyridin-5-yl]-1,3-thiazol-2-yl}propanoyl]-1,2-diazinane-3-carboxylate (230 mg, 70% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{39}H_{53}N_7O_7S$ 763.4; found 764.3.

Step 9.

To a mixture of methyl (3S)-1-[(2S)-2-{[(tert-butoxy)carbonyl]amino}-3-{4-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-{2-[(1 S)-1-methoxyethyl]pyridin-3-yl}pyrrolo[3,2-b]pyridin-5-yl]-1,3-thiazol-2-yl}propanoyl]-1,2-diazinane-3-carboxylate (230 mg, 0.3 mmol) in DCE (3 mL) under an atmosphere of N_2 was added Me_3SnOH (300 mg). The mixture was heated to 65° C. and stirred for 16 h, then concentrated under reduced pressure. The residue was diluted with EtOAc (20 mL), washed with H_2O (20 mL) and brine (10 mL), dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to give (3S)-1-[(2S)-2-{[(tert-butoxy)carbonyl]amino}-3-{4-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-{2-[(1 S)-1-methoxyethyl]pyridin-3-yl}pyrrolo[3,2-b]pyridin-5-yl]-1,3-thiazol-2-yl}propanoyl]-1,2-diazinane-3-carboxylic acid (200 mg) as a foam, which was used directly in the next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{38}H_{51}N_7O_7S$ 749.4; found 750.3.

Step 10.

To a mixture of (3S)-1-[(2S)-2-{[(tert-butoxy)carbonyl]amino}-3-{4-[1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-{2-[(1 S)-1-methoxyethyl]pyridin-3-yl}pyrrolo[3,2-b]pyridin-5-yl]-1,3-thiazol-2-yl}propanoyl]-1,2-diazinane-3-carboxylic acid (245 mg, 0.32 mmol) in DCM (50 mL) at 0-5° C. were added HOBT (432 mg, 3.2 mmol), EDCI HCl (1.8 g, 9.6 mmol) and DIPEA (1.65 g, 12.8 mmol). The mixture was warmed to room temperature and stirred for 16 h, then concentrated under reduced pressure. The residue was diluted with EtOAc (20 mL) and H_2O (20 mL) and the aqueous and organic layers were partitioned. The organic layer was washed with H_2O (30 mL×3), brine (30 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-TLC to give tert-butyl ((6^{3}S,4S,Z)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-thiazola-1(5,3)-pyrrolo[3,2-b]pyridina-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (100 mg, 43% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{38}H_{49}N_7O_6S$ 731.4; found 732.3.

Step 11.

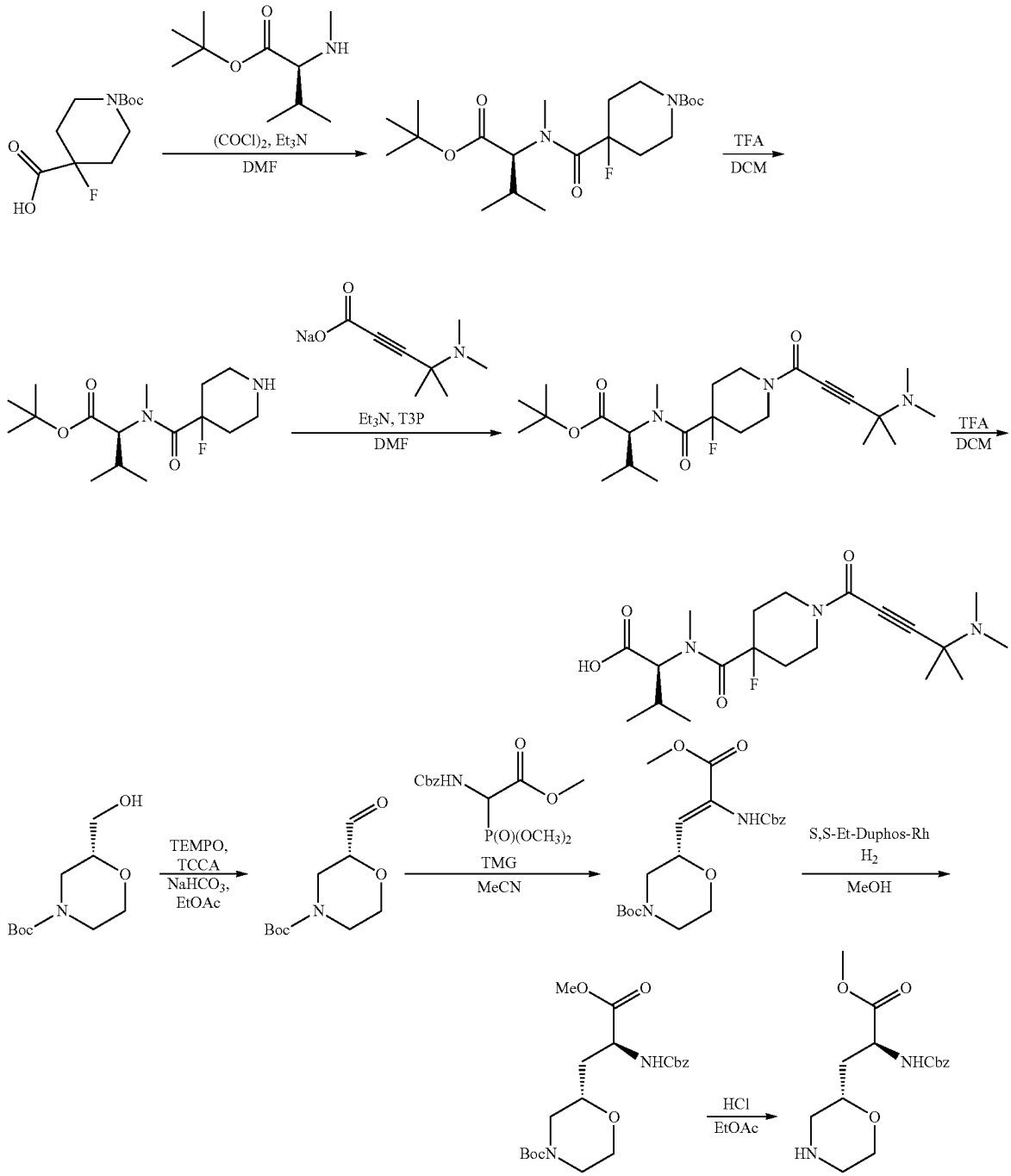
A mixture of tert-butyl ((6^{3}S,4S,Z)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-thiazola-1(5,3)-pyrrolo[3,2-b]pyridina-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (80 mg, 0.11 mmol) in DCM (0.6 mL) and TFA (0.2 mL) was stirred for 1 h. The mixture was concentrated under reduced pressure to give (6^{3}S,4S,Z)-4-amino-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-thiazola-1(5,3)-pyrrolo[3,2-b]pyridina-6(1,3)-pyridazinacycloundecaphane-5,7-dione (72 mg, 95% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{33}H_{41}N_7O_4S$ 631.3; found 632.3.

Step 12.

To a mixture of (6^{3}S,4S,Z)-4-amino-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-thiazola-1(5,3)-pyrrolo[3,2-b]pyridina-6(1,3)- pyridazinacycloundecaphane-5,7-dione (120 mg, 0.39 mmol) and DIPEA (335 mg, 2.6 mmol) in DMF (1 mL) at 0° C. was added HATU (60 mg, 0.16 mmol). The mixture was stirred at 0° C. for 1 h, then diluted with H_2O (110 mL) and extracted with EtOAc (80 mL×2). The combined organic layers were washed with H_2O (100 mL), brine (100 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-TLC to give (3S)-1-acryloyl-N-((2S)-1-(((6^{3}S,4S,Z)-1^1-ethyl-1^2-(2-((S)-

1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-2(4,2)-thiazola-1(5,3)-pyrrolo[3,2-b]pyridina-6(1,3)-pyridazinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-N-methylpyrrolidine-3-carboxamide (1.8 mg, 2% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{47}H_{61}N_9O_7S$ 895.4; found 896.3; ^{1}H NMR (400 MHz, CD_3OD) δ 8.72 (d, J=4.5 Hz, 1H), 7.98-7.77 (m, 3H), 7.72 (dd, J=12.0, 8.6 Hz, 1H), 7.54 (dd, J=7.6, 4.8 Hz, 1H), 6.67-6.54 (m, 1H), 6.26 (m, 1H), 5.79-5.58 (m, 2H), 4.83-4.75 (m, 1H), 4.39-4.16 (m, 4H), 4.02 (dd, J=28.0, 10.6 Hz, 2H), 3.89-3.65 (m, 6H), 3.50 (m, 4H), 3.34 (d, J=6.2 Hz, 3H), 3.12 (d, J=4.0 Hz, 2H), 3.00 (s, 1H), 2.73 (m, 1H), 2.48-2.37 (m, 1H), 2.31-2.07 (m, 4H), 1.88 (d, J=11.2 Hz, 1H), 1.71 (d, J=12.8 Hz, 1H), 1.44 (m, 7H), 0.97 (dd, J=6.2, 4.4 Hz, 3H), 0.92-0.84 (m, 8H), 0.41 (d, J=6.2 Hz, 3H).

Example 647. Synthesis of 1-(4-(dimethylamino)-4-methylpent-2-ynoyl)-N-((2S)-1-(((2^{2}S,6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-4-fluoro-N-methylpiperidine-4-carboxamide



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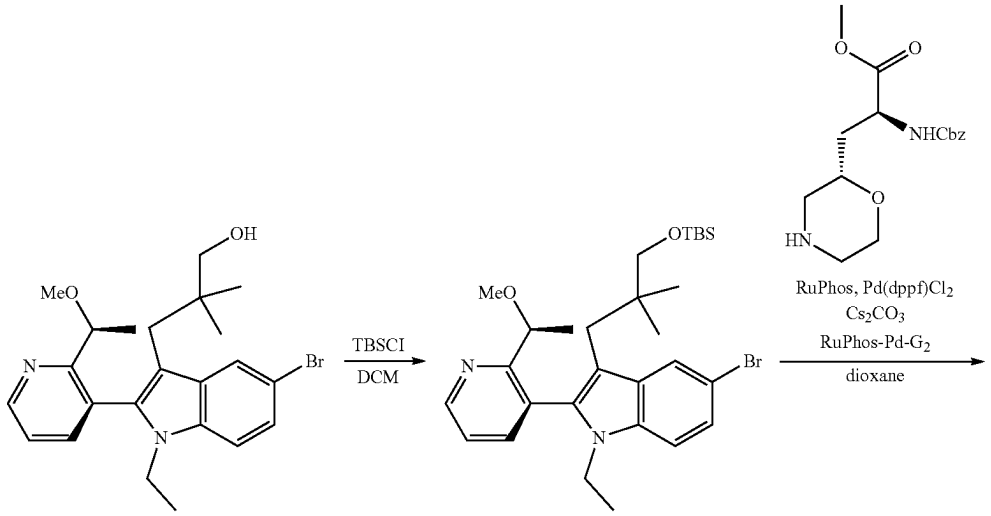
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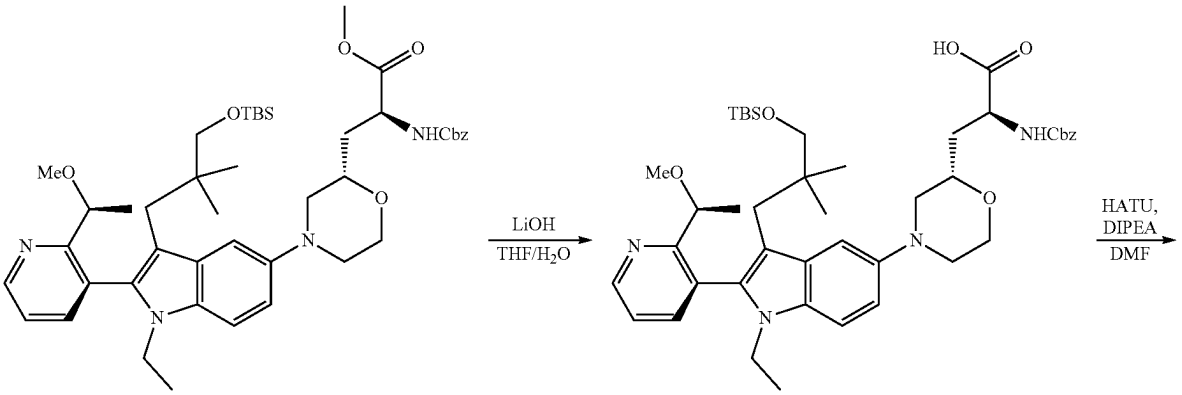
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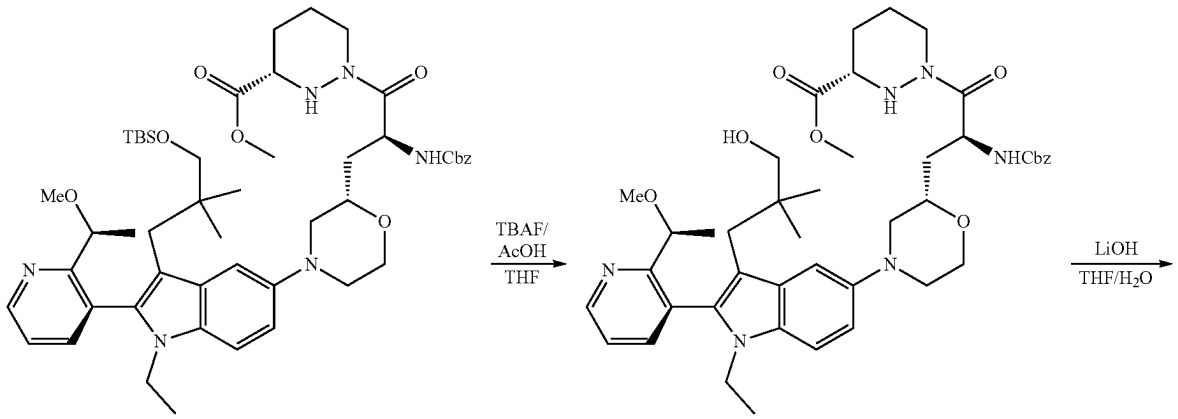
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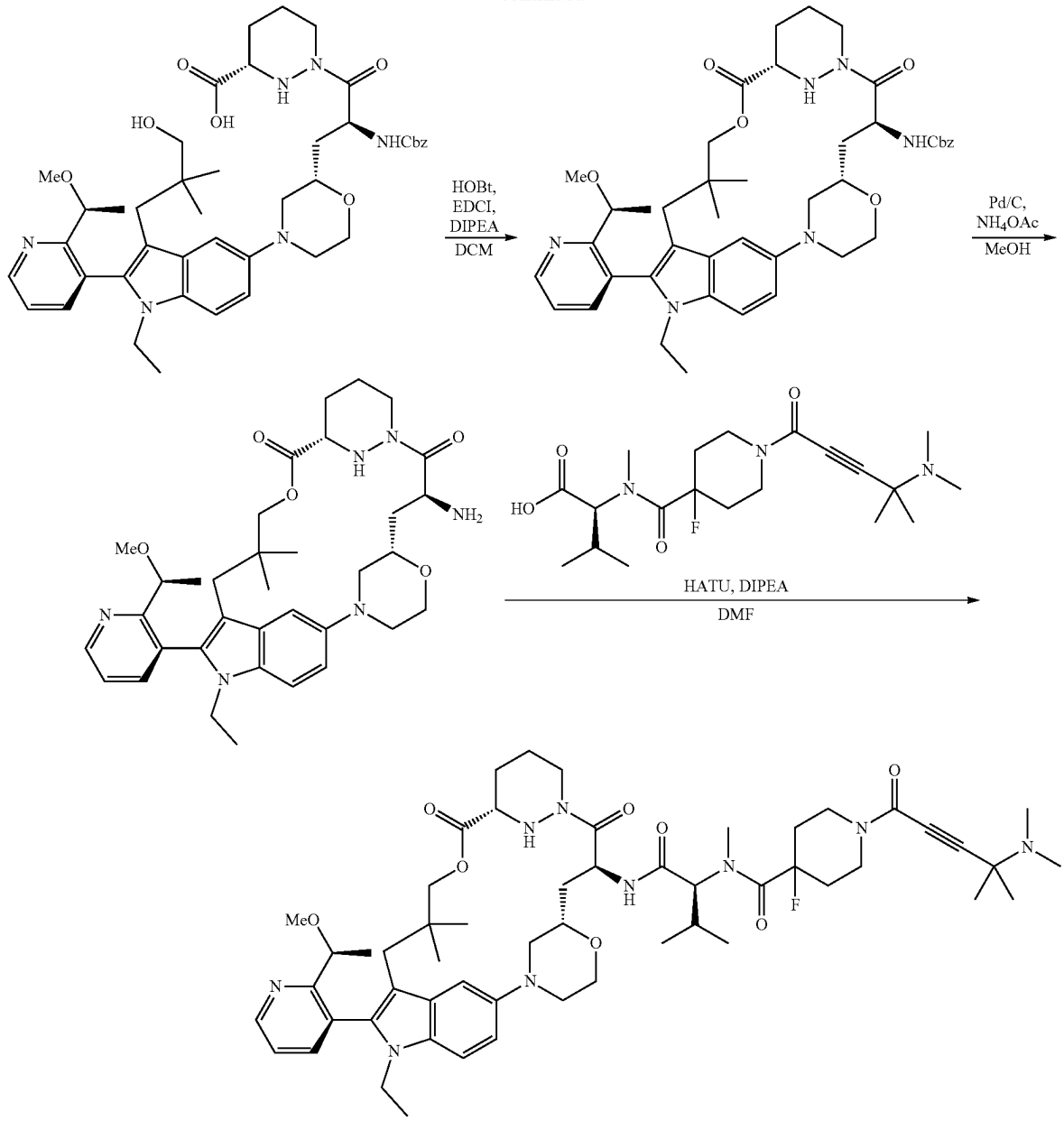


Step 1.

A mixture of 1-[(tert-butoxy)carbonyl]-4-fluoropiperidine-4-carboxylic acid (2.0 g, 8.1 mmol) in DCM (20 mL) was added oxalic dichloride (1.34 g, 10.5 mmol) and DMF (30 mg, 0.4 mmol). The resulting solution was stirred at room temperature for 1 h. Et_3N (3.2 g, 3.2 mmol) and (2S)-3-methyl-2-(methylamino)butanoic acid (1.25 g, 9.5 mmol) were added and the mixture was stirred at room temperature for 1 h. H_2O (100 mL) was added and the mixture was extracted with EtOAc (50 mL×3). The combined organic layers were concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give tert-butyl (S)-4-((1-(tert-butoxy)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)-4-fluoropiperidine-1-carboxylate (1.34 g, 45% yield) as a solid. LCMS (ESI): m/z [M+Na] calc'd for $C_{21}H_{37}FN_2O_5Na$ 439.3; found 439.3.

Step 2.

A mixture of tert-butyl (S)-4-((1-(tert-butoxy)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)-4-fluoropiperidine-1-carboxylate (290 mg, 0.70 mmol) in DCM (4 mL) and TFA (2 mL) was stirred at room temperature for 2 h, then concentrated under reduced pressure to give N-(4-fluoropiperidine-4-carbonyl)-N-methyl-L-valine, which was used directly in the next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{12}H_{21}FN_2O_3$ 260.2; found 261.2.

Step 3.

To a solution of the tert-butyl N-(4-fluoropiperidine-4-carbonyl)-N-methyl-L-valinate (1.7 g, 5.3 mmol), sodium 4-(dimethylamino)-4-methylpent-2-ynoate (1.67 g, 9.4 mmol) and Et_3N (2.73 g, 36.9 mmol) in DMF (20 mL) stirred at 5° C. was added T3P (4.11 g, 10.7 mmol, 50 wt % in EtOAc). The reaction mixture was stirred at 5° C. for 1 h. The resulting mixture was quenched with H_2O (100 mL) and extracted with EtOAc (50 mL×3). The combined organic

layers were concentrated and purified by silica gel column chromatography to give tert-butyl N-(1-(4-(dimethylamino)-4-methylpent-2-ynoyl)-4-fluoropiperidine-4-carbonyl)-N-methyl-L-valinate (1.6 g, 74.0% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{24}H_{40}FN_3O_4$ 453.3; found 454.2.

Step 4.

To a solution of tert-butyl N-(1-(4-(dimethylamino)-4-methylpent-2-ynoyl)-4-fluoropiperidine-4-carbonyl)-N-methyl-L-valinate (50 mg, 0.11 mmol) in DCM (2 mL) was added TFA (1 mL). The reaction mixture was stirred at 20° C. for 2 h, then concentrated under reduced pressure to afford crude N-(1-(4-(dimethylamino)-4-methylpent-2-ynoyl)-4-fluoropiperidine-4-carbonyl)-N-methyl-L-valine. It was used for the next step directly without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{20}H_{32}FN_3O_4$ 397.2; found 398.3.

Step 5.

To a solution of tert-butyl (2R)-2-(hydroxymethyl)morpholin-4-yl formate (50 g, 230 mmol) in EtOAc (1 L) was added TEMPO (715 mg, 4.6 mmol) and $NaHCO_3$ (58 g, 690 mmol) at 20° C. The mixture was cooled to −50° C., then TCCA (56 g, 241 mmol) in EtOAc (100 mL) was added dropwise over 30 min. The reaction mixture was warmed to 5° C. for 2 h, then quenched with 10% $Na_2S_2O_3$ (200 mL) and stirred for 20 min. The resulting mixture was filtered and the organic phase was separated from filtrate. The aqueous phase was extracted with EtOAc (100 mL×2). The combined organic layers were washed with H_2O (100 mL) and brine (100 mL), and dried over anhydrous Na_2SO_4. The organic layer was concentrated under reduced pressure to afford tert-butyl (2R)-2-formylmorpholin-4-yl formate (50 g, crude) as an oil.

Step 6.

To a solution of tert-butyl (2R)-2-formylmorpholin-4-yl formate (49 g, 153 mmol) and methyl 2-{[(benzyloxy)carbonyl]amino}-2-(dimethoxyphosphoryl)acetate (60 g, 183 mmol) in CAN (300 mL) was added tetramethylguanidine (35 g, 306 mmol) at 0-10° C. The reaction mixture was stirred at 10° C. for 30 min then warmed to 20° C. for 2 h. The reaction mixture was diluted with DCM (200 mL) and washed with Citric acid (10%, 200 mL) and 10% $NaHCO_3$ aqueous solution (200 mL). The organic phase was concentrated under reduced pressure, and purified by silica gel column chromatography to afford tert-butyl (S,Z)-2-(2-(((benzyloxy)carbonyl)amino)-3-methoxy-3-oxoprop-1-en-1-yl)morpholine-4-carboxylate (36 g, 90% yield) as solid. LCMS (ESI): m/z [M+Na] calc'd for $C_{21}H_{28}N_2O_4$ 420.2; found: 443.1.

Step 7.

To a solution of tert-butyl (S,Z)-2-(2-(((benzyloxy)carbonyl)amino)-3-methoxy-3-oxoprop-1-en-1-yl)morpholine-4-carboxylate (49 g, 0.12 mol) in MeOH (500 mL) was added (S,S)-Et-DUPHOS-Rh (500 mg, 0.7 mmol). The mixture was stirred at 25° C. under an H_2 (60 psi) atmosphere for 48 h. The reaction was concentrated and purified by chromatography to give tert-butyl (S)-2-((S)-2-(((benzyloxy)carbonyl)amino)-3-methoxy-3-oxopropyl)morpholine-4-carboxylate (44 g, 89.8% yield) as solid. LCMS (ESI): m/z [M+Na] calc'd for $C_{21}H_{30}N_2O_7$ 422.2; found: 445.2.

Step 8.

To a stirred solution of tert-butyl (S)-2-((S)-2-(((benzyloxy)carbonyl)amino)-3-methoxy-3-oxopropyl)morpholine-4-carboxylate (2.2 g, 5.2 mmol) in EtOAc (2 mL) was added HCl/EtOAc (25 mL) at 15° C. The reaction was stirred at 15° C. for 2 h, then concentrated under reduced pressure to afford methyl (S)-2-(((benzyloxy)carbonyl)amino)-3-((S)-morpholin-2-yl)propanoate (1.51 g, 90.4% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{16}H_{22}N_2O_5$ 322.1; found 323.2.

Step 9.

To a solution of 3-(5-bromo-1-ethyl-2-{2-[(1 S)-1-methoxyethyl]pyridin-3-yl}indol-3-yl)-2,2-dimethylpropan-1-ol (100 g, 0.22 mol) and 1H-imidazole (30.6 g, 0.45 mol) in DCM (800 mL) was added TBSCl (50.7 g, 0.34 mol) in DCM (200 mL) at 0° C. The reaction was stirred at 25° C. for 2 h. The resulting solution was washed with H_2O (300 mL×3) and brine (200 mL×2), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified with silica gel column chromatography to give (S)-5-bromo-3-(3-((tert-butyldimethylsilyl)oxy)-2,2-dimethylpropyl)-1-ethyl-2-(2-(1-methoxyethyl)pyridin-3-yl)-1H-indole (138 g, 90% yield) as an solid. LCMS (ESI): m/z [M+H] calc'd for $C_{29}H_{43}BrN_2O_2Si$ 558.2; found 559.2.

Step 10.

To a stirred solution of Intermediate 1 (50 g, 89.3 mmol) in dioxane (500 mL) was added methyl (2S)-2-{[(benzyloxy)carbonyl]amino}-3-[(2S)-morpholin-2-yl]propanoate from step 1 (31.7 g, 98.2 mmol), RuPhos (16.7 g, 35.7 mmol), Di-mu-chlorobis(2-amino-1,1-biphenyl-2-yl-C,N)dipalladium(II) (2.8 g, 4.4 mmol) and cesium carbonate (96 g, 295 mmol) followed by RuPhos-Pd-G2 (3.5 g, 4.4 mmol) at 105° C. under an N_2 atmosphere. The reaction mixture was stirred for 6 h at 105° C. under an N_2 atmosphere. The resulting mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by prep-TLC chromatography to afford methyl (2S)-2-{[(benzyloxy)carbonyl]amino}-3-[(2S)-4-(3-{3-[(tert-butyldimethylsilyl)oxy]-2,2-dimethylpropyl}-1-ethyl-2-{2-[(1 S)-1-methoxyethyl]pyridin-3-yl}indol-5-yl)morpholin-2-yl]propanoate (55 g, 73% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{45}H_{64}N_4O_7Si$ 800.5; found 801.5.

Step 11.

To a solution of methyl (2S)-2-{[(benzyloxy)carbonyl]amino}-3-[(2S)-4-(3-{3-[(tert-butyldimethylsilyl)oxy]-2,2-dimethylpropyl}-1-ethyl-2-{2-[(1 S)-1-methoxyethyl]pyridin-3-yl}indol-5-yl)morpholin-2-yl]propanoate (10 g, 12 mmol) in THF (270 mL) was added LiOH (1.3 g, 31 mmol) in H_2O (45 mL) at 20° C. The reaction was stirred at 20° C. for 2 h, then treated with 1N HCl to adjust pH to 4-5 at 0~5° C. The resulting mixture was extracted with EtOAc (50 mL×2). The combined organic layers were washed with brine and dried over anhydrous Na_2SO_4. The organic phase was then concentrated under reduced pressure to afford (2S)-2-{[(benzyloxy)carbonyl]amino}-3-[(2S)-4-(3-{3-[(tert-butyldimethylsilyl)oxy]-2,2-dimethylpropyl}-1-ethyl-2-{2-[(1 S)-1-methoxyethyl]pyridin-3-yl}indol-5-yl)morpholin-2-yl]propanoic acid (9.5 g, 97% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{44}H_{62}N_4O_7Si$ 786.4; found 787.4.

Step 12.

To a stirred solution of (2S)-2-{[(benzyloxy)carbonyl]amino}-3-[(2S)-4-(3-{3-[(tert-butyldimethylsilyl)oxy]-2,2-dimethylpropyl}-1-ethyl-2-{2-[(1 S)-1-methoxyethyl]pyridin-3-yl}indol-5-yl)morpholin-2-yl]propanoic acid (10 g, 12.7 mmol) in DMF (150 mL), was added methyl (S)-hexahydropyridazine-3-carboxylate (2 g, 14 mmol), then cooled to 0° C., DIPEA (32.8 g, 254 mmol) was added followed by HATU (9.7 g, 25.4 mmol) at 0~5° C. The reaction mixture was stirred at 0~5° C. for 1 h. The resulting mixture was diluted with EtOAc (500 mL) and H_2O (200 mL). The organic layer was separated and washed with H_2O (100 mL×2) and brine (100 mL), dried over anhydrous sodium sulfate. The solution was filtered and concentrated under reduced pressure, and the residue was purified by silica gel column chromatography to afford methyl (S)-1-((S)-2-(((benzyloxy)carbonyl)amino)-3-((S)-4-(3-(3-((tert-butyldimethylsilyl)oxy)-2,2-dimethylpropyl)-1-ethyl-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1H-indol-5-yl)morpholin-2-yl)propanoyl)hexahydropyridazine-3-carboxylate (8 g, 70% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{50}H_{72}N_6O_8Si$ 912.5; found 913.4.

Step 13.

A solution of methyl (S)-1-((S)-2-(((benzyloxy)carbonyl)amino)-3-((S)-4-(3-(3-((tert-butyldimethylsilyl)oxy)-2,2-dimethylpropyl)-1-ethyl-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1H-indol-5-yl)morpholin-2-yl)propanoyl)hexahydropyridazine-3-carboxylate (8.5 g, 9 mmol) in THF (8 mL) was added a mixture of tetrabutylammonium fluoride (1M in THF, 180 mL, 180 mmol) and AcOH (11 g, 200 mmol) at 20° C. The reaction mixture was stirred at 75° C. for 3 h. The resulting mixture was diluted with EtOAc (150 mL) and washed with H_2O (20 mL×6). The organic phase was concentrated under reduced pressure to give methyl (S)-1-((S)-2-(((benzyloxy)carbonyl)amino)-3-((S)-4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1H-indol-5-yl)morpholin-2-yl)propanoyl)hexahydropyridazine-3-carboxylate (7.4 g, 100% yield) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{44}H_{58}N_6O_8$ 799.4; found 798.4.

Step 14.

To a solution of methyl (S)-1-((S)-2-(((benzyloxy)carbonyl)amino)-3-((S)-4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1H-indol-5-yl)morpholin-2-yl)propanoyl)hexahydropyridazine-3-carboxylate (8 g, 10 mmol) in THF (200 mL) was added lithium hydroxide (600 mg, 25 mmol) in H_2O (30 mL). The reaction mixture was stirred at 20° C. for 1 h, then treated with 1N HCl to adjust pH to 4-5 at 0~5° C., and extracted with EtOAc (500 mL×2). The organic phase was washed with brine, and concentrated under reduced pressure to afford (S)-1-((S)-2-(((benzyloxy)carbonyl)amino)-3-((S)-4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1H-indol-5-yl)morpholin-2-yl)propanoyl)hexahydropyridazine-3-carboxylic acid (8 g, crude) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{43}H_{56}N_6O_8$ 784.4; found 785.4.

Step 15.

To a stirred solution of (S)-1-((S)-2-(((benzyloxy)carbonyl)amino)-3-((S)-4-(1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1H-indol-5-yl)morpholin-2-yl)propanoyl)hexahydropyridazine-3-carboxylic acid (8 g, 10.2 mmol) and DIPEA (59 g, 459 mmol) in DCM (800 mL) was added EDCI (88 g, 458 mmol) and HOBT (27.6 g, 204 mmol) at 25° C. under an argon atmosphere. The reaction mixture was stirred at 25° C. for 16 h. The resulting mixture was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography to afford benzyl ((2^{2}S,6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (5 g, 66% yield) as a solid; LCMS (ESI): m/z [M+H] calc'd for $C_{43}H_{54}N_6O_7$ 766.4; found 767.4.

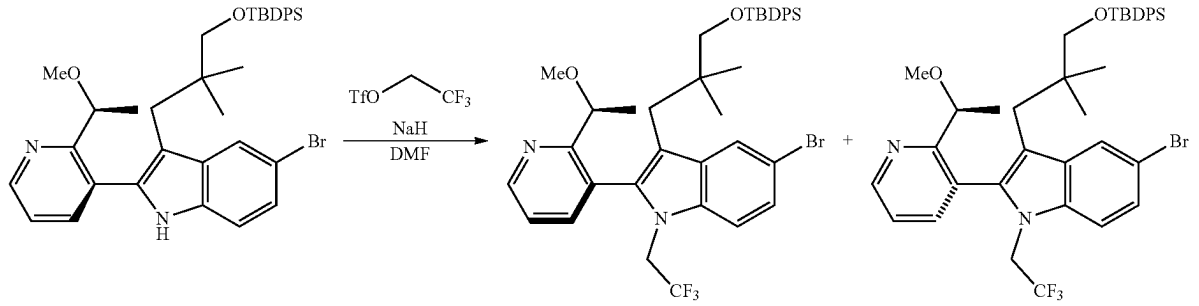
Step 16.

To a solution of benzyl ((2^{2}S,6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (400 mg, 0.5 mmol) in MeOH (20 mL) was added Pd/C (200 mg) and ammonium acetate (834 mg, 16 mmol) at 20° C. under an H_2 atmosphere and the mixture was stirred for 2 h. Then resulting mixture was filtered and concentrated under reduced pressure. The residue was redissolved in DCM (20 mL) and washed with H_2O (5 mL×2), then concentrated under reduced pressure to afford (2^{2}S,6^{3}S,4S)-4-amino-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (320 mg, 97% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{35}H_{48}N_6O_5$ 632.4; found 633.3.

Step 17.

To a solution of the (2^{2}S,6^{3}S,4S)-4-amino-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (50 mg, 0.079 mmol), N-(1-(4-(dimethylamino)-4-methylpent-2-ynoyl)-4-fluoropiperidine-4-carbonyl)-N-methyl-L-valine (47 mg, 0.12 mmol) in DMF (2 mL) stirred at 0° C. was added HATU (36 mg, 0.09 mmol) and DIPEA (153 mg, 1.2 mmol) dropwise. The reaction was stirred at 0° C. for 1 h. The resulting mixture was purified by reverse phase to afford 1-(4-(dimethylamino)-4-methylpent-2-ynoyl)-N-((2S)-1-(((2^{2}S,6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-4-fluoro-N-methylpiperidine-4-carboxamide (11.9 mg, 13.9% yield) as a solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.71 (dd, J=4.8, 1.7 Hz, 1H), 7.86 (d, J=7.8 Hz, 1H), 7.51 (dd, J=7.8, 4.8 Hz, 1H), 7.39 (d, J=8.9 Hz, 1H), 7.15-7.04 (m, 2H), 5.67 (d, J=8.8 Hz, 1H), 4.62 (d, J=11.2 Hz, 1H), 4.46 (d, J=12.4 Hz, 1H), 4.39-4.27 (m, 2H), 4.23 (d, J=6.1 Hz, 1H), 4.17-4.08 (m, 1H), 3.93 (s, 2H), 3.86 (s, 1H), 3.84-3.76 (m, 2H), 3.74-3.65 (m, 2H), 3.63-3.51 (m, 2H), 3.27-3.23 (m, 1H), 3.22-3.11 (m, 6H), 3.0-2.89 (m, 2H), 2.85-2.75 (m, 2H), 2.74-2.55 (m, 2H), 2.36 (d, J=8.2 Hz, 6H), 2.32-2.21 (m, 2H), 2.20-2.02 (m, 5H), 1.92 (d, J=12.5 Hz, 2H), 1.69 (dd, J=43.8, 12.6 Hz, 2H), 1.46 (dt, J=8.0, 4.9 Hz, 9H), 1.03 (d, J=3.5 Hz, 3H), 0.90 (dd, J=48.3, 6.5 Hz, 6H), 0.77 (d, J=3.0 Hz, 3H), 0.69 (s, 3H). LCMS (ESI): m/z [M+H] calc'd for $C_{55}H_{78}FN_9O_8$ 1011.6; found 1012.5.

Example A375. Synthesis of (2R)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-N-((2^{2}S,6^{3}S,4S)-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methylbutanamide



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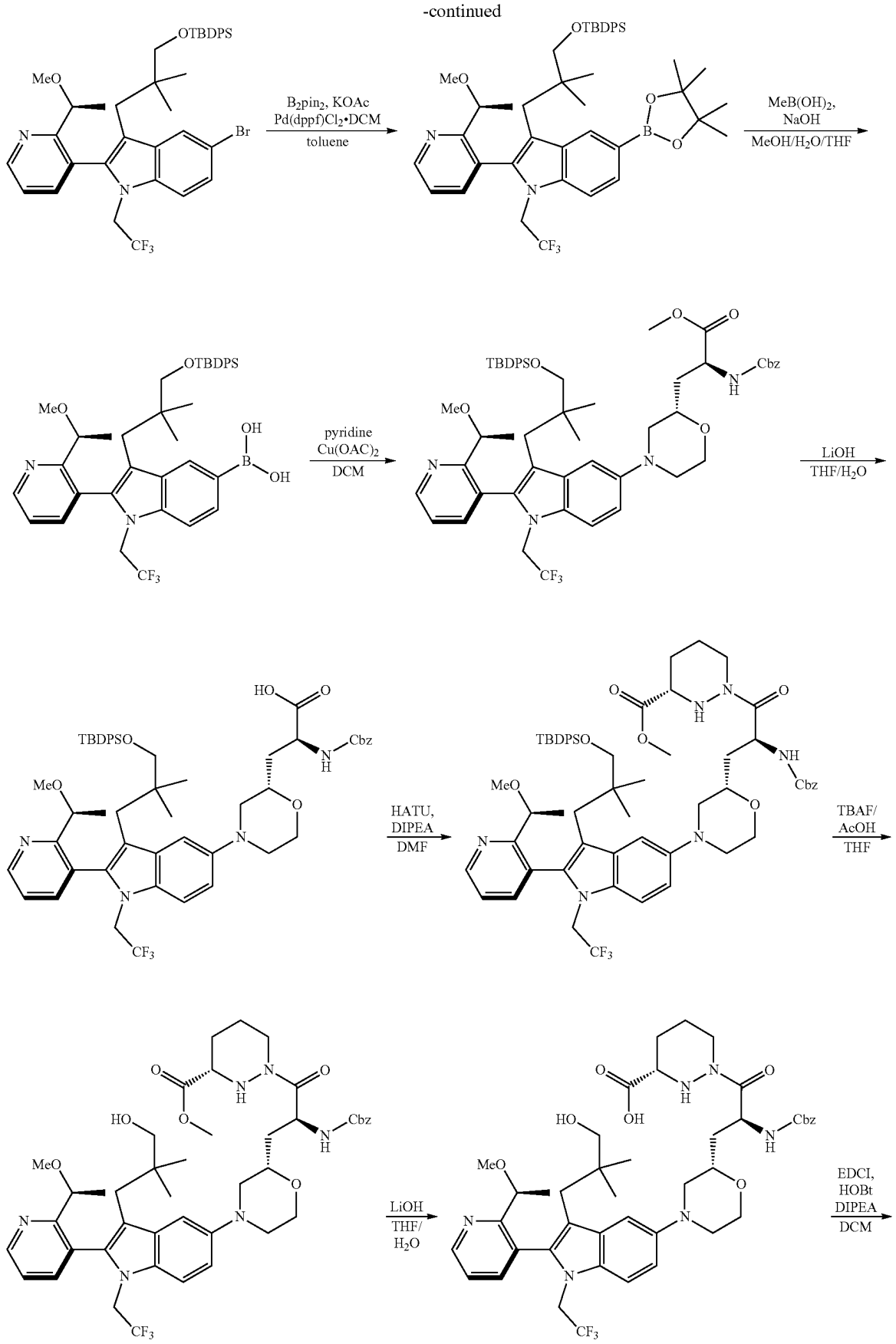
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pyridine
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DCM
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LiOH
THF/H2O
HO
HATU,
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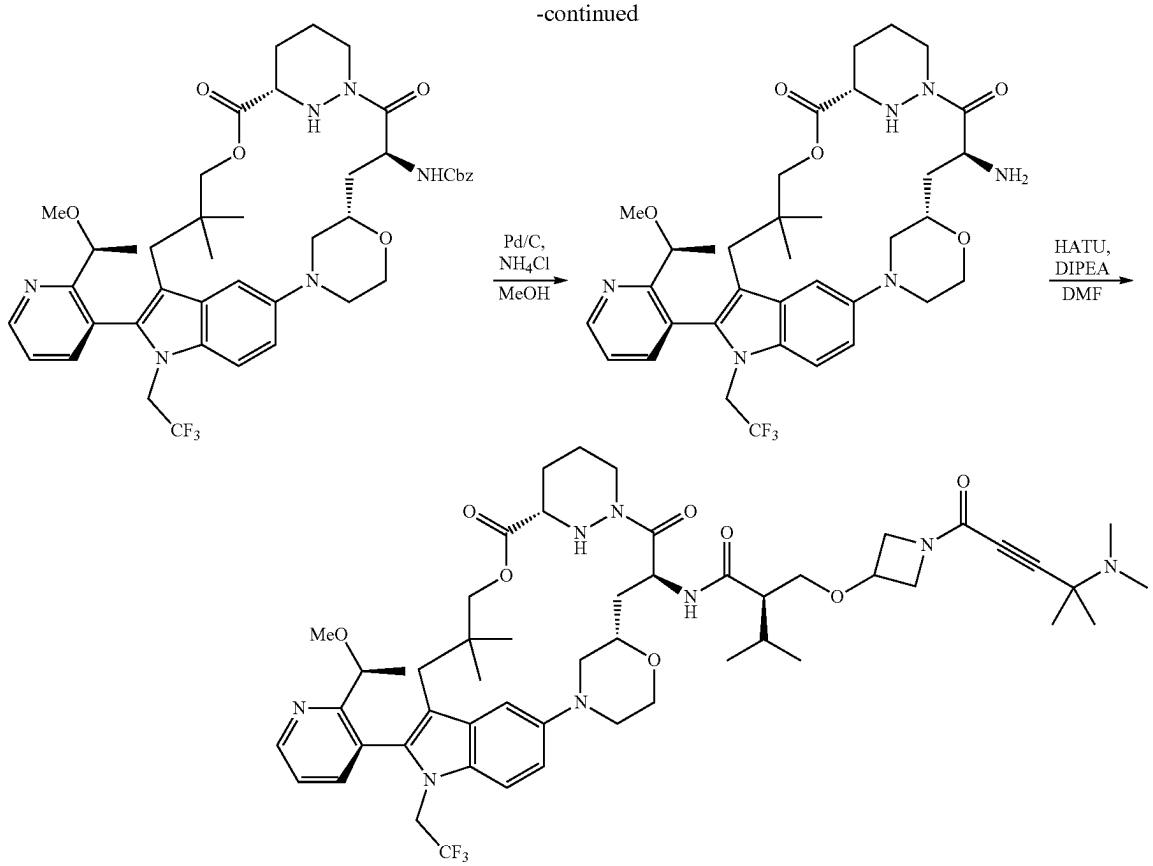


Step 1.

To a mixture of 5-5bromo-3-{3-[(tert-butyldiphenylsiyl)oxy]-2,2-dimethylpropyl}-2-{2-[(1 S)-1-methoxyethyl]pyridin-3-yl}-1H-indole (10.0 g, 15.2 mmol) in anhydrous DMF (120 mL) at 0° C. under an atmosphere of N_2 was added NaH, 60% dispersion in oil (1.2 g, 30.4 mmol) and 2,2,2-trifluoroethyl trifluoromethanesulfonate (35.4 g, 152 mmol). The mixture was stirred at 0° C. for 1 h, then saturated NH_4Cl (30 mL) added and the mixture extracted with EtOAc (100 mL×3). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give (S)-5-bromo-3-(3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-(1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1H-indole (8 g) as an oil and the other atropisomer (6 g, 48% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{39}H_{44}BrF_3N_2O_2Si$ 736.2; found 737.1.

Step 2.

To a mixture of (S)-5-bromo-3-(3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-(1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1H-indole (7.2 g, 9.7 mmol) in toulene (80 mL) under an atmosphere of N_2 was added 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (2.7 g, 10.6 mmol), KOAc (1.9 g, 19.4 mmol) and Pd(dppf)Cl_2 DCM (0.8 g, 0.1 mmol). The mixture was heated to 90° C. and stirred for 8 h, then saturated NH_4Cl (30 mL) added and the mixture extracted with EtOAc (40 mL×3). The combined organic layers were dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give (S)-3-(3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-(1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-(2,2,2-trifluoroethyl)-1H-indole (6.1 g, 64% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{45}H_{56}BF_3N_2O_4Si$ 784.4; found 785.3.

Step 3.

To a mixture of (S)-3-(3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-(1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-(2,2,2-trifluoroethyl)-1H-indole (33 g, 42 mmol) in THF (120 mL) and MeOH (330 mL) at 0° C. under an atmosphere of N_2 was added $MeB(OH)_2$ (50.4 g, 841 mmol), then a mixture of NaOH (33.6 g, 841 mmol) in H_2O (120 mL). The mixture was warmed to room temperature and stirred for 16 h, then concentrated under reduced pressure. H_2O (500 mL) was added to the residue and the mixture extracted with EtOAc (300 mL×3). The combined organic layers were washed with brine (300 mL), H_2O (300 mL), then concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give (S)-(3-(3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-(1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1H-indol-5-yl) boronic acid (20 g, 68% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{39}H_{46}BF_3N_2O_4Si$ 702.3; found 703.3.

Step 4.

Note: Three reactions were run in parallel—the yield reflects the sum of the products.

A mixture of (S)-(3-(3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-(1-methoxyethyl)pyridin-3-yl)-1-(2,2,

2-trifluoroethyl)-1H-indol-5-yl)boronic acid (1.85 g, 5.6 mmol) and methyl (S)-2-(((benzyloxy)carbonyl)amino)-3-((S)-morpholin-2-yl)propanoate in DCM (150 mL) under air was added pyridine (1.35 g, 16.9 mmol) and $Cu(OAc)_2$ (2.0 g, 11.3 mmol). The mixture was stirred for 48 h, then concentrated under reduced pressure. H_2O (300 mL) was added to the residue and the mixture was extracted with EtOAc (300 mL×2). The combined organic layers were washed with brine (100 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was silica gel column chromatography to give methyl (S)-2-(((benzyloxy)carbonyl)amino)-3-((S)-4-(3-(3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1H-indol-5-yl)morpholin-2-yl)propanoate (9.2 g, 55% yield) as a solid. LCMS (ESI): m/z [M/2+H] calc'd for $C_{55}H_{65}F_3N_4O_7Si$ 490.2; found 490.3.

Step 5.

To a mixture of methyl (S)-2-(((benzyloxy)carbonyl)amino)-3-((S)-4-(3-(3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1H-indol-5-yl)morpholin-2-yl)propanoate (10.8 g, 11.0 mmol) in THF (50 mL) was added LiOH (528 mg, 22 mmol) in H_2O (10 mL). The mixture was stirred for 1 h, then cooled to 0-5° C. and acidified to pH~7 using 2N HCl (10 mL). The mixture was extracted with DCM (100 mL×2) and the combined organic layers were dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to give (S)-2-(((benzyloxy)carbonyl)amino)-3-((S)-4-(3-(3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1H-indol-5-yl)morpholin-2-yl)propanoic acid (10.6 g, 100% yield) as a solid. LCMS (ESI): m/z [M/2+H] calc'd for $C_{54}H_{63}F_3N_4O_7Si$ 483.2; found 483.3.

Step 6.

To a mixture of (S)-2-(((benzyloxy)carbonyl)amino)-3-((S)-4-(3-(3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1H-indol-5-yl)morpholin-2-yl)propanoic acid (10.6 g, 11.0 mmol) and methyl (3S)-1,2-diazinane-3-carboxylate (15.8 g, 22.0 mmol) in DMF (150 mL) at 0° C. was added DIPEA (28.4 g, 220 mmol) and HATU (8.4 g, 22.0 mmol). The mixture was stirred at 0~5° C. for 1 h, then EtOAc (500 mL) was added and the mixture was washed with H_2O (200 mL×2), brine (100 mL), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give methyl (S)-1-((S)-2-(((benzyloxy)carbonyl)amino)-3-((S)-4-(3-(3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1H-indol-5-yl)morpholin-2-yl)propanoyl)hexahydropyridazine-3-carboxylate (11 g, 90% yield) as a solid. LCMS (ESI): m/z [M/2+H] calc'd for $C_{60}H_{73}F_3N_6O_8Si$ 546.3; found 546.3.

Step 7.

To a mixture of methyl (S)-1-((S)-2-(((benzyloxy)carbonyl)amino)-3-((S)-4-(3-(3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1H-indol-5-yl)morpholin-2-yl)propanoyl)hexahydropyridazine-3-carboxylate (11.0 g, 10.1 mmol) in THF (10 mL) was added a mixture of AcOH (21.2 g, 353 mmol) and 1M TBAF in THF (300 mL, 300 mmol). The mixture was heated to 80° C. and stirred for 16 h, then concentrated under reduced pressure. EtOAc (800 mL) was added to the residue and the mixture was washed with H_2O (80 mL×6), concentrated under reduced pressure and the residue was purified by preparative-HPLC to give methyl (S)-1-((S)-2-(((benzyloxy)carbonyl)amino)-3-((S)-4-(3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1H-indol-5-yl)morpholin-2-yl)propanoyl)hexahydropyridazine-3-carboxylate (7.9 g, 91% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{44}H_{55}F_3N_6O_8$ 852.4; found 853.3.

Step 8.

To a mixture of methyl (S)-1-((S)-2-(((benzyloxy)carbonyl)amino)-3-((S)-4-(3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1H-indol-5-yl)morpholin-2-yl)propanoyl)hexahydropyridazine-3-carboxylate (7.9 g, 9.3 mmol) in THF (50 mL) was added LiOH (443 mg, 18.5 mmol) in H_2O (10 mL). The mixture was stirred for 1 h, then cooled to 0-5° C. and acidified to ~pH 7 with 2N HCl (9 mL). The mixture was extracted with DCM (100 mL×2) and the combined organic layers were dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure to give (S)-1-((S)-2-(((benzyloxy)carbonyl)amino)-3-((S)-4-(3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1H-indol-5-yl)morpholin-2-yl)propanoyl)hexahydropyridazine-3-carboxylic acid (7.6 g, 98% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{43}H_{53}F_3N_6O_8$ 838.4; found 839.3.

Step 9.

To a mixture of (S)-1-((S)-2-(((benzyloxy)carbonyl)amino)-3-((S)-4-(3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1H-indol-5-yl)morpholin-2-yl)propanoyl)hexahydropyridazine-3-carboxylic acid (7.6 g, 9.0 mmol) and DIPEA (52.3 g, 405 mmol) in DCM (800 mL) under an atmosphere of Ar was added EDCI (77.6 g, 405 mmol) and HOBT (12 g, 90 mmol). The mixture was stirred for 16 h, then concentrated under reduced pressure. The residue was diluted with EtOAc (500 mL), washed with H_2O (100 mL×2) and filtered. The organic layer was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give benzyl ((2^{2}S,6^{3}S,4S)-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (6.1 g, 74% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{43}H_{51}F_3N_6O_7$ 820.4; found 821.3.

Step 10.

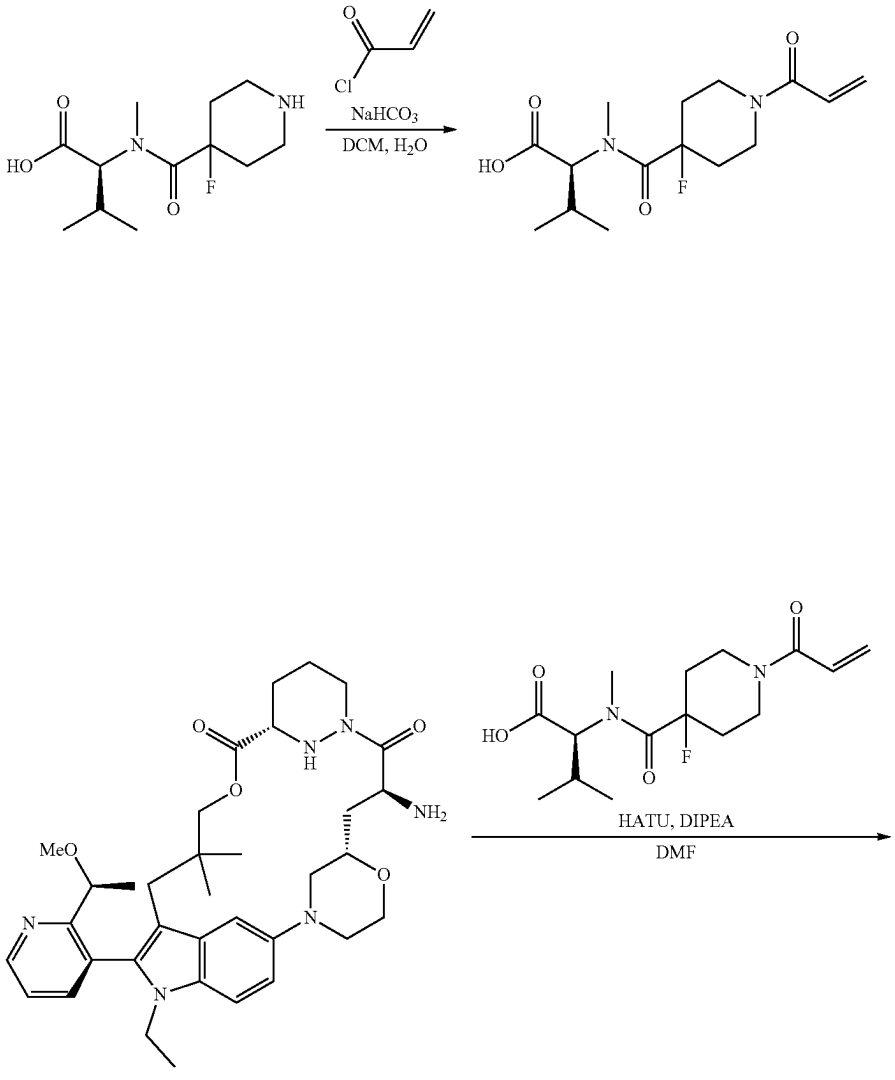
To a mixture of benzyl ((2^{2}S,6^{3}S,4S)-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (700 mg, 0.85 mmol) in MeOH (30 mL) was added 10% Pd on C (317 mg) and NH_4Cl (909 mg). The mixture was stirred under an atmosphere of H_2 (1 atm) for 16 h, then filtered through Celite and the filter cake was washed with MeOH (150 mL). The filtrate was concentrated under reduced pressure, DCM (20 mL) was added to the residue and the mixture was washed with saturated $NaHCO_3$ (20 mL×3). The organic layer was concentrated under reduced pressure to give (2^{2}S,6^{3}S,4S)-4-amino-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)- pyridazinacycloundecaphane-5,7-dione (660 mg, 95% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{35}H_{45}F_3N_6O_5$ 686.3; found 687.3; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.80 (dd, J=4.7, 1.7 Hz, 1H), 7.66 (d, J=7.4 Hz, 1H), 7.43-7.30 (m, 2H), 7.12-7.01 (m, 2H), 4.90-4.83 (m,

1H), 4.68 (d, J=12.5 Hz, 1H), 4.57 (dd, J=16.2, 8.1 Hz, 1H), 4.24 (q, J=6.1 Hz, 1H), 4.08 (d, J=10.6 Hz, 1H), 3.97-3.82 (m, 4H), 3.80-3.68 (m, 2H), 3.55 (d, J=11.6 Hz, 1H), 3.21 (d, J=9.4 Hz, 1H), 2.93 (dd, J=19.9, 9.3 Hz, 3H), 2.66 (t, J=11.6 Hz, 1H), 2.47 (d, J=14.5 Hz, 1H), 2.19-2.04 (m, 4H), 1.96 (d, J=13.6 Hz, 2H), 1.80-1.71 (m, 2H), 1.66-1.59 (m, 1H), 1.47 (d, J=6.1 Hz, 3H), 0.88 (s, 3H), 0.42 (s, 3H).

Step 11.

To a mixture of (2^2S,6^3S,4S)-4-amino-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (300 mg, 0.4 mmol), (2R)-2-[({1-[4-(dimethylamino)-4-methylpent-2-ynoyl]azetidin-3-yl}oxy)methyl]-3-methylbutanoic acid (157 mg, 0.48 mmol) and DIPEA (569.0 mg, 0.4 mmol) in DMF (5 mL) at 0° C. was added HATU (217 mg, 0.57 mmol). The mixture was stirred at 0° C. for 0.5 h, then diluted with H_2O and extracted with EtOAc (20 mL×3). The combined organic layers were washed with brine (20 mL×3), dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-TLC to give (2R)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-N-((2^2S,6^3S,4S)-12-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1'-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methylbutanamide (200 mg, 46% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{52}H_{71}F_3N_8O_8$ 992.5; found 993.4; ^{1}H NMR (400 MHz, CD_3OD) δ 8.74 (m, 1H), 8.00 (m, 1H), 7.85 (d, J=7.7 Hz, 1H), 7.60-7.43 (m, 2H), 7.14 (t, J=9.5 Hz, 2H), 5.63 (s, 1H), 5.06 (m, 1H), 4.64 (s, 1H), 4.52-4.31 (m, 3H), 4.27-4.05 (m, 3H), 3.97 (m, 1H), 3.92-3.66 (m, 6H), 3.59 (m, 2H), 3.46 (m, 2H), 3.25 (d, J=5.3 Hz, 3H), 3.07-2.89 (m, 2H), 2.86-2.59 (m, 3H), 2.38-2.32 (m, 3H), 2.28 (s, 3H), 2.13 (m, 2H), 2.03-1.51 (m, 6H), 1.50-1.41 (m, 6H), 1.38 (d, J=7.5 Hz, 3H), 0.98 (t, J=8.7 Hz, 6H), 0.89 (t, J=6.4 Hz, 3H), 0.54 (d, J=8.4 Hz, 3H).

Example A722. Synthesis of 1-acryloyl-N-((2S)-1-(((6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-4-fluoro-N-methylpiperidine-4-carboxamide



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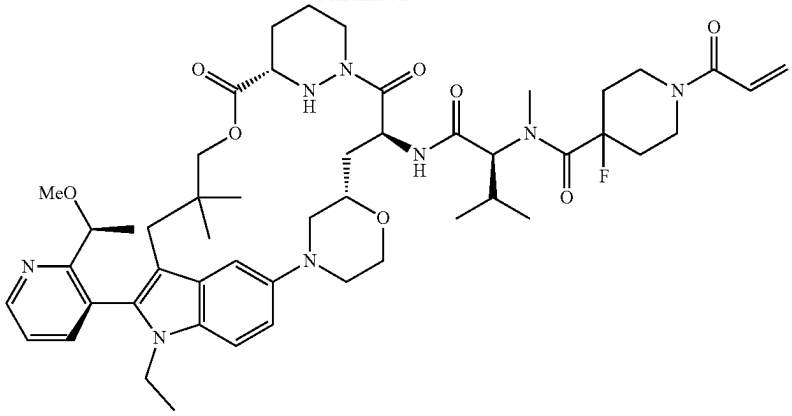


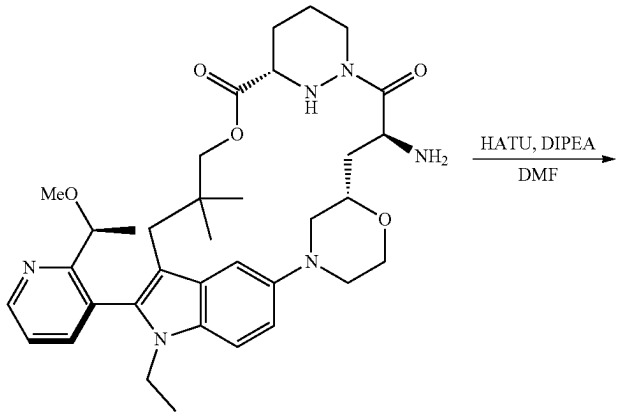
Step 1.

To a mixture of N-(4-fluoropiperidine-4-carbonyl)-N-methyl-L-valine (190 mg, 0.73 mmol) and $NaHCO_3$ (306 mg, 3.6 mmol) in DCM (2 mL) and H_2O (1 mL) at −10° C. was added prop-2-enoyl chloride (132 mg, 1.45 mmol). The mixture was stirred at 0-5° C. for 1 h, then diluted with DCM (20 mL) and washed with H_2O (20 mL×2), brine (20 mL) and the organic layer dried over Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give N-(1-acryloyl-4-fluoropiperidine-4-carbonyl)-N-methyl-L-valine (120 mg, 52% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{15}H_{23}FN_2O_4$ 314.2; found 315.2.

Step 2.

To a mixture of (6^3S,4S)-4-amino-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (153 mg, 0.24 mmol), N-(1-acryloyl-4-fluoropiperidine-4-carbonyl)-N-methyl-L-valine (106 mg, 0.34 mmol) in DMF (2 mL) at 5° C. was added HATU (110 mg, 0.29 mmol) and DIPEA (468 mg, 3.6 mmol) dropwise. The mixture was stirred at 5° C. for 1 h, then purified by preparative-HPLC to give 1-acryloyl-N-((2S)-1-(((6^3S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)- pyridazinacycloundecaphane-4-yl) amino)-3-methyl-1-oxobutan-2-yl)-4-fluoro-N-methylpiperidine-4-carboxamide (69.5 mg, 29% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{50}H_{69}FN_8O_8$ 928.5; found 929.4; ^{1}H NMR (400 MHz, CD_3OD) δ 8.71 (d, J=3.2 Hz, 1H), 7.86 (d, J=7.7 Hz, 1H), 7.51 (dd, J=7.7, 4.8 Hz, 1H), 7.39 (d, J=8.9 Hz, 1H), 7.18-7.02 (m, 2H), 6.80 (dd, J=16.8, 10.7 Hz, 1H), 6.23 (d, J=16.8 Hz, 1H), 5.77 (d, J=10.6 Hz, 1H), 5.67 (d, J=6.5 Hz, 1H), 4.61 (d, J=11.1 Hz, 1H), 4.44 (t, J=15.1 Hz, 2H), 4.23 (q, J=6.1 Hz, 1H), 4.18-4.10 (m, 1H), 4.09-4.01 (m, 1H), 3.99-3.83 (m, 3H), 3.83-3.65 (m, 4H), 3.58-3.46 (m, 2H), 3.27 (s, 1H), 3.21-3.11 (m, 6H), 3.00-2.91 (m, 2H), 2.85-2.75 (m, 2H), 2.73-2.64 (m, 1H), 2.62-2.54 (m, 1H), 2.36-2.21 (m, 2H), 2.19-2.01 (m, 5H), 1.92 (d, J=12.7 Hz, 2H), 1.79-1.57 (m, 2H), 1.44 (d, J=6.2 Hz, 3H), 1.04 (t, J=6.3 Hz, 3H), 0.98-0.81 (m, 6H), 0.76 (s, 3H), 0.68 (s, 3H).

Example A377. Synthesis of (2R)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-N-(((2^2S,6^3S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methylbutanamide



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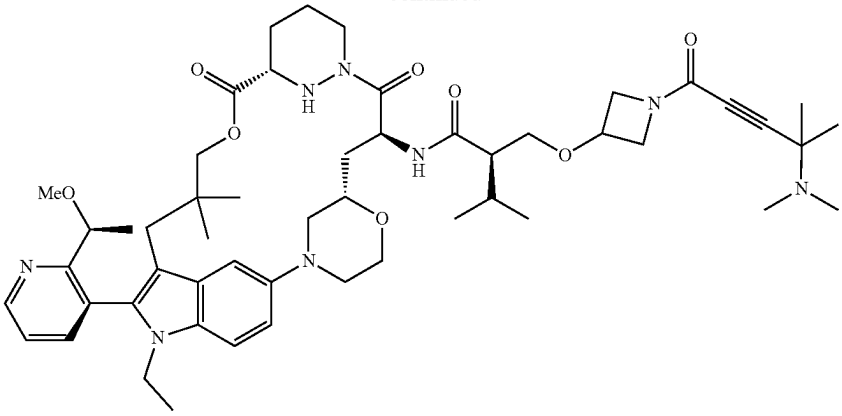
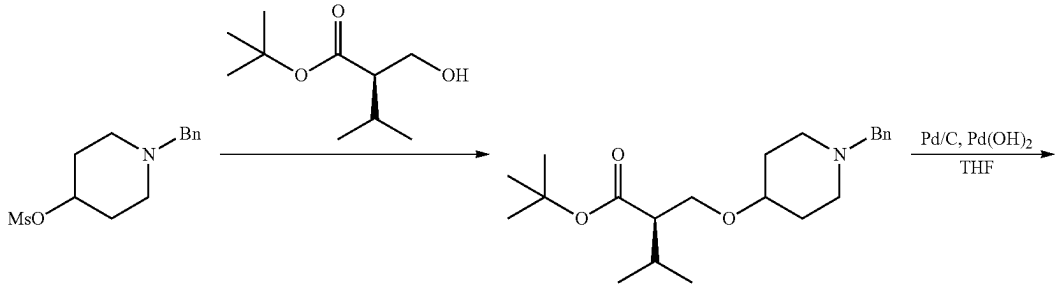


Step 1.

(2R)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-N-((2^{2}S,6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl) pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methylbutanamide was synthesized in a manner similar to (2R)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-N-((2^{2}S,6^{3}S,4S)-12-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methylbutanamide except (2^{2}S,6^{3}S,4S)-4-amino-1^2-(2-((S)-1-methoxyethyl) pyridin-3-yl)-10,10-dimethyl-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione was substituted with (2^{2}S,6^{3}S,4S)-4-amino-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione and 3-({1-[4-(dimethylamino)-4-methylpent-2-ynoyl]azetidin-3-yl}oxy)propanoic acid was substituted with (2R)-2-[({1-[4-(dimethylamino)-4-methylpent-2-ynoyl]azetidin-3-yl}oxy)methyl]-3-methylbutanoic acid to give the desired product (25.6 mg, 26% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{52}H_{74}N_8O_8$ 938.6; found 939.5; ^{1}H NMR (400 MHz, CD_3OD) δ 8.72 (dd, J=4.8, 1.5 Hz, 1H), 7.97 (dd, J=20.0, 6.8 Hz, 1H), 7.87 (dd, J=5.8, 2.6 Hz, 1H), 7.54-7.51 (m, 1H), 7.41 (d, J=8.9 Hz, 1H), 7.14 (dd, J=36.0, 10.4 Hz, 2H), 5.65 (s, 1H), 4.49-4.33 (m, 3H), 4.27-4.08 (m, 4H), 3.96 (d, J=8.6 Hz, 2H), 3.87 (dd, J=10.8, 3.6 Hz, 2H), 3.79 (dd, J=10.8, 7.7 Hz, 3H), 3.69-3.58 (m, 3H), 3.43 (dd, J=23.9, 11.7 Hz, 2H), 3.17 (d, J=21.9 Hz, 3H), 3.00-2.95 (m, 1H), 2.70 (t, J=14.0 Hz, 8H), 2.34-2.24 (m, 1H), 2.05 (d, J=34.4 Hz, 3H), 1.92-1.82 (m, 2H), 1.69-1.62 (m, 5H), 1.57 (d, J=11.5 Hz, 3H), 1.45 (d, J=6.2 Hz, 3H), 1.33 (d, J=12.6 Hz, 1H), 1.05-0.93 (m, 10H), 0.80 (d, J=9.8 Hz, 3H), 0.64 (d, J=12.2 Hz, 2H).

Example A643. Synthesis of (2R)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)piperidin-4-yl)oxy)methyl)-N-((2^{2}S,6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methylbutanamide



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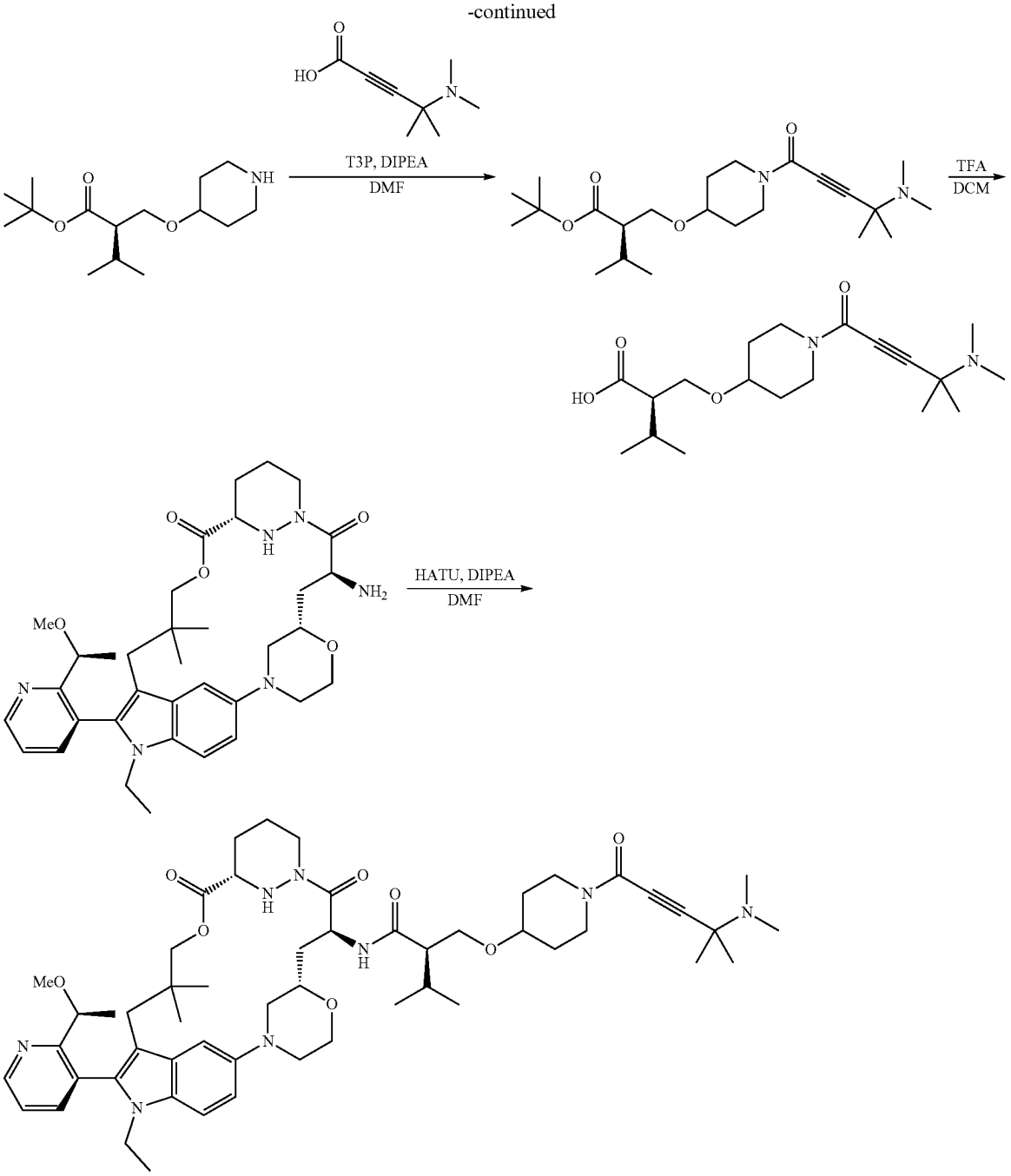


Step 1.

A mixture of 1-(1-methylphenyl)piperidin-4-yl methanesulfonate (2 g, 7.4 mmol) and tert-butyl (2R)-2-(hydroxymethyl)-3-methylbutanoate (1.39 g, 7.4 mmol) was stirred at 120° C. for 1 h, then purified by silica gel column chromatography to give tert-butyl (R)-2-(((1-benzylpiperidin-4-yl)oxy)methyl)-3-methylbutanoate (800 mg, 28% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{22}H_{35}NO_3$ 361.3; found 362.3.

Step 2.

A mixture of tert-butyl (R)-2-(((1-benzylpiperidin-4-yl)oxy)methyl)-3-methylbutanoate (700 mg, 1.9 mmol), 10% wet Pd/C (411 mg, 3.9 mmol) and 20% wet $Pd(OH)_2/C$ (542 mg, 3.9 mmol) in THF (30 mL) was stirred under an atmosphere of H_2 (15 psi) for 16 h. The mixture was filtered and the filtrate was concentrated under reduced pressure to give tert-butyl (R)-3-methyl-2-((piperidin-4-yloxy)methyl)butanoate (440 mg, 80% yield) as a an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{15}H_{29}NO_3$ 271.2; found 272.2.

Step 3.

To a mixture of tert-butyl (R)-3-methyl-2-((piperidin-4-yloxy)methyl)butanoate (440 mg, 1.6 mmol) 4-(dimethylamino)-4-methylpent-2-ynoic acid (3.77 g, 24.3 mmol) and DIPEA (2.09 g, 16.2 mmol) in DMF (50 mL) at 0° C. was added T3P (2.57 g, 8.1 mmol). The mixture was stirred at 0° C. for 1 h, then poured into H_2O (50 mL) and extracted with

EtOAc (50 mL×3). The combined organic layers were washed with brine, concentrated under reduced pressure and the residue purified by silica gel column chromatography to give tert-butyl (R)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)piperidin-4-yl)oxy)methyl)-3-methylbutanoate (190 mg, 27% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{23}H_{40}N_2O_4$ 408.3; found 409.4.

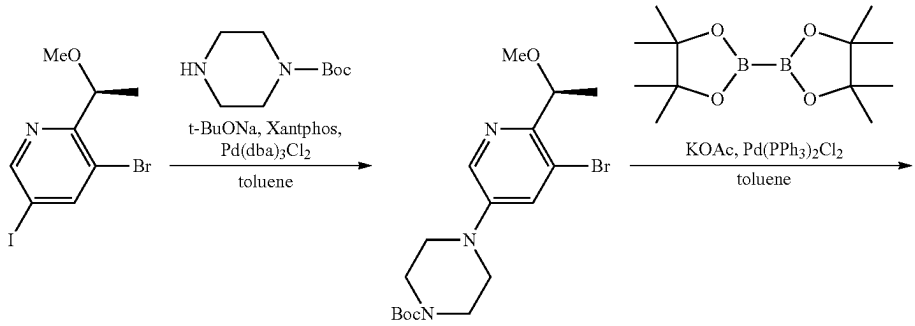
Step 4.

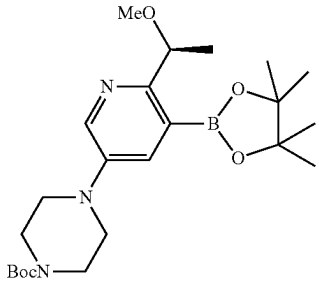
To a mixture of tert-butyl (R)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)piperidin-4-yl)oxy)methyl)-3-methylbutanoate (180 mg, 0.47 mmol) in DCM (2 mL) was added TFA (1 mL). The mixture was stirred at room temperature for 1 h, then concentrated under reduced pressure to give (R)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)piperidin-4-yl)oxy)methyl)-3-methylbutanoic acid (170 mg, 98% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{19}H_{32}N_2O_4$ 352.2; found 353.2.

Step 5.

(2R)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)piperidin-4-yl)oxy)methyl)-N-((2^{2}S,6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methylbutanamide was synthesized in a manner similar to (2R)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-N-((2^{2}S,6^{3}S,4S)-12-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methylbutanamide except (2^{2}S,6^{3}S,4S)-4-amino-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione was substituted with (2^{2}S,6^{3}S,4S)-4-amino-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-morpholina-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione and 3-({1-[4-(dimethylamino)-4-methylpent-2-ynoyl]azetidin-3-yl}oxy)propanoic acid was substituted with (2R)-2-[({1-[4-(dimethylamino)-4-methylpent-2-ynoyl]piperidin-4-yl}oxy)methyl]-3-methylbutanoic acid. (101 mg, 42% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{54}H_{78}N_8O_8$ 966.6; found 969.5; ^{1}H NMR (400 MHz, CD_3OD) δ 8.70 (d, J=4.8 Hz, 1H), 7.81-7.76 (m, 1H), 7.56-7.46 (m, 1H), 7.43-7.34 (m, 1H), 7.24-7.01 (m, 2H), 5.66-5.54 (m, 1H), 4.50-4.40 (m, 1H), 4.31-4.22 (m, 1H), 4.19-4.08 (m, 1H), 4.02-3.82 (m, 4H), 3.80-3.53 (m, 10H), 3.47-3.34 (m, 2H), 3.26-3.15 (m, 3H), 2.98-2.57 (m, 5H), 2.37-2.30 (m, 3H), 2.27-2.18 (m, 4H), 2.15-2.02 (m, 2H), 2.00-1.80 (m, 4H), 1.78-1.71 (m, 2H), 1.68-1.55 (m, 3H), 1.49-1.37 (m, 6H), 1.35-1.28 (m, 3H), 1.05-0.92 (m, 9H), 0.85-0.72 (m, 3H), 0.68-0.51 (m, 3H).

Example A328. Synthesis of two atropisomers of (3S)-1-acryloyl-N-((2S)-1-(((6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-N-methylpyrrolidine-3-carboxamide





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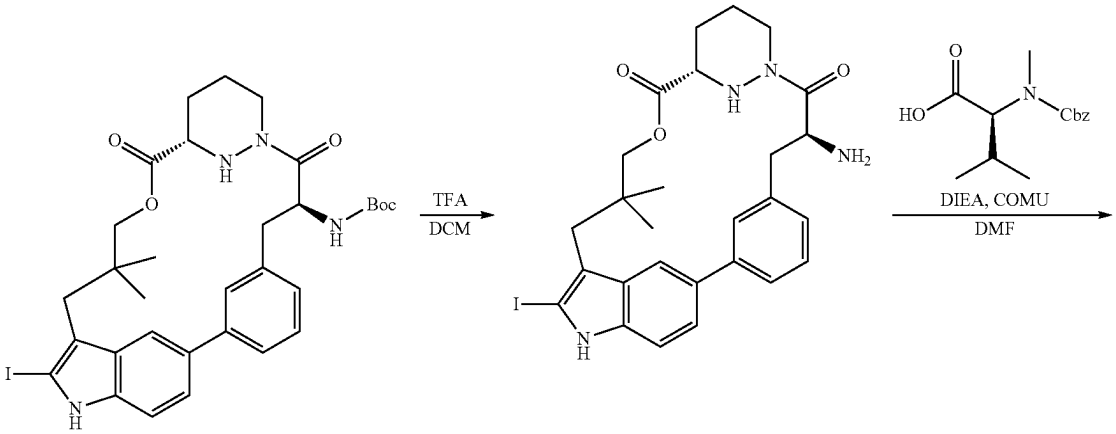
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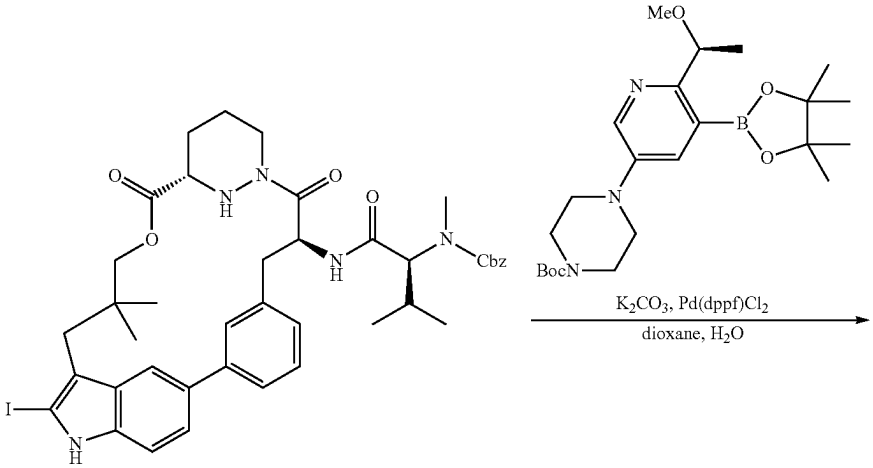
DCM
DIEA, COMU
DMF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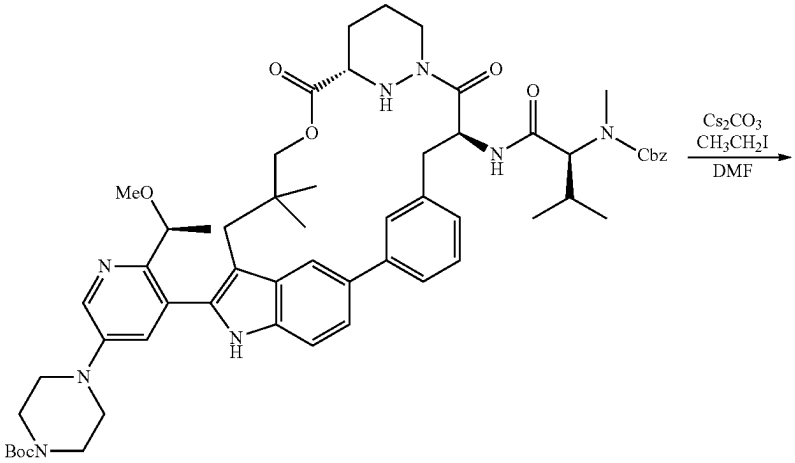
dioxane, H_2O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DMF

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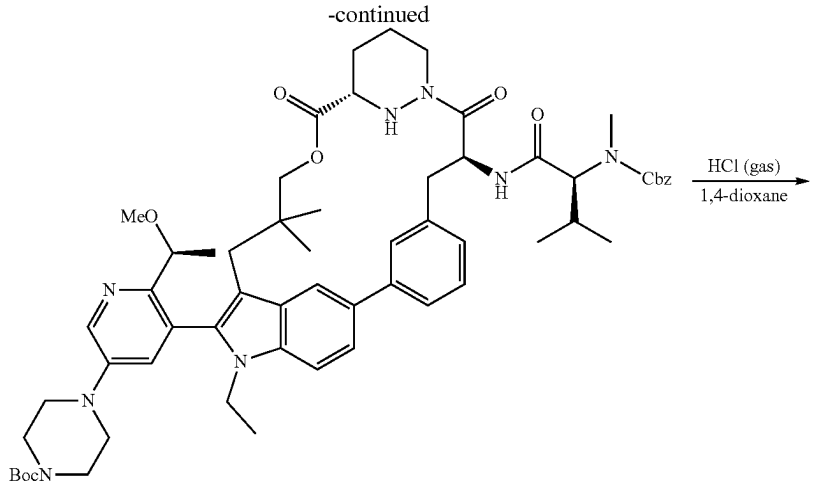
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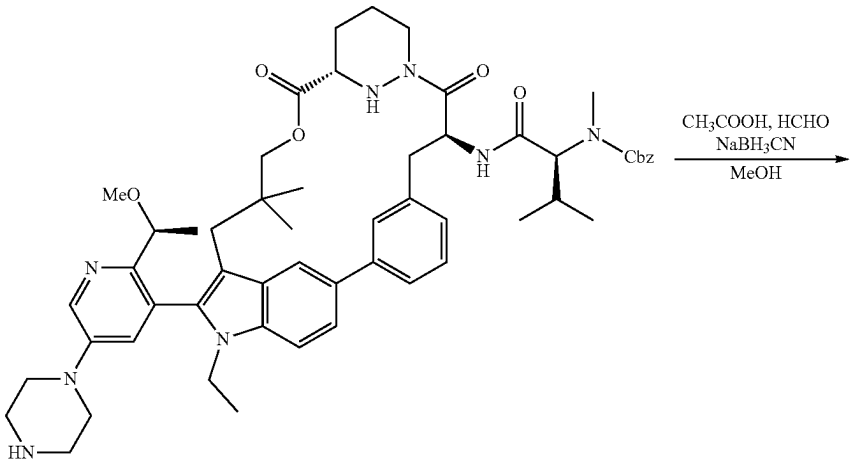
1,4-dioxane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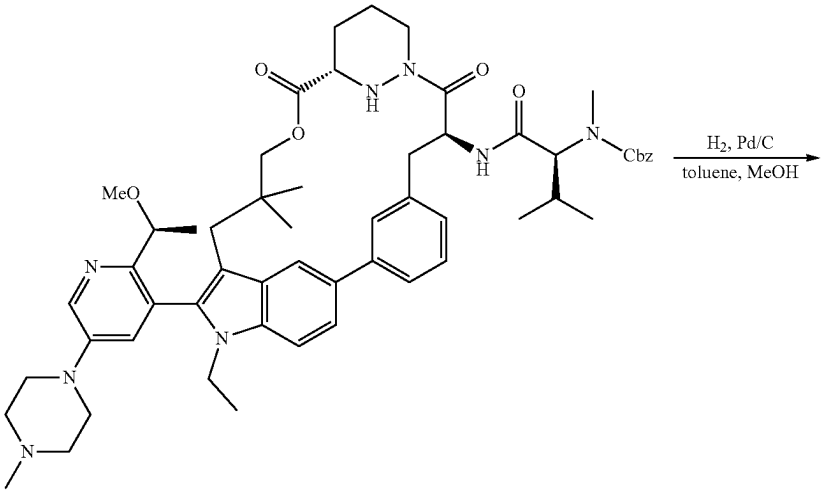
NaBH3CN
MeOH


H2, Pd/C
toluene, MeOH

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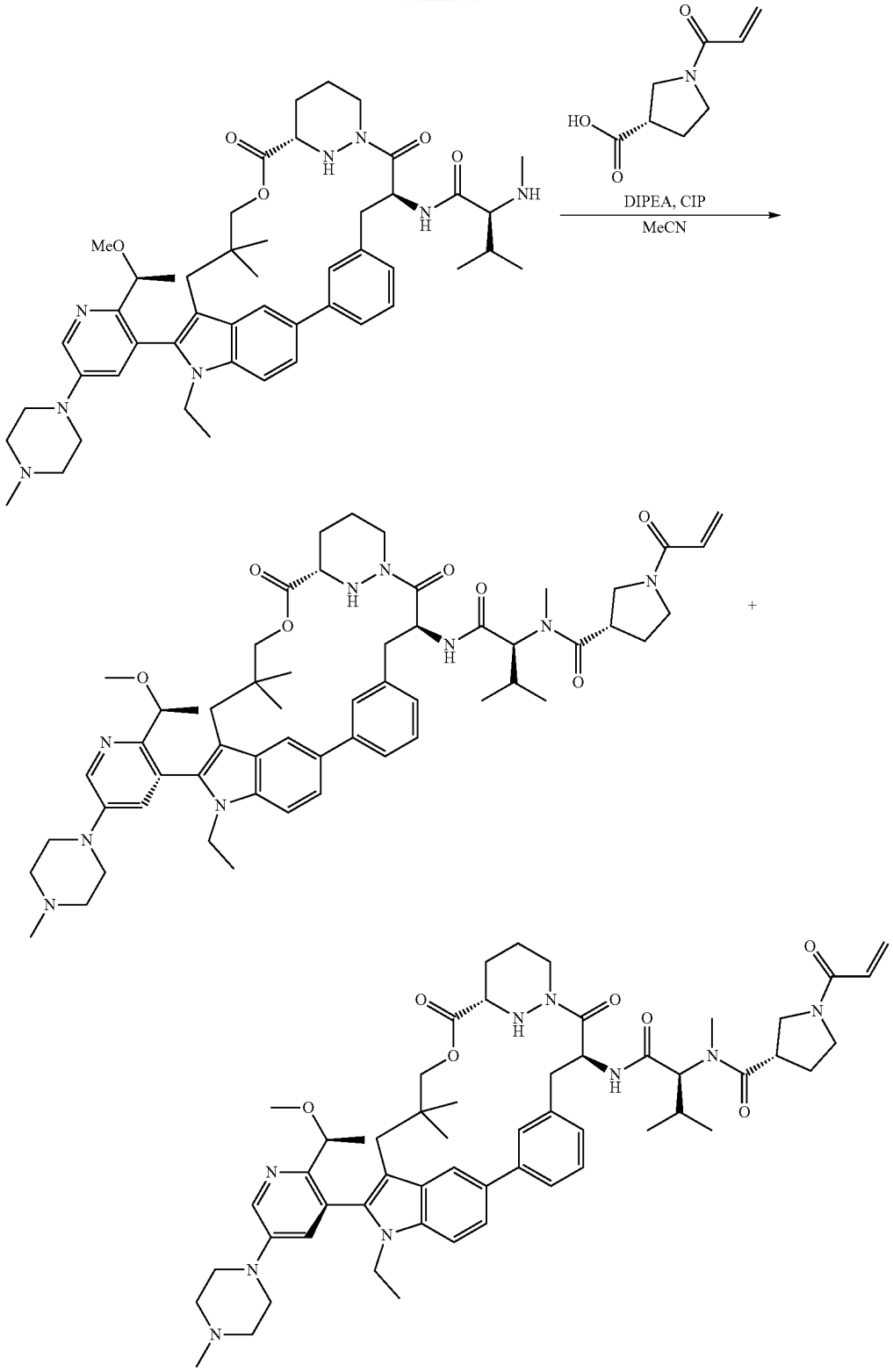


assumed

Step 1.

To a mixture of 3-bromo-5-iodo-2-[(1S)-1-methoxyethyl] pyridine (2.20 g, 6.4 mmol) and tert-butyl piperazine-1-carboxylate (1.20 g, 6.4 mmol) in toluene (50 mL) under an atmosphere of Ar were added tBuONa (0.74 g, 7.7 mmol) and portion-wise addition of $Pd_2(dba)_3$ (0.59 g, 0.64 mmol),

followed by portion-wise addition of Xantphos (0.74 g, 1.3 mmol). The mixture was heated to 100° C. and stirred for 16 h then H_2O added and the mixture extracted with EtOAc (400 mL×3). The combined organic layers were washed with brine (150 mL×3), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-HPLC to give tert-butyl 4-[5-bromo-6-[(1S)-1-methoxyethyl]pyridin-3-yl]piperazine-1-carboxylate (1.7 g, 61% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{17}H_{26}BrN_3O_3$ 399.1; found 400.1.

Step 2.

A mixture of tert-butyl 4-[5-bromo-6-[(1 S)-1-methoxyethyl]pyridin-3-yl]piperazine-1-carboxylate (1.76 g, 4.4 mmol) and 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (1.67 g, 6.6 mmol) in toluene (18 mL) under an atmosphere of Ar were added KOAc (0.95 g, 9.7 mmol) and $Pd(PPh_3)_2Cl_2$ (0.31 g, 0.44 mmol) in portions. The mixture was heated to 80° C. and stirred for 16 h, then diluted with H_2O and the mixture extracted with EtOAc (500 mL×3). The combined organic layers were washed with brine (100 mL×3), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give tert-butyl 4-[6-[(1 S)-1-methoxyethyl]-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-3-yl]piperazine-1-carboxylate (1.4 g, 68% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{23}H_{38}BN_3O_5$ 447.4; found 448.2.

Step 3.

To a mixture of tert-butyl ((6^{3}S,4S)-1^2-iodo-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (1.0 g, 1.5 mmol) in DCM (10 mL) at 0 'C under an atmosphere of N_2 was added TFA (5.0 mL, 67.3 mmol) in portions. The mixture was stirred at 0° C. for 1 h then concentrated under reduced pressure and dried azeotropically with toluene (3 mL×3) to give (6^{3}S,4S)-4-amino-1^2-iodo-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (1.0 g), which was used directly in the next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{27}H_{31}IN_4O_3$ 586.1; found 587.3.

Step 4.

To a mixture of (6^{3}S,4S)-4-amino-1^2-iodo-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione (1.0 g, 1.7 mmol) in DMF (15 mL) at 0° C. under an atmosphere of N_2 were added DIPEA (2.20 g, 17.0 mmol) and (2S)-2-[[(benzyloxy)carbonyl](methyl)amino]-3-methylbutanoic acid (0.90 g, 3.4 mmol) in portions, followed by COMU (1.10 g, 2.6 mmol) in portions over 10 min. The mixture was stirred at 0° C. for 1.5 h, then diluted with H_2O and the mixture was extracted with EtOAc (300 mL×3). The combined organic layers were washed with brine (100 mL×3), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-HPLC to give benzyl ((2S)-1-(((6^{3}S,4S)-1^2-iodo-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate (790 mg, 53% yield) as a solid.

Step 5.

To a mixture of benzyl ((2S)-1-(((6^{3}S,4S)-1^2-iodo-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate (480 mg, 0.58 mmol) and tert-butyl 4-[6-[(1S)-1-methoxyethyl]-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-3-yl]piperazine-1-carboxylate (309 mg, 0.69 mmol) in 1,4-dioxane (8.0 mL) and H_2O (1.6 mL) under an atmosphere of Ar were added K_2CO_3 (199 mg, 1.4 mmol) and $Pd(dppf)Cl_2$ (42 mg, 0.06 mmol) in portions. The mixture was heated to 70° C. and stirred for 16 h, then diluted with H_2O and extracted with EtOAc (200 mL×3). The combined organic layers were washed with brine (150 mL×3), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by silica gel column chromatography to give tert-butyl 4-(5-((6^{3}S,4S)-4-((S)-2-(((benzyloxy)carbonyl)(methyl)amino)-3-methylbutanamido)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-1^2-yl)-6-((S)-1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (335 mg, 51% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{58}H_{74}N_8O_9$ 1026.6; found 1027.4.

Step 6.

To a mixture of tert-butyl 4-(5-((6^{3}S,4S)-4-((S)-2-(((benzyloxy)carbonyl)(methyl)amino)-3-methylbutanamido)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-1^2-yl)-6-((S)-1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (335 mg, 0.33 mmol) in DMF (5 mL) at 0° C. under an atmosphere of N_2 were added Cs_2CO_3 (234 mg, 0.72 mmol) and iodoethane (102 mg, 0.65 mmol) in portions. The mixture was warmed to room temperature and stirred for 16 h, then diluted with H_2O and the mixture extracted with EtOAc (100 mL×3). The combined organic layers were washed with brine (50 mL×3), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-TLC to give tert-butyl 4-(5-((6^{3}S,4S)-4-((S)-2-(((benzyloxy)carbonyl)(methyl)amino)-3-methylbutanamido)-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-1^2-yl)-6-((S)-1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (320 mg, 84% yield) as a light yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{60}H_{78}N_8O_9$ 1054.6; found 1055.8.

Step 7.

A mixture of tert-butyl 4-(5-((6^{3}S,4S)-4-((S)-2-(((benzyloxy)carbonyl)(methyl)amino)-3-methylbutanamido)-1^1-ethyl-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-1^2-yl)-6-((S)-1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (320 mg) in xx M HCl in 1,4-dioxane (3.0 mL) at 0° C. under an atmosphere of N_2 was stirred at room temperature for 2 h, then concentrated under reduced pressure to give benzyl ((2S)-1-(((6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)-5-(piperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate, which was used directly in the next step further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{55}H_{70}N_8O_7$ 954.5; found 955.3.

Step 8.

To a mixture of benzyl ((2S)-1-(((6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)-5-(piperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycl-

oundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate (320 mg, 0.34 mmol) and HCHO (60 mg, 2.0 mmol) in MeOH (3.0 mL) at 0° C. under an atmosphere of N_2 were added $NaCNBH_3$ (42 mg, 0.67 mmol) and AcOH (60 mg, 1.0 mmol) in portions. The mixture was warmed to room temperature and stirred for 2 h, then diluted with H_2O and the mixture extracted with DCM/MeOH (5:1) (200 mL×3). The combined organic layers were washed with brine (100 mL×3), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-HPLC to give benzyl ((2S)-1-(((6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate (160 mg, 59% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{56}H_{72}N_8O_7$ 968.6; found 969.6.

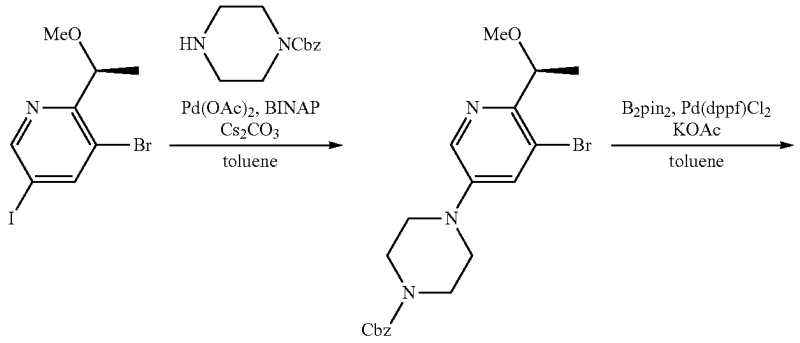
Step 9.

To a mixture of benzyl ((2S)-1-(((6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate (160 mg, 0.17 mmol) in toluene (10 mL) and MeOH (1.0 mL) was added Pd/C (130 mg, 1.2 mmol) in portions. The mixture was evacuated and re-filled with H_2 (×3), then stirred under an atmosphere of H_2 for 16 h. The mixture was filtered and the filtrate was concentrated under reduced pressure to give (2S)—N-((6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)- benzenacycloundecaphane-4-yl)-3-methyl-2-(methylamino)butanamide (140 mg), which was used directly in the next step without further purification. LCMS (ESI): m/z [M+H] calc'd for $C_{48}H_{66}N_8O_5$ 834.5; found 835.5.

Step 10.

To a mixture of (2S)—N-((6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)-3-methyl-2-(methylamino)butanamide (140 mg, 0.17 mmol) in ACN (2.0 mL) at 0° C. under an atmosphere of N_2 were added DIPEA (433 mg, 3.35 mmol), (3S)-1-(prop-2-enoyl)pyrrolidine-3-carboxylic acid (57 mg, 0.34 mmol) in portions and CIP (70 mg, 0.25 mmol) in portions over 10 min. The mixture was stirred at 0° C. for 1.5 h, then H_2O added and the mixture extracted with EtOAc (150 mL×3). The combined organic layers were washed with brine (100 mL×3), dried over anhydrous Na_2SO_4 and filtered. The filtrate was concentrated under reduced pressure and the residue was purified by preparative-HPLC to give two atropisomers of (3S)-1-acryloyl-N-((2S)-1-(((6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-N-methylpyrrolidine-3-carboxamide (40 mg, 24% yield) as a solid and (20 mg, 12% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{56}H_{75}N_9O_7$ 985.6; found 986.7; ^{1}H NMR (400 MHz, DMSO-d$_6$) 8.47 (t, J=2.1 Hz, 1H), 8.00 (d, J=4.7 Hz, 1H), 7.78-7.59 (m, 3H), 7.58-7.48 (m, 1H), 7.42-7.30 (m, 1H), 7.23 (dq, J=8.0, 4.0, 3.5 Hz, 1H), 7.15-7.03 (m, 1H), 6.75-6.50 (m, 1H), 6.18 (dt, J=16.8, 2.7 Hz, 1H), 5.70 (tt, J=9.3, 2.7 Hz, 1H), 5.48-5.23 (m, 1H), 5.06 (dd, J=31.1, 12.3 Hz, 1H), 4.74 (dd, J=11.0, 4.3 Hz, 1H), 4.33-4.15 (m, 2H), 4.01 (ddd, J=36.1, 12.6, 7.6 Hz, 2H), 3.91-3.56 (m, 6H), 3.52-3.39 (m, 2H), 3.31-3.28 (m, 2H), 3.24 (d, J=5.7 Hz, 4H), 3.06 (s, 4H), 2.93 (d, J=9.8 Hz, 2H), 2.81 (d, J=5.4 Hz, 3H), 2.47-2.43 (m, 4H), 2.22 (s, 4H), 2.09 (tq, J=12.0, 7.4, 6.6 Hz, 3H), 1.81 (s, 1H), 1.74 (d, J=11.7 Hz, 1H), 1.56 (d, J=11.7 Hz, 1H), 1.20 (dd, J=6.3, 1.5 Hz, 3H), 1.10 (td, J=7.2, 2.4 Hz, 3H), 1.00-0.86 (m, 6H), 0.86-0.72 (m, 3H), 0.54 (d, J=3.5 Hz, 3H) and LCMS (ESI): m/z [M−H] calc'd for $C_{56}H_{75}N_9O_7$ 985.6; found 984.4; ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 8.46 (d, J=3.0 Hz, 2H), 7.98 (s, 1H), 7.89-7.83 (m, 1H), 7.76-7.57 (m, 3H), 7.24 (s, 2H), 7.07 (s, 1H), 6.70-6.58 (m, 1H), 6.17 (d, J=16.5 Hz, 1H), 5.73-5.67 (m, 1H), 5.36-5.30 (m, 1H), 4.31-3.97 (m, 6H), 3.83-3.77 (m, 2H), 3.74-3.49 (m, 6H), 3.48-3.41 (m, 1H), 3.40-3.37 (m, 2H) 3.28-3.24 (m, 4H), 3.07 (s, 3H), 2.88-2.82 (m, 1H), 2.80-2.64 (m, 7H), 2.49-2.44 (m, 4H), 2.22 (s, 3H), 2.04 (d, J=26.1 Hz, 3H), 1.85-1.79 (m, 1H), 1.67-1.55 (m, 2H), 1.35 (d, J=6.1 Hz, 3H), 1.27-1.22 (m, 1H), 1.05-0.93 (m, 4H), 0.89 (d, J=6.9 Hz, 2H), 0.79 (d, J=12.4 Hz, 5H), 0.73 (d, J=6.5 Hz, 1H), 0.56 (s, 3H).

Example A542. The synthesis of 1-(4-(dimethylamino)-4-methylpent-2-ynoyl)-4-fluoro-N-((2S)-1-(((6^{3}S,4S)-1^2-(2-((S)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-N-methylpiperidine-4-carboxamide



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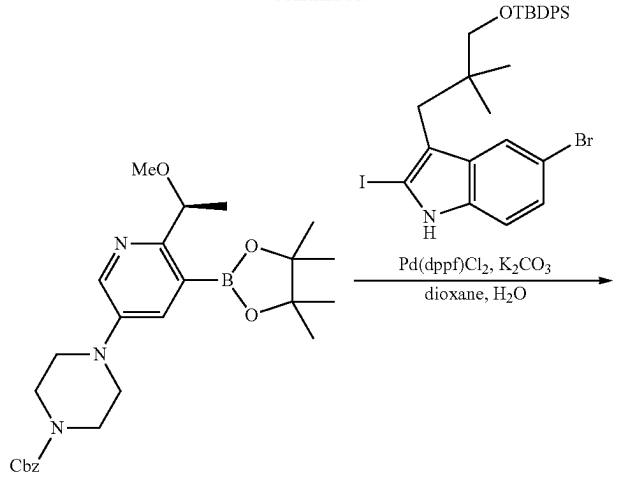
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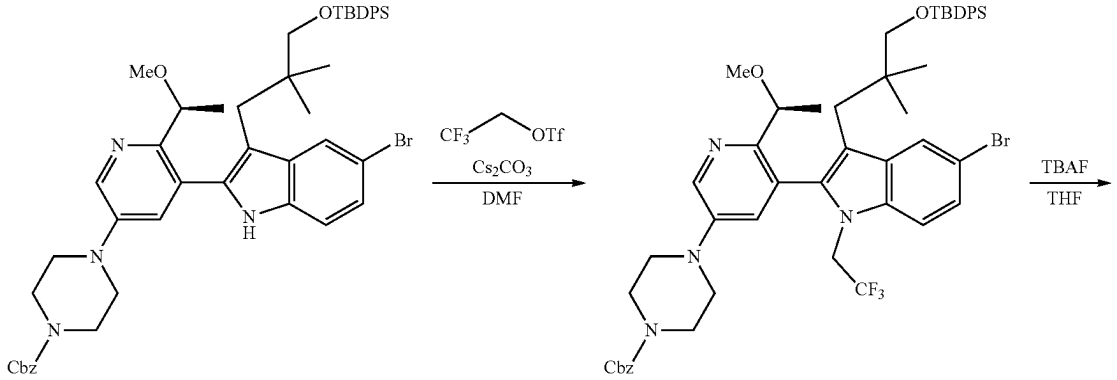
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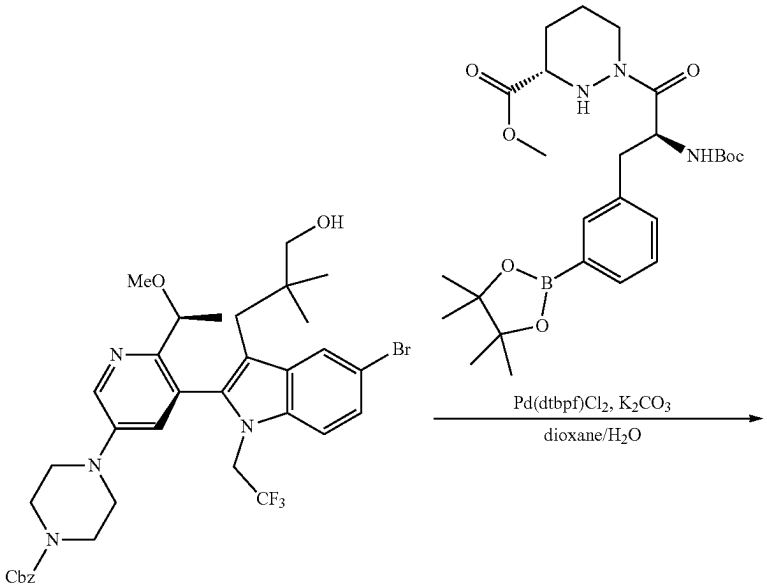
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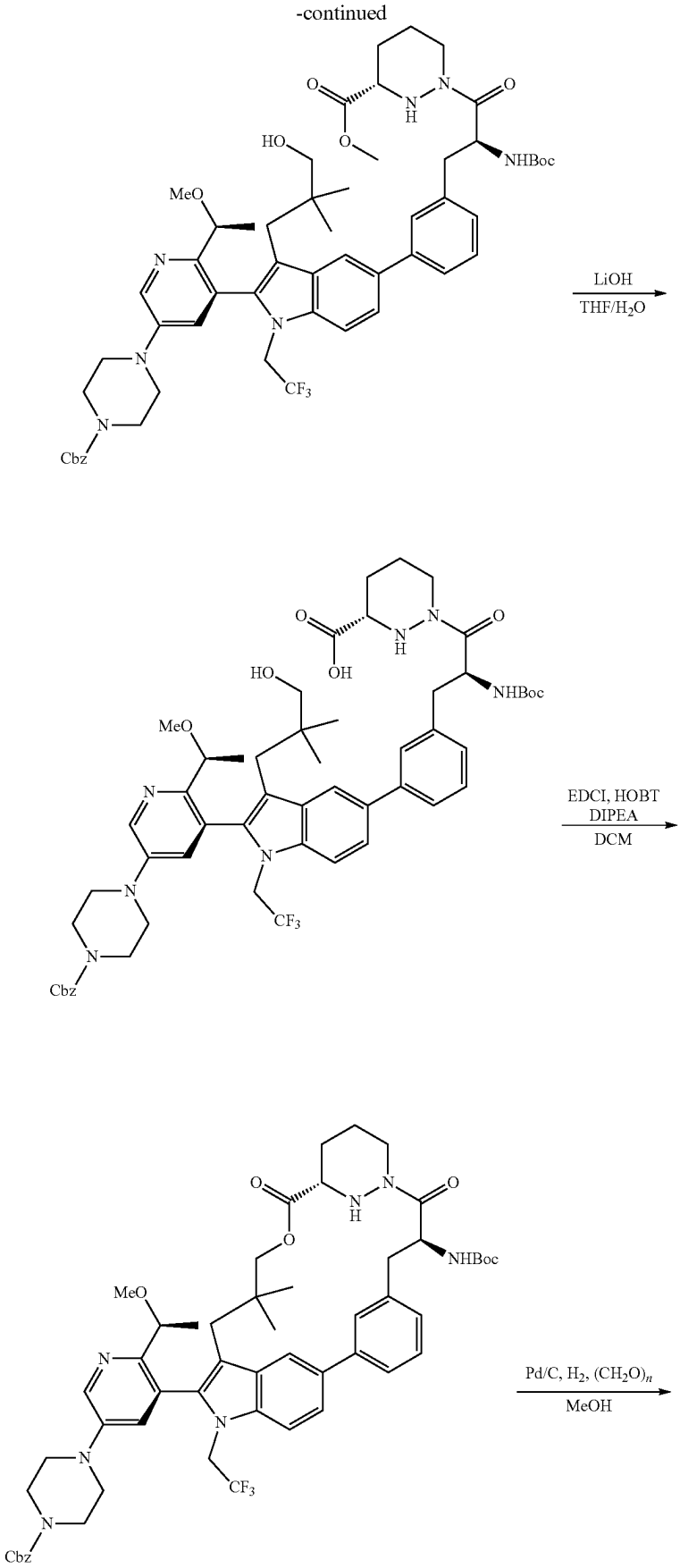

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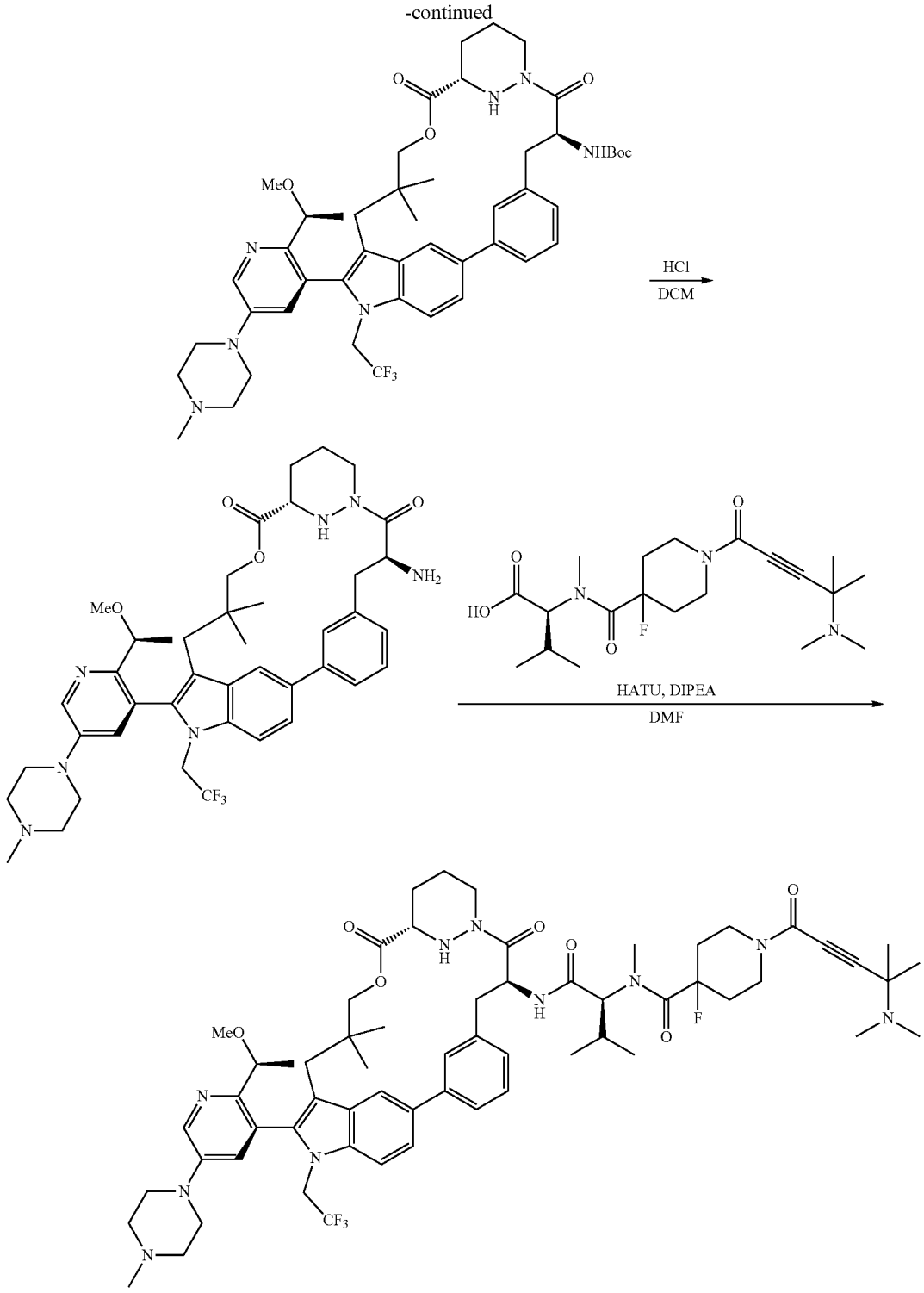


Step 1.

To a solution of (S)-3-bromo-5-iodo-2-(1-methoxyethyl) pyridine (15 g, 43.86 mmol), and benzyl piperazine-1-carboxylate (8.7 g, 39.48 mmol) in toluene (150 mL) at 0° C., were added cesium carbonate (71.46 g, 219.32 mmol), BINAP (0.55 g, 0.88 mmol) and palladium acetate (0.49 g, 2.19 mmol) in portions. The reaction mixture was stirred at 90° C. for 12 h under an argon atmosphere. The resulting mixture was cooled down to room temperature, filtered and the filter cake was washed with EtOAc (150 mL×3). The filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford benzyl (S)-4-(5-bromo-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (16 g, 84% yield) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{44}H_{58}N_6O_7$ 433.1; found 434.0.

Step 2.

To a stirred solution of benzyl (S)-4-(5-bromo-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (22.7 g, 52.26 mmol), bis(pinacolato)diboron (19.91 g, 78.4 mmol) in toluene (230 mL) at 0° C., were added potassium acetate (12.82 g, 130.66 mmol) and Pd(dppf)Cl_2·DCM (4.26 g, 5.23 mmol) in portions. The reaction mixture was stirred at 90° C. for 6 h under an argon atmosphere. The resulting mixture was filtered and the filter cake was washed with EtOAc (200 mL×3). The filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford benzyl (S)-4-(6-(1-methoxyethyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-3-yl)piperazine-1-carboxylate (14.7 g, 58% yield) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{26}H_{36}BN_3O_5$ 481.3; found 482.3.

Step 3.

To a stirred solution of 5-bromo-3-(3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-iodo-1H-indole (17.46 g, 27 mmol) in 1,4-dioxane (150 mL) and H_2O (30 mL) at 0° C., were added potassium carbonate (9.33 g, 67.51 mmol) and Pd(dppf)Cl_2 DCM (2.2 g, 2.7 mmol) in portions, followed by benzyl (S)-4-(6-(1-methoxyethyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) pyridin-3-yl) piperazine-1-carboxylate (13 g, 27 mmol). The reaction mixture was stirred at 70° C. for 12 h under an argon atmosphere. The resulting mixture was cooled to room temperature and quenched with H_2O, then extracted with EtOAc (200 mL×3). The organic phase was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford benzyl (S)-4-(5-(5-bromo-3-(3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-1H-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (20 g, 84.7% yield) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{49}H_{57}BN_4O_4Si$ 873.2; found 873.3.

Step 4.

To a mixture of benzyl (S)-4-(5-(5-bromo-3-(3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-1H-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (19 g, 21.74 mmol) and Cs_2CO_3 (49.58 g, 152.17 mmol) in DMF (190 mL) at 0° C. under argon atmosphere, was dropwise added 2,2,2-trifluoroethyl trifluoromethanesulfonate (50.46 g, 217.39 mmol). The reaction mixture was stirred at room temperature for 12 h under an argon atmosphere, then quenched with H_2O, extracted with EtOAc (200 mL×3). The combined organic layers were washed with brine (200 mL×3), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford benzyl (S)-4-(5-(5-bromo-3-(3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-1-(2,2,2-trifluoroethyl)-1H-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (17.6 g, 84.7% yield) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{51}H_{58}BF_3N_4O_4Si$ 954.2; found 955.3.

Step 5.

To a solution of benzyl (S)-4-(5-(5-bromo-3-(3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-1-(2,2,2-trifluoroethyl)-1H-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl) piperazine-1-carboxylate (18 g, 18.83 mmol), was added TBAF in THF (180.0 mL) at 0° C. The reaction mixture was stirred at 40° C. for 12 h under an argon atmosphere, then quenched with cold H_2O. The resulting mixture was extracted with EtOAc (200 mL×3). The combined organic layers were washed with brine (200 mL×3), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford benzyl (S)-4-(5-(5-bromo-3-(3-hydroxy-2,2-dimethylpropyl)-1-(2,2,2-trifluoroethyl)-1H-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (7.8 g, 57.7% yield) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{35}H_{40}BrF_3N_4O_4$ 716.2; found 717.1.

Step 6.

A solution of benzyl (S)-4-(5-(5-bromo-3-(3-hydroxy-2,2-dimethylpropyl)-1-(2,2,2-trifluoroethyl)-1H-indol-2-yl)-6-(1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (1 g, 1.39 mmol) in 1,4-dioxane (10 mL) and H_2O (2 mL), was added methyl (S)-1-((S)-2-((tert-butoxycarbonyl)amino)-3-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)propanoyl)hexahydropyridazine-3-carboxylate (1.08 g, 2.09 mmol), potassium carbonate (481.47 mg, 3.48 mmol) and Pd(dtbpf)Cl_2 (181.64 mg, 0.28 mmol) in portions at 0° C. The reaction mixture was stirred at 70° C. for 3 h under an argon atmosphere. The resulting mixture was cooled to room temperature, then quenched with H_2O and extracted with EtOAc (50 mL×3). The combined organic layers were washed with brine (20 mL×3), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by silica gel chromatography to afford methyl (S)-1-((S)-3-(3-(2-(5-(4-((benzyloxy)carbonyl)piperazin-1-yl)-2-((S)-1-methoxyethyl)pyridin-3-yl)-3-(3-hydroxy-2,2-dimethylpropyl)-1-(2,2,2-trifluoroethyl)-1H-indol-5-yl)phenyl)-2-((tert-butoxycarbonyl)amino)propanoyl)hexahydropyridazine-3-carboxylate (1.1 g, 77% yield) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{55}H_{68}F_3N_7O_9$ 1027.5; found 1028.3.

Step 7.

To a solution of methyl (S)-1-((S)-3-(3-(2-(5-(4-((benzyloxy)carbonyl)piperazin-1-yl)-2-((S)-1-methoxyethyl)pyridin-3-yl)-3-(3-hydroxy-2,2-dimethylpropyl)-1-(2,2,2-trifluoroethyl)-1H-indol-5-yl)phenyl)-2-((tert-butoxycarbonyl)amino)propanoyl)hexahydropyridazine-3-carboxylate (1.1 g, 1.07 mmol) in THF (8 mL) and H_2O (2 mL) at 0° C., was dropwise added LiOH (2.2 mL, 1M aqueous) under an argon atmosphere. The reaction mixture was stirred for 2 h then concentrated under reduced pressure. The residue was acidified to pH 5 with citric acid (1M) and extracted with EtOAc (20 mL×3). The combined organic layers were concentrated under reduced pressure. The residue was purified by reverse phase chromatography to afford (S)-1-((S)-3-(3-(2-(5-(4-((benzyloxy)carbonyl)piperazin-1-yl)-2-((S)-1-methoxyethyl)pyridin-3-yl)-3-(3-hydroxy-2,2-dimethylpropyl)-1-(2,2,2-trifluoroethyl)-1H-indol-5-yl)phenyl)-2-((tert-butoxycarbonyl)amino)propanoyl)hexahydropyridazine-3-carboxylic acid (750 mg, 69% yield) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{54}H_{66}F_3N_7O_9$ 1013.5; found 1014.3.

Step 8.

To a solution of (S)-1-((S)-3-(3-(2-(5-(4-((benzyloxy)carbonyl)piperazin-1-yl)-2-((S)-1-methoxyethyl)pyridin-3-yl)-3-(3-hydroxy-2,2-dimethylpropyl)-1-(2,2,2-trifluoroethyl)-1H-indol-5-yl)phenyl)-2-((tert-butoxycarbonyl)amino)propanoyl)hexahydropyridazine-3-carboxylic acid (0.75 g, 0.74 mmol) in DCM (75 mL) at 0° C., were added in portions HOBT (0.5 g, 3.7 mmol), DIPEA (3.82 g, 29.58 mmol), and EDCI (4.25 g, 22.19 mmol) at 0° C. The reaction mixture was stirred at room temperature for 12 h under an argon atmosphere. The resulting mixture was concentrated under reduced pressure and extracted with EtOAc (100 mL×3). The combined organic layers were washed with brine (50 mL×3), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by silica gel chromatography to afford benzyl 4-(5-((6^{3}S,4S)-4-((tert-butoxycarbonyl)amino)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-

hexahydro-1^1H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)- benzenacycloundecaphane-1^2-yl)-6-((S)-1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (0.5 g, 67.9% yield) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{54}H_{64}F_3N_7O_8$ 995.5; found 996.3.

Step 9.

To a mixture of benzyl 4-(5-(((6^3S,4S)-4-((tert-butoxycarbonyl)amino)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)- benzenacycloundecaphane-1^2-yl)-6-((S)-1-methoxyethyl)pyridin-3-yl)piperazine-1-carboxylate (500 mg, 0.5 mmol) in MeOH (15 mL) at 0° C., was added paraformaldehyde (135.64 mg, 1.5 mmol), and Pd/C (750 mg) in portions. The reaction mixture was stirred at room temperature for 12 h under a hydrogen atmosphere. The resulting mixture was filtered and the filter cake was washed with EtOAc (50 mL×5). The filtrate was concentrated under reduced pressure. The residue was purified by silica gel chromatography to afford tert-butyl ((6^3S,4S)-1^2-(2-((S)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)carbamate (350 mg, 79.6% yield) as an solid. LCMS (ESI): m/z [M+H] calc'd for $C_{47}H_{60}F_3N_7O_8$ 875.5; found 876.5.

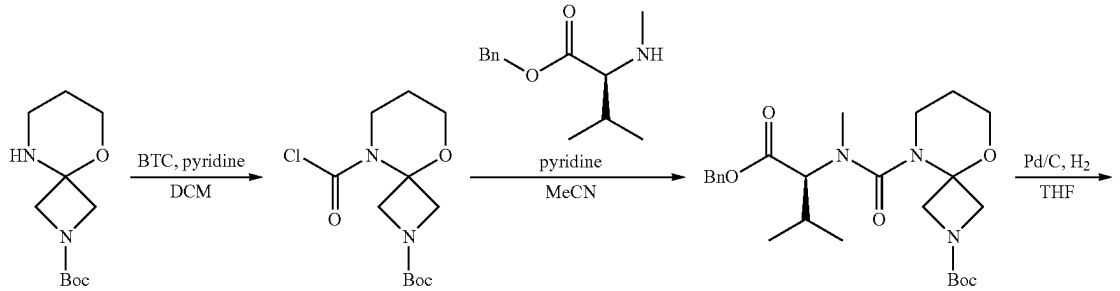
Step 10.

To a solution of tert-butyl ((6^3S,4S)-1^2-(2-((S)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl) carbamate (300 mg, 0.34 mmol) in DCM (2 mL) at 0° C., was dropwise added HCl in 1,4-dioxane (1 mL, 4M, 4 mmol). The reaction mixture was stirred at room temperature for 2 h, then concentrated under reduced pressure to give (6^3S,4S)-4-amino-1^2-(2-((S)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione hydrochloride (350 mg, crude) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{42}H_{52}F_3N_7O_4$ 775.4; found 766.4.

Step 11.

To a solution of (6^3S,4S)-4-amino-1^2-(2-((S)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-5,7-dione hydrochloride (150 mg, 0.19 mmol) and N-(1-(4-(dimethylamino)-4-methylpent-2-ynoyl)-4-fluoropiperidine-4-carbonyl)-N-methyl-L-valine (154 mg, 0.39 mmol) in DMF (2 mL) at 0° C., was dropwise added a mixture of DIPEA (1 g, 7.72 mmol) and HATU (110 mg, 0.29 mmol) in DMF (0.2 mL). The reaction mixture was stirred at 0° C. for 2 h under an argon atmosphere, then quenched with H_2O. The resulting mixture was extracted with EtOAc (20 mL×3). The combined organic phase was washed with brine (10 mL×3), dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by reverse phase chromatography to afford 1-(4-(dimethylamino)-4-methylpent-2-ynoyl)-4-fluoro-N-((2S)-1-(((6^3S,4S)-1^2-(2-((S)-1-methoxyethyl)-5-(4-methylpiperazin-1-yl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-benzenacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-N-methylpiperidine-4-carboxamide (39.5 mg, 17% yield) as solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.48 (d, J=2.9 Hz, 1H), 8.32 (t, J=7.3 Hz, 1H), 7.97 (s, 1H), 7.82-7.69 (m, 3H), 7.66 (t, J=7.8 Hz, 1H), 7.33-7.07 (m, 3H), 5.50 (dd, J=16.7, 8.6 Hz, 1H), 5.33 (t, J=9.2 Hz, 1H), 5.16 (d, J=12.2 Hz, 1H), 4.94-4.80 (m, 1H), 4.64 (d, J=10.8 Hz, 1H), 4.33-4.16 (m, 3H), 4.12-4.02 (m, 2H), 3.71-3.50 (m, 3H), 3.25 (s, 3H), 3.21-3.16 (m, 3H), 3.14-3.05 (m, 1H), 2.96 (t, J=4.7 Hz, 4H), 2.84 (s, 1H), 2.82-2.72 (m, 2H), 2.59-2.53 (m, 1H), 2.47-2.40 (m, 4H), 2.22 (d, J=2.9 Hz, 9H), 2.18-2.12 (m, 2H), 2.11-1.99 (m, 3H), 1.87-1.78 (m, 1H), 1.74-1.62 (m, 1H), 1.59-1.48 (m, 1H), 1.40-1.32 (m, 9H), 1.01 (t, J=7.7 Hz, 1H), 0.89 (s, 5H), 0.83 (d, J=6.3 Hz, 1H), 0.77 (d, J=6.6 Hz, 2H), 0.38 (s, 3H). LCMS (ESI): m/z [M+H] calc'd for $C_{44}H_{58}N_6O_7$ 1154.6; found 1155.7.

Example A735. Synthesis of 2-acryloyl-N-((2S)-1-(((6^3S,4S,Z)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl) pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl) amino)-3-methyl-1-oxobutan-2-yl)-N-methyl-5-oxa-2,9-diazaspiro[3.5]nonane-9-carboxamide



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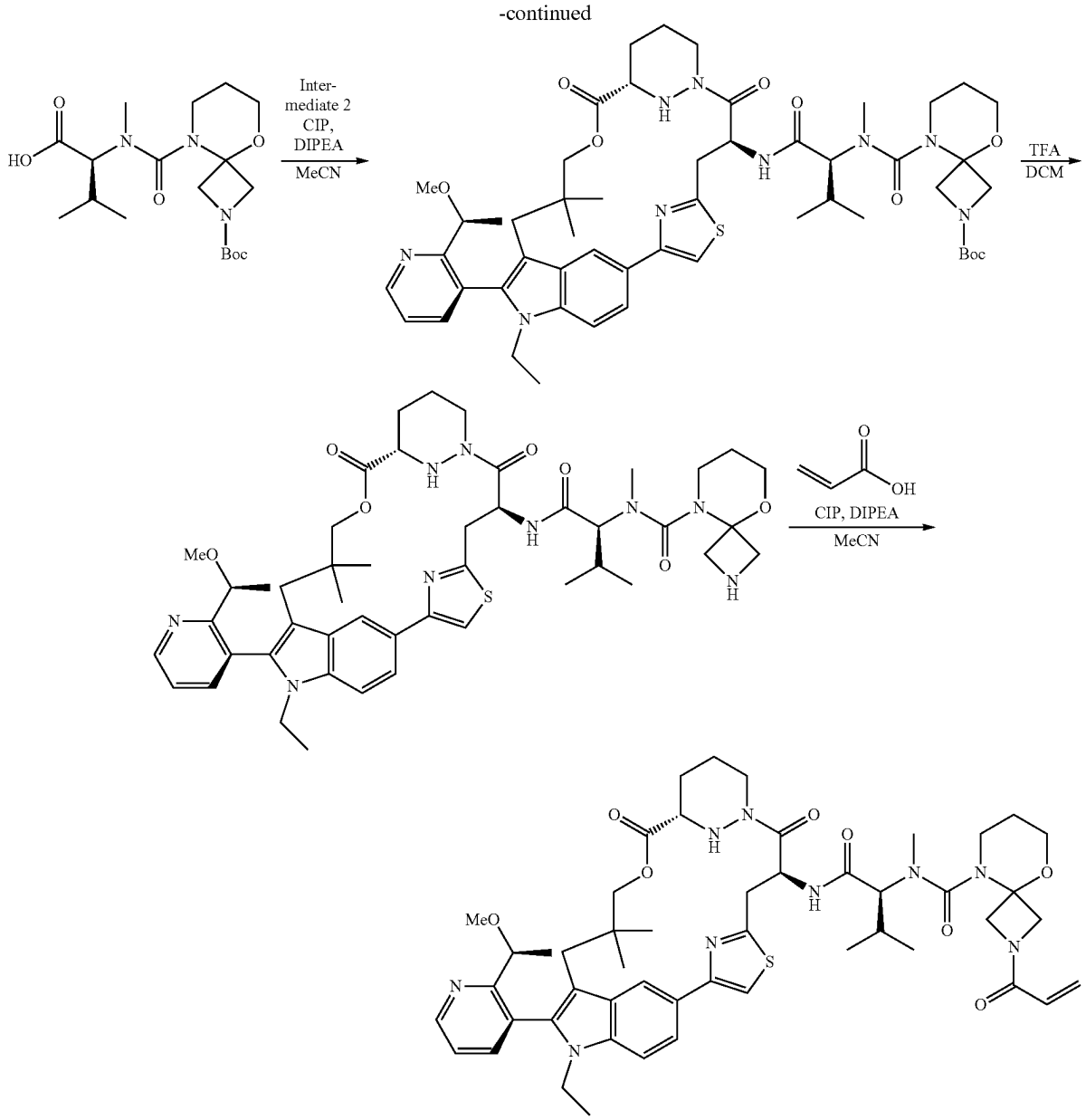


Step 1.

To a stirred mixture of BTC (425.2 mg, 1.448 mmol) in DCM (10 mL) was added dropwise pyridine (1.04 g, 13.16 mmol) and tert-butyl 5-oxa-2,9-diazaspiro[3.5]nonane-2-carboxylate (1 g, 4.39 mmol), the reaction mixture was stirred at room temperature for 2 h. The resulting mixture was concentrated under reduced pressure to give crude tert-butyl 9-(chlorocarbonyl)-5-oxa-2,9-diazaspiro[3.5]nonane-2-carboxylate.

Step 2.

To a stirred solution of tert-butyl 9-(chlorocarbonyl)-5-oxa-2,9-diazaspiro[3.5]nonane-2-carboxylate (2.5 g, crude) in MeCN (20 mL) were added dropwise pyridine (1.04 g, 13.16 mmol) and benzyl (2S)-3-methyl-2-(methylamino) butanoate (970.66 mg, 4.38 mmol) at room temperature. The reaction mixture was stirred at 80° C. for 12 h and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford tert-butyl (S)-9-((1-(benzyloxy)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)-5-oxa-2,9-diazaspiro[3.5]nonane-2-carboxylate (783 mg, 37.6% yield, two steps) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{25}H_{37}N_3O_6$ 475.3; found 476.3.

Step 3.

A solution of tert-butyl (S)-9-((1-(benzyloxy)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)-5-oxa-2,9-diazaspiro[3.5]nonane-2-carboxylate (783 mg, 1.65 mmol) and 10 wt % palladium on carbon (226.29 mg) in THF (10 mL) was stirred for 2 h at 50° C. under a hydrogen atmosphere. The resulting mixture was cooled to room temperature, filtered and the filter cake was washed with MeCN (10 mL×3). The filtrate was concentrated under reduced pressure to give N-(2-(tert-butoxycarbonyl)-5-oxa-2,9-diazaspiro[3.5]nonane-9-carbonyl)-N-methyl-L-valine (591 mg, 98.8% yield) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{18}H_{31}N_3O_6$ 385.2; found 386.3.

Step 4.

To a stirred solution of intermediate 2 (731 mg, 1.16 mmol) and DIPEA (2.25 g, 17.38 mmol) in MeCN (50 mL) was added CIP (644.31 mg, 2.32 mmol) and N-(2-(tert-butoxycarbonyl)-5-oxa-2,9-diazaspiro[3.5]nonane-9-carbonyl)-N-methyl-L-valine (446.68 mg, 1.16 mmol) at room temperature. The reaction mixture was stirred for 2 h then concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford tert-butyl 9-(((2S)-1-(((6^{3}S,4S,Z)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)-5-oxa-2,9-diazaspiro[3.5]nonane-2-carboxylate (752 mg, 65% yield) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{52}H_{71}N_9O_9S$ 997.5; found 996.6.

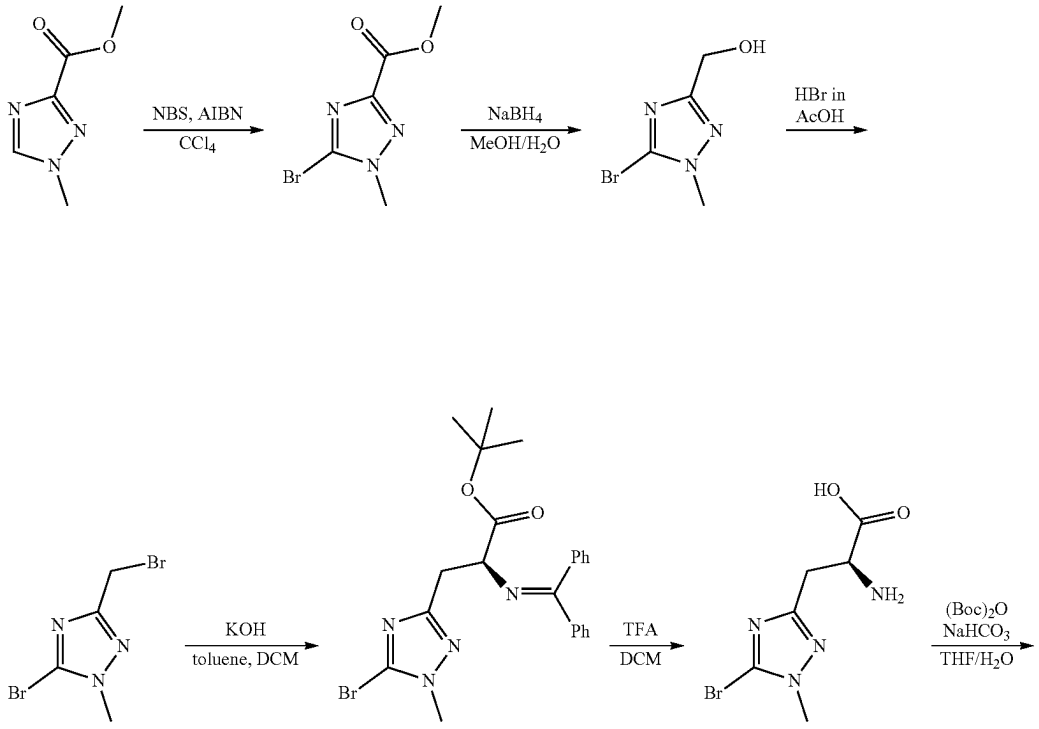
Step 5.

To a stirred solution of tert-butyl 9-(((2S)-1-(((6^{3}S,4S,Z)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamoyl)-5-oxa-2,9-diazaspiro[3.5]nonane-2-carboxylate (752 mg, 0.75 mmol) in DCM (40 mL) was added TFA (10 mL) in portions at room temperature. The reaction mixture was stirred for 2 h, then concentrated under reduced pressure. To the residue was added saturated aqueous sodium bicarbonate (100 mL) and DCM (100 mL). The aqueous layer was separated and extracted with DCM (100 mL×2). The combined organic phase was dried over anhydrous sodium sulfate, filtrated and concentrated under reduced pressure to afford N-((2S)-1-(((6^{3}S,4S,Z)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-N-methyl-5-oxa-2,9-diazaspiro[3.5]nonane-9-carboxamide (587 mg, 87% yield) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{47}H_{63}N_9O_7S$ 897.5; found 898.4.

Step 6.

A stirred solution of N-((2S)-1-(((6^{3}S,4S,Z)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)- pyridazinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-N-methyl-5-oxa-2,9-diazaspiro[3.5]nonane-9-carboxamide (586 mg, 0.65 mmol) in MeCN (10 mL) was added acrylic acid (47 mg, 0.65 mmol), DIPEA (421 mg, 3.26 mmol), CIP (362 mg, 1.3 mmol). The reaction mixture was stirred for 12 h and concentrated under reduced pressure. The residue was purified by reverse phase chromatography to afford 2-acryloyl-N-((2S)-1-(((6^{3}S,4S,Z)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(4,2)-thiazola-1(5,3)-indola-6(1,3)- pyridazinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-N-methyl-5-oxa-2,9-diazaspiro[3.5]nonane-9-carboxamide (194 mg, 27% yield) as a solid. ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 8.78 (dd, J=4.8, 1.7 Hz, 1H), 8.48 (d, J=1.6 Hz, 2H), 7.85-7.65 (m, 3H), 7.65-7.42 (m, 2H), 6.30 (mm, 1H), 6.10 (m, J=17.0, 1H), 5.78-5.50 (m, J=10.3, 2H), 5.10 (dd, 1H), 4.40-3.80 (m, 14H), 3.60-3.10 (m, 10H), 2.94 (d, J=14.5 Hz, 1H), 2.85 (s, 4H), 2.42 (dd, 1H), 2.07 (dd, 2H), 1.80 (s, 2H), 1.55 (s, 3H), 1.32 (d, 3H), 0.95-0.75 (m, 12H), 0.33 (s, 3H). LCMS (ESI): m/z [M+H] calc'd for $C_{50}H_{65}N_9O_8S$ 951.5; found 952.6.

Example A720. Synthesis of (2R)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-N-((6^{3}S,4S,Z)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-2^1,10,10-trimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H,2^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,3)-triazolacycloundecaphane-4-yl)-3-methylbutanamide



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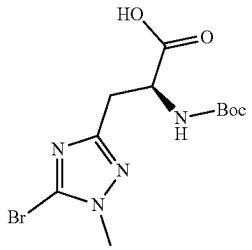

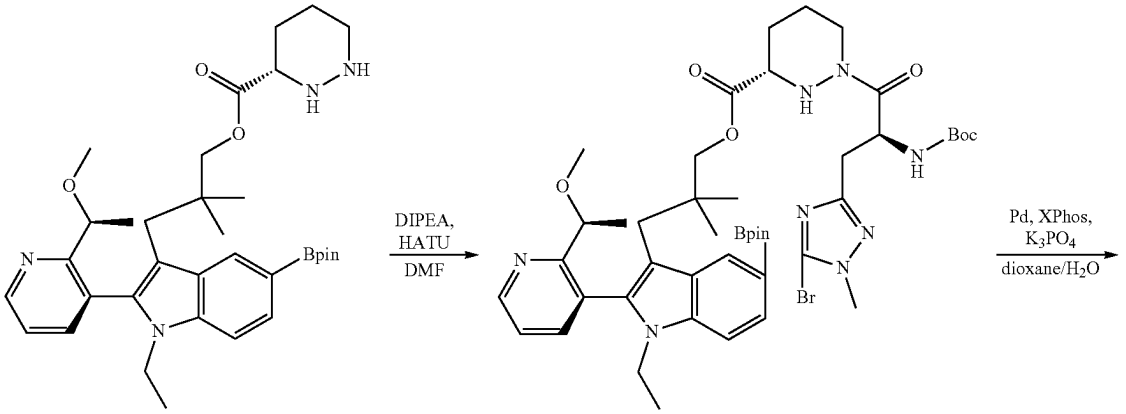
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HATU
DMF
Pd, XPhos,
K_3PO_4
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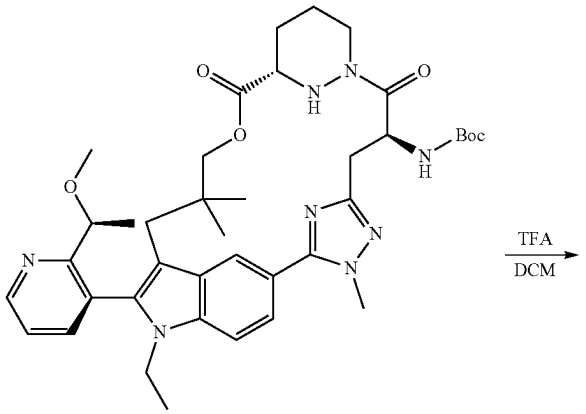
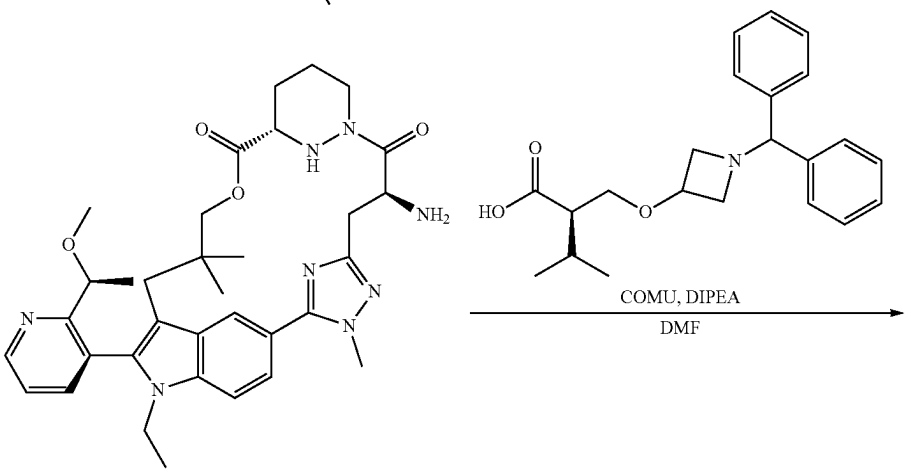

TFA
DCM


COMU, DIPEA
DMF

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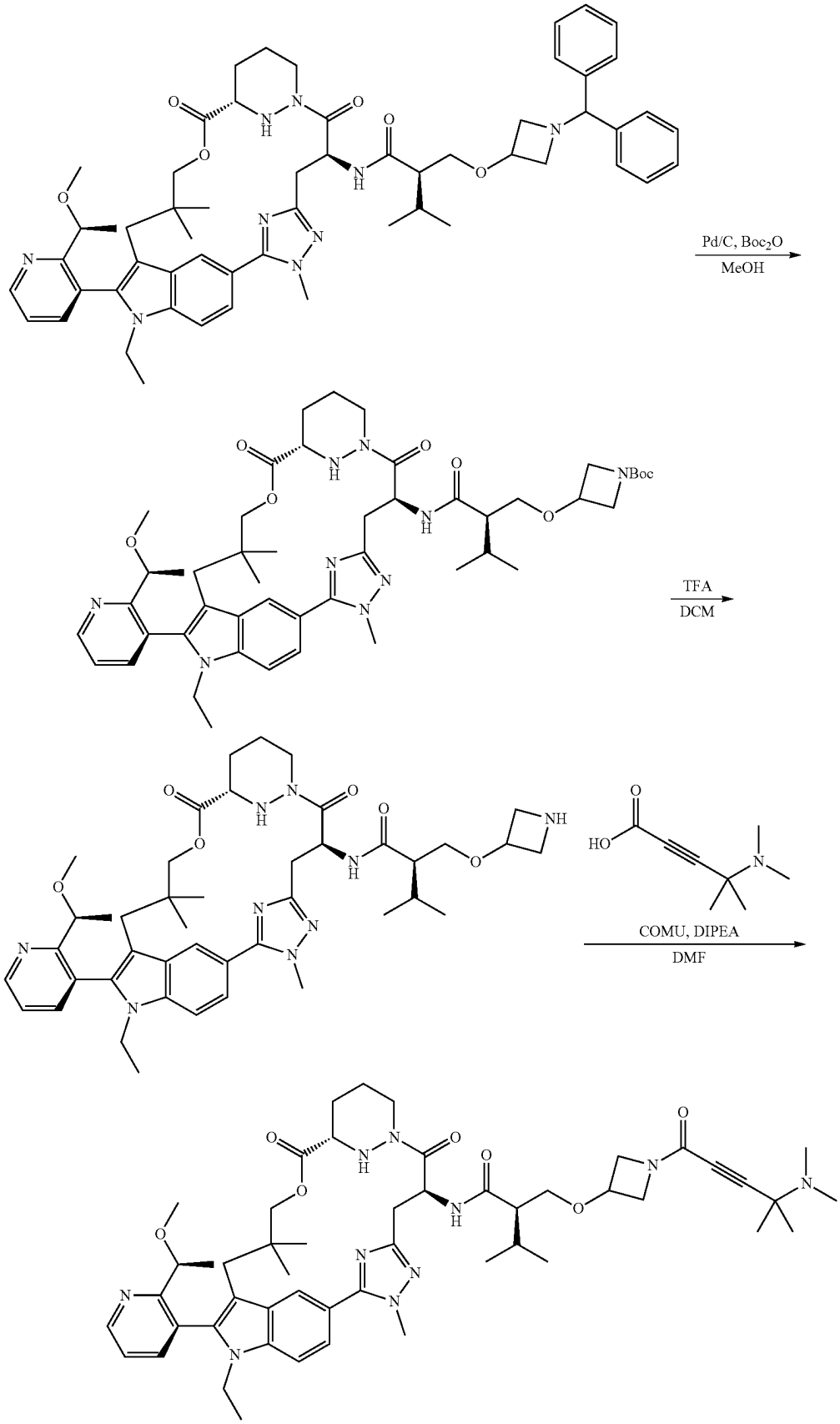


Step 1.

To a stirred solution of methyl 1-methyl-1,2,4-triazole-3-carboxylate (7.0 g, 49.60 mmol) in CCl_4 (70. mL) was added NBS (13.24 g, 74.40 mmol) and AIBN (11.40 g, 69.44 mmol) in portions at 25° C. under an argon atmosphere. The resulting mixture was stirred for 24 h at 80° C. The resulting mixture was filtered, the filtrate was cooled to 20° C. and kept at 20° C. for 30 min. The resulting mixture was filtered. The filter cake was washed with H_2O (3×50 mL) and pet. ether (3×100 mL). The filter cake was dried under reduced pressure. This resulted in methyl 5-bromo-1-methyl-1,2,4-triazole-3-carboxylate (10 g, crude) as a light yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_5H_6BrN_3O_2$ 219.0; found 219.9.

Step 2.

To a stirred solution of methyl 5-bromo-1-methyl-1,2,4-triazole-3-carboxylate (10.0 g, 45.50 mmol) in MeOH (150.0 mL) and H_2O (30.0 mL) was added $NaBH_4$ (6.88 g, 181.80 mmol) in portions at −5° C. under a nitrogen atmosphere. The resulting mixture was stirred for 2 h at 0~10° C. Desired product could be detected by LCMS. The reaction was quenched with brine (100 mL) at 0° C. The resulting mixture was extracted with pet. ether (100 mL). The aqueous layer was separated and filtered. The filter cake was washed with MeOH (2×50 mL). The filtrate was concentrated under reduced pressure to afford (5-bromo-1-methyl-1,2,4-triazol-3-yl)methanol (6 g, crude) as a light yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_4H_6BrN_3O$ 191.98; found 192.0.

Step 3.

A solution of (5-bromo-1-methyl-1,2,4-triazol-3-yl) methanol (6.0 g) and HBr in AcOH (144.0 mL) was stirred for overnight at 80° C. The mixture was neutralized to pH 9 with saturated $NaHCO_3$ (aq.). The resulting mixture was extracted with EtOAc (3×60 mL). The combined organic layers were washed with brine (3×100 mL), dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. This resulted in 5-bromo-3-(bromomethyl)-1-methyl-1,2,4-triazole (6 g, crude) as a white solid. LCMS (ESI): m/z [M+H] calc'd for $C_4H_5Br_2N_3$ 253.89; found 253.8.

Step 4.

To a stirred mixture of 5-bromo-3-(bromomethyl)-1-methyl-1,2,4-triazole (6.0 g, 23.54 mmol) and tert-butyl 2-[(diphenylmethylidene)amino]acetate (6.95 g, 23.54 mmol) in toluene (42 mL) and DCM (18.0 mL) was added (2R,4R,5S)-1-(anthracen-9-ylmethyl)-5-ethenyl-2-[(S)-(prop-2-en-1-yloxy)(quinolin-4-yl)methyl]-1-azabicyclo[2.2.2]octan-1-ium bromide (1.43 g, 2.35 mmol) in portions at 0° C. under argon atmosphere. The resulting mixture was stirred and KOH (60 mL) in H_2O was added. The resulting mixture was stirred for 24 h at −10° C. under an argon atmosphere. Desired product could be detected by LCMS. The reaction was quenched with sat. NH_4Cl (aq.) at 0° C. The resulting mixture was extracted with EtOAc (3×100 mL). The combined organic layers were washed with brine (1×200 mL), dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by Prep-TLC to afford tert-butyl (2S)-3-(5-bromo-1-methyl-1,2,4-triazol-3-yl)-2-[(diphenylmethylidene)amino]propanoate (5 g, 38.6% yield) as a yellow oil. LCMS (ESI): m/z [M+H] calc'd for $C_{21}H_{21}BrN_4O_2$ 469.12; found 469.1.

Step 5.

To a stirred solution of tert-butyl (2S)-3-(5-bromo-1-methyl-1,2,4-triazol-3-yl)-2-[(diphenylmethylidene)amino]propanoate (5.0 g, 10.65 mmol) in DCM (50.0 mL) was added TFA (25.0 mL) dropwise at 0° C. under argon atmosphere. The resulting mixture was stirred for 16 h at room temperature under an argon atmosphere. The resulting mixture was concentrated under reduced pressure to afford (2S)-2-amino-3-(5-bromo-1-methyl-1,2,4-triazol-3-yl)propanoic acid (6 g, crude) as a brown oil. LCMS (ESI): m/z [M+H] calc'd for $C_6H_9BrN_4O_2$ 249.00; found 249.0.

Step 6.

To a stirred solution of (2S)-2-amino-3-(5-bromo-1-methyl-1,2,4-triazol-3-yl)propanoic acid (6.0 g, 24.09 mmol) in THF (36.0 mL) was added $NaHCO_3$ (10.14 g, 120.69 mmol), Boc_2O (7.89 g, 36.14 mmol) in portions at 0° C. under an argon atmosphere. The resulting mixture was stirred for 16 h at room temperature. Desired product could be detected by LCMS. The resulting mixture was concentrated under reduced pressure. The mixture was purified by reverse phase chromatography to afford (2S)-3-(5-bromo-1-methyl-1,2,4-triazol-3-yl)-2-[(tert-butoxycarbonyl)amino]propanoic acid (3 g, 33.9% yield) as a white solid. LCMS (ESI): m/z [M+H] calc'd for $C_1H_{17}BrN_4O_4$ 349.05; found 349.0.

Step 7.

To a stirred solution of 2-[[(2M)-1-ethyl-2-[2-[(1 S)-1-methoxyethyl]pyridin-3-yl]-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)indol-3-yl]methyl]-2-methylpropyl (3S)-1,2-diazinane-3-carboxylate (1.0 g, 1.65 mmol) in DMF (10.0 mL) was added DIPEA (4.28 g, 33.08 mmol), (2S)-3-(5-bromo-1-methyl-1,2,4-triazol-3-yl)-2-[(tert-butoxycarbonyl)amino]propanoic acid (0.69 g, 1.98 mmol) and HATU (0.75 g, 1.99 mmol) in portions at 0° C. The resulting mixture was stirred for 2 h at 20° C. under an argon atmosphere. Desired product could be detected by LCMS. The resulting mixture was quenched with H_2O (100 mL) and extracted with EtOAc (3×30 mL). The combined organic layers were washed with brine (3×100 mL), dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by reverse phase chromatography to afford 2-[[(2M)-1-ethyl-2-[2-[(1 S)-1-methoxyethyl]pyridin-3-yl]-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)indol-3-yl]methyl]-2-methylpropyl (3S)-1-[(2S)-3-(5-bromo-1-methyl-1,2,4-triazol-3-yl)-2-[(tert-butoxycarbonyl)amino]propanoyl]-1,2-diazinane-3-carboxylate (800 mg, 46.5% yield) as a light yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{45}H_{64}BBrN_8O_8$ 935.42; found 935.2.

Step 8.

To a stirred solution of 2-[[(2M)-1-ethyl-2-[2-[(1 S)-1-methoxyethyl]pyridin-3-yl]-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)indol-3-yl]methyl]-2-methylpropyl (3S)-1-[(2S)-3-(5-bromo-1-methyl-1,2,4-triazol-3-yl)-2-[(tert-butoxycarbonyl)amino]propanoyl]-1,2-diazinane-3-carboxylate (800.0 mg, 0.86 mmol) in dioxane (10.0 mL) were added K_3PO_4 (0.45 g, 2.12 mmol), XPhos (122.26 mg, 0.27 mmol), XPhos Pd G3 (0.22 g, 0.27 mmol) and H_2O (2.0 mL) at room temperature. The resulting mixture was stirred for 3 h at 75° C. under an argon atmosphere. Desired product could be detected by LCMS. The resulting mixture was extracted with EtOAc (3×100 mL). The combined organic layers were washed with brine (3×60 mL), dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by reverse phase chromatography to afford tert-butyl ((6^{3}S,4S,Z)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-2^1,10,10-trimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H,2^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,3)-triazolacycloundecaphane-4-yl)carbamate (400 mg, 56.8%

yield) as a light yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{39}H_{52}N_8O_6$ 729.41; found 729.3.

Step 9.

To a solution of tert-butyl ((6^{3}S,4S,Z)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-2^1,10,10-trimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H,2^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,3)-triazolacycloundecaphane-4-yl)carbamate (400.0 mg, 0.56 mmol) in DCM (1 mL) was added TFA (0.5 mL). The reaction was stirred for 1 h at room temperature under an argon atmosphere. After concentration, the mixture was neutralized to pH 8 with saturated $NaHCO_3$ (aq., 20 mL). The mixture was extracted with DCM (3×20 mL). The organic layers were dried over Na_2SO_4 and concentrated to afford (6^{3}S,4S,2)-4-amino-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-2^1,10,10-trimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H,2^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,3)-triazolacycloundecaphane-5,7-dione (500 mg, crude) as a light yellow solid. ESI-MS m/z=629.3 [M+H]$^+$; Calculated MW:628.3. LCMS (ESI): m/z [M+H] calc'd for $C_{34}H_{44}N_8O_4$ 629.36; found 629.3.

Step 10.

To a stirred solution of (6^{3}S,4S,2)-4-amino-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-2^1,10,10-trimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H,2^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,3)- triazolacycloundecaphane-5,7-dione (170.0 mg, 0.27 mmol) and (R)-2-(((1-benzhydrylazetidin-3-yl)oxy)methyl)-3-methylbutanoic acid (114.68 mg, 0.32 mmol) in DMF (5 mL) were added DIPEA (698.86 mg, 5.41 mmol) and HATU (123.36 mg, 0.32 mmol) dropwise at 0° C. under an air atmosphere. The resulting mixture was stirred for 2 h at 0° C. The resulting mixture was diluted with 25 mL H_2O. The resulting mixture was extracted with EtOAc (3×25 mL). The combined organic layers were washed with brine (3×25 mL), dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure to afford (2R)-2-(((1-benzhydrylazetidin-3-yl)oxy)methyl)-N-((6^{3}S,4S,Z)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-2^1,10,10-trimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H,2^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,3)-triazolacycloundecaphane-4-yl)-3-methylbutanamide (180 mg, crude) as an off-white oil. LCMS (ESI): m/z [M+H] calc'd for $C_{56}H_{69}N_9O_6$ 964.54; found 964.4.

Step 11.

To a stirred solution of (2R)-2-(((1-benzhydrylazetidin-3-yl)oxy)methyl)-N-((6^{3}S,4S,Z)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-2^1,10,10-trimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H,2^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,3)-triazolacycloundecaphane-4-yl)-3-methylbutanamide (180.0 mg, 0.19 mmol) and Pd/C (90.0 mg, 0.85 mmol) in MeOH (10 mL) was added Boc_2O (81.48 mg, 0.37 mmol) at room temperature under a hydrogen atmosphere. The resulting mixture was stirred overnight at room temperature. The resulting mixture was filtered, the filter cake was washed with MeOH (3×10 mL). The filtrate was concentrated under reduced pressure to afford tert-butyl 3-((2R)-2-(((6^{3}S,4S,Z)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-2^1,10,10-trimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H,2^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,3)-triazolacycloundecaphane-4-yl)carbamoyl)-3-methylbutoxy)azetidine-1-carboxylate (80 mg, 47.7% yield) as an off-white solid. LCMS (ESI): m/z [M+H] calc'd for $C_{48}H_{67}N_9O_8$ 898.52; found 898.4.

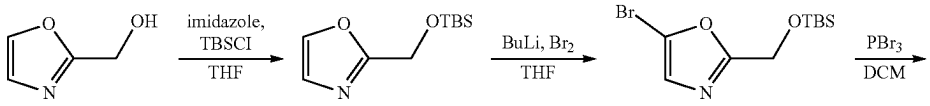
Step 12.

To a stirred solution of tert-butyl 3-((2R)-2-(((6^{3}S,4S,Z)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-2^1,10,10-trimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H,2^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,3)-triazolacycloundecaphane-4-yl)carbamoyl)-3-methylbutoxy)azetidine-1-carboxylate in DCM (2 mL) was added TFA (1.0 mL) dropwise at 0° C. under an air atmosphere. The resulting mixture was stirred for 1 h at 0° C. The resulting mixture was concentrated under reduced pressure to afford (2R)-2-((azetidin-3-yloxy)methyl)-N-((6^{3}S,4S,Z)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-2^1,10,10-trimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H,2^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,3)-triazolacycloundecaphane-4-yl)-3-methylbutanamide (85 mg, crude) as a yellow green oil.

Step 13.

To a stirred solution of (2R)-2-((azetidin-3-yloxy)methyl)-N-((6^{3}S,4S,Z)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-2^1,10,10-trimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H,2^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,3)-triazolacycloundecaphane-4-yl)-3-methylbutanamide (80.0 mg, 0.10 mmol) and 4-(dimethylamino)-4-methylpent-2-ynoic acid (38.90 mg, 0.25 mmol) in DMF (2 mL) were added DIPEA (518.27 mg, 4.01 mmol) and COMU (51.52 mg, 0.12 mmol) in portions at 0° C. The reaction mixture was stirred under an air atmosphere for 2 h. The crude product (150 mg) was purified by reverse phase chromatography to afford (2R)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-N-((6^{3}S,4S,Z)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-2^1,10,10-trimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H,2^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(5,3)-triazolacycloundecaphane-4-yl)-3-methylbutanamide (15.3 mg, 16.3% yield) as an off-white solid. ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 8.77 (dd, J=4.8, 1.7 Hz, 1H), 8.15 (d, J=1.7 Hz, 1H), 8.07 (d, J=8.1 Hz, 1H), 7.85-7.78 (m, 1H), 7.70 (d, J=8.6 Hz, 1H), 7.58-7.48 (m, 2H), 5.82 (s, 1H), 4.95 (d, J=11.7 Hz, 1H), 4.41-4.30 (m, 5H), 4.30 (d, J=8.2 Hz, 2H), 4.25 (d, J=5.6 Hz, 4H), 4.10 (td, J=17.1, 16.1, 9.1 Hz, 2H), 3.99-3.82 (m, 3H), 3.71-3.60 (m, 1H), 3.54-3.43 (m, 3H), 3.39 (s, 2H), 3.22 (d, J=1.6 Hz, 1H), 2.92 (d, J=13.6 Hz, 1H), 2.86-2.77 (m, 2H), 2.45 (s, 6H), 2.37 (q, J=7.7 Hz, 1H), 2.17 (d, J=6.6 Hz, 2H), 2.03 (d, J=10.2 Hz, 2H), 1.78-1.66 (m, 3H), 1.47 (t, J=10.9 Hz, 6H), 1.35-1.28 (m, 12H), 0.32 (s, 3H). LCMS (ESI): m/z [M+H] calc'd for $C_{51}H_{70}N_{10}O_7$ 935.55; found 935.3.

Example A692. Synthesis of (3S)-1-(4-(dimethylamino)-4-methylpent-2-ynoyl)-N-((2S)-1-(((6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(5,2)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-N-methylpyrrolidine-3-carboxamide



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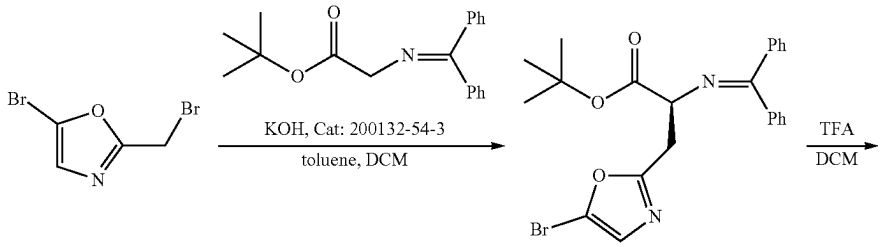
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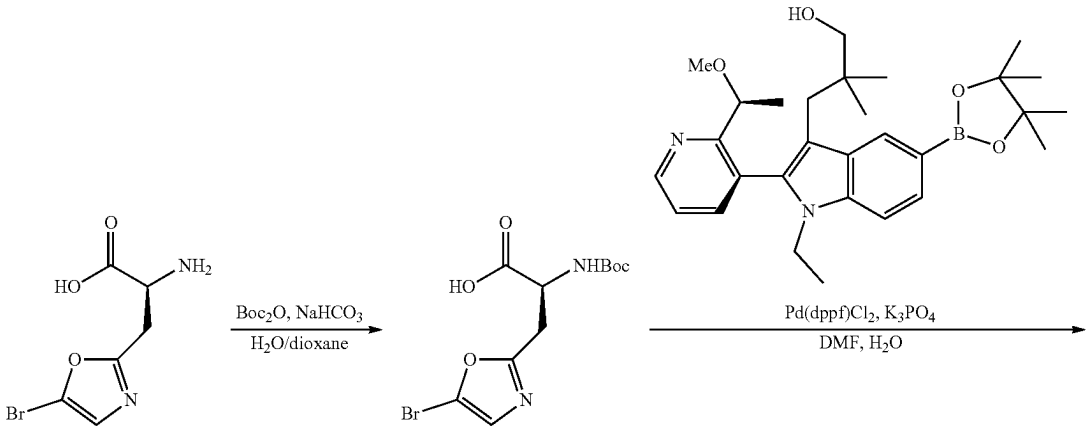
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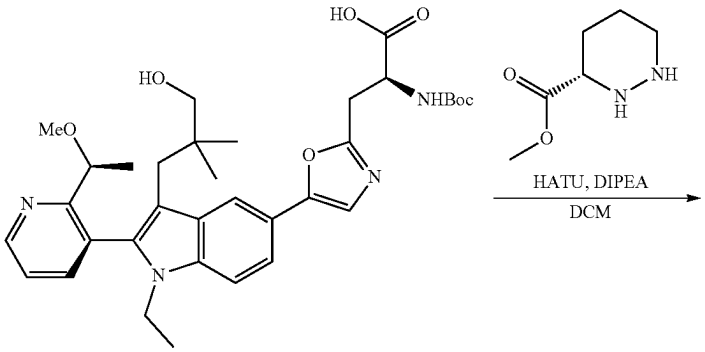
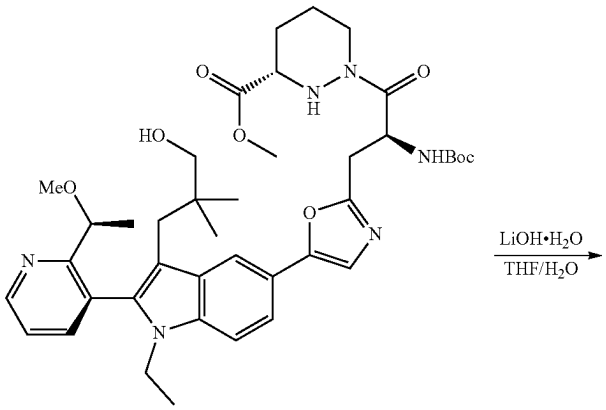
H2O/dioxane
Pd(dppf)Cl2, K3PO4
DMF, H2O


HATU, DIPEA
DCM


LiOH•H2O
THF/H2O

1081 **1082**

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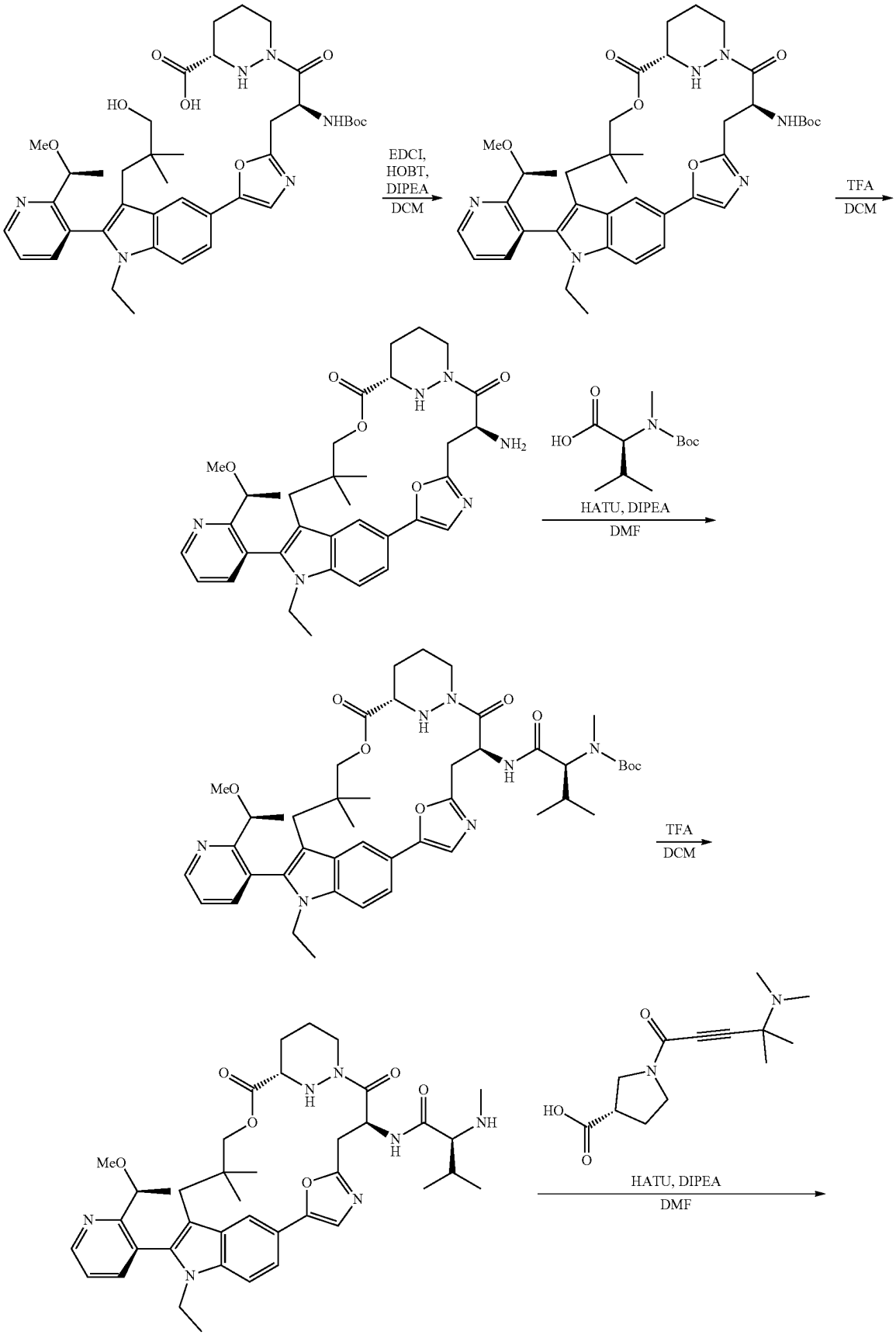

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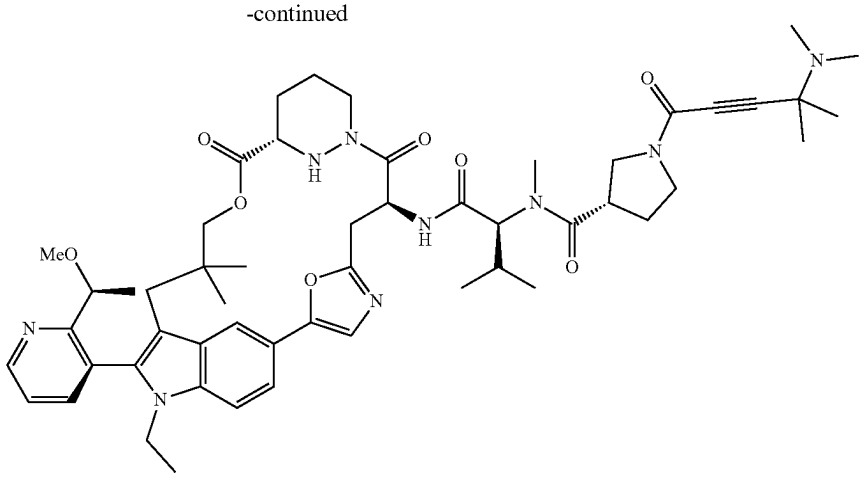


Step 1.

To a solution of 1,3-oxazol-2-ylmethanol (5.0 g, 50.46 mmol) in THF (75 mL), were added imidazole (8.59 mg, 0.13 mmol), and TBSCI (11.41 mg, 0.08 mmol) at 0° C. The resulting solution was stirred for 5 h then concentrated under reduced pressure. The crude material was purified by silica gel column chromatography to afford 2-[[(tert-butyldimethylsilyl)oxy]methyl]-1,3-oxazole (10 g, 92.8% yield) as colorless oil. LCMS (ESI): m/z [M+H] calc'd for $C_{10}H_{19}NO_2Si$ 214.13; found 214.3.

Step 2.

To a solution of 2-[[(tert-butyldimethylsilyl)oxy]methyl]-1,3-oxazole (10.0 g, 46.87 mmol) in THF (150.0 mL, 1851.45 mmol) at −78° C. was added n-BuLi (22.4 mL, 56.25 mmol) over 10 min and stirred for 30 min at −78° C. under an argon atmosphere. Then the solution of Br_2 (3.6 mL, 70.31 mmol) in THF (10 mL) was added over 10 min to the solution at −78° C. The resulting solution was slowly warmed to room temperature and stirred for 2 h. The resulting mixture was diluted with NH_4Cl/H_2O (100 mL) and extracted with EtOAc (3×100 mL). The combined organic layers were dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure and purified by silica gel column chromatography to afford 5-bromo-2-[[(tert-butyldimethylsilyl)oxy]methyl]-1,3-oxazole (5.3 g, 38.6% yield) as yellow oil. LCMS (ESI): m/z [M+H] calc'd for $C_{10}H_{18}BrNO_2Si$ 292.04; found 292.0.

Step 3.

To a solution of 5-bromo-2-[[(tert-butyldimethylsilyl) oxy]methyl]-1,3-oxazole (4.0 g, 13.69 mmol) in DCM (60.0 mL) was added PBr_3 (7.41 g, 27.37 mmol) at 0° C. under an argon atmosphere. The resulting solution was stirred for 4 h then diluted with $NaHCO_3/H_2O$ (30 mL). The mixture was extracted with EtOAc (3×40 mL). The organic layers were concentrated under reduced pressure and purified by silica gel column chromatography to afford 5-bromo-2-(bromomethyl)-1,3-oxazole (2.5 g, 75.7% yield) as yellow oil. LCMS (ESI): m/z [M+H] calc'd for $C_4H_3Br_2NO$ 239.87; found 241.9.

Step 4.

A mixture of 5-bromo-2-(bromomethyl)-1,3-oxazole (9.0 g, 37.36 mmol), Cat:200132-54-3 (2.26 g, 3.74 mmol), DCM (45.0 mL), toluene (90.0 mL), KOH (20.96 g, 373.63 mmol), H_2O (42 mL),and tert-butyl 2-[(diphenylmethylidene)amino]acetate (13.24 g, 44.82 mmol) at 0° C. was stirred for 4 h then diluted with H_2O (30 mL). The mixture was extracted with DCM (3×40 mL). The organic layers were concentrated under reduced pressure and purified by reverse phase column chromatography to afford tert-butyl (2S)-3-(5-bromo-1,3-oxazol-2-yl)-2-[(diphenylmethylidene)amino]propanoate (4.8 g, 28.2% yield) as a yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{23}H_{23}BrN_2O_3$ 455.10; found 457.1.

Step 5.

A mixture of tert-butyl (2S)-3-(5-bromo-1,3-oxazol-2-yl)-2-[(diphenylmethylidene)amino]propanoate (1.20 g, 2.64 mmol), DCM (10.0 mL, 157.30 mmol), and TFA (5.0 mL, 67.32 mmol) at 0° C. was stirred for 2 h then concentrated under reduced pressure to afford (S)-3-(5-bromooxazol-2-yl)-2-((2,2,2-trifluoroacetyl)-14-azaneyl)propanoic acid (0.5 g, 81.3% yield) as a yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_6H_7BrN_2O_3$ 234.97; found 237.0.

Step 6.

A mixture of (S)-3-(5-bromooxazol-2-yl)-2-((2,2,2-trifluoroacetyl)-14-azaneyl)propanoic acid (500.0 mg, 2.13 mmol), Boc_2O (928.56 mg, 4.26 mmol), dioxane (2.50 mL), H_2O (2.50 mL), and $NaHCO_3$ (714.84 mg, 8.51 mmol) at 0° C. was stirred for 3 h. The resulting solution was purified by reverse phase column chromatography to afford (2S)-3-(5-bromo-1,3-oxazol-2-yl)-2-[(tert-butoxycarbonyl)amino] propanoic acid (0.65 g, 91.1% yield) as a yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{11}H_{15}BrN_2O_5$ 335.02; found 334.8.

Step 7.

To a solution of (2S)-3-(5-bromo-1,3-oxazol-2-yl)-2-[(tert-butoxycarbonyl)amino]propanoic acid (500.0 mg, 1.49 mmol) and 3-[(2M)-1-ethyl-2-[2-[(1S)-1-methoxyethyl]pyridin-3-yl]-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)indol-3-yl]-2,2-dimethylpropan-1-ol (808.16 mg, 1.64 mmol) in DMF (5.0 mL) and H_2O (1.0 mL) were added K_3PO_4 (791.67 mg, 3.73 mmol) and $Pd(dppf)Cl_2$ (109.16 mg, 0.15 mmol). The resulting mixture was stirred for 2 h at 70° C. under an argon atmosphere. The mixture was purified by reverse phase column chromatography to afford (2S)-2-[(tert-butoxycarbonyl)amino]-3-[5-[(2M)-1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-[(1 S)-1-methoxyethyl] pyridin-3-yl]indol-5-yl]-1,3-oxazol-2-yl]propanoic acid (600 mg, 64.79% yield) as a light brown solid. LCMS (ESI): m/z [M+H] calc'd for $C_{34}H_{44}N_4O_7$ 621.33; found 621.3.

Step 8.

To a stirred mixture of methyl (3S)-1,2-diazinane-3-carboxylate (627.10 mg, 4.350 mmol) and (2S)-2-[(tert-butoxy-

carbonyl)amino]-3-[5-[(2M)-1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-[(1S)-1-methoxyethyl]pyridin-3-yl]indol-5-yl]-1,3-oxazol-2-yl]propanoic acid (900.0 mg, 1.45 mmol) in DCM (10.0 mL) were added HATU (661.54 mg, 1.74 mmol) and DIPEA (3747.71 mg, 29.00 mmol) at 0° C. The resulting mixture was stirred for 2 h. Desired product could be detected by LCMS. The resulting mixture was concentrated under reduced pressure and the crude material was purified by silica gel column chromatography to afford methyl (3S)-1-[(2S)-2-[(tert-butoxycarbonyl)amino]-3-[5-[(2M)-1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-[(1S)-1-methoxyethyl]pyridin-3-yl]indol-5-yl]-1,3-oxazol-2-yl]propanoyl]-1,2-diazinane-3-carboxylate (900 mg, 83.11%) as a brown yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{40}H_{54}N_6O_8$ 747.41; found 747.2.

Step 9.

To a stirred mixture of methyl (3S)-1-[(2S)-2-[(tert-butoxycarbonyl)amino]-3-[5-[(2M)-1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-[(1S)-1-methoxyethyl]pyridin-3-yl]indol-5-yl]-1,3-oxazol-2-yl]propanoyl]-1,2-diazinane-3-carboxylate (2000.0 mg, 2.68 mmol) in THF (18. mL) and H_2O (6.0 mL) was added LiOH·H_2O (337.10 mg, 8.03 mmol) at 0° C. The resulting mixture was stirred for 2 h. Desired product could be detected by LCMS. The reaction was quenched with H_2O at 0° C. and adjusted to pH 6 with 1N HCl solution. The resulting mixture was extracted with EtOAc (3×50 mL). The combined organic layers were washed with brine (1×10 mL), dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure to afford (3S)-1-[(2S)-2-[(tert-butoxycarbonyl)amino]-3-[5-[(2M)-1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-[(1 S)-1-methoxyethyl]pyridin-3-yl]indol-5-yl]-1,3-oxazol-2-yl]propanoyl]-1,2-diazinane-3-carboxylic acid (1300 mg, 66.2% yield) as a yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{39}H_{52}N_6O_8$ 733.39; found 733.3.

Step 10.

To a stirred mixture of (3S)-1-[(2S)-2-[(tert-butoxycarbonyl)amino]-3-[5-[(2M)-1-ethyl-3-(3-hydroxy-2,2-dimethylpropyl)-2-[2-[(1 S)-1-methoxyethyl]pyridin-3-yl]indol-5-yl]-1,3-oxazol-2-yl]propanoyl]-1,2-diazinane-3-carboxylic acid (1.2 g, 1.64 mmol) and DIPEA (8.5 g, 65.50 mmol) in DCM (120.0 mL) were added HOBT (1.8 g, 13.10 mmol) and EDCI (7.8 g, 40.93 mmol) at 0° C. The resulting mixture was stirred for 2 h. Desired product could be detected by LCMS. The mixture was concentrated under reduced pressure and purified by silica gel column chromatography to afford tert-butyl ((6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(5,2)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (660 mg, 56.4% yield) as a brown yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{39}H_{50}N_6O_7$ 715.38; found 715.3.

Step 11.

A mixture of tert-butyl ((6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(5,2)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (20.0 mg, 0.028 mmol) and TFA (3.0 mL) in DCM (6.0 mL) at 0° C. was stirred for 2 h. Desired product could be detected by LCMS. The resulting mixture was concentrated under reduced pressure to afford (6^{3}S,4S)-4-amino-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(5,2)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (160 mg, crude) as a yellow green solid. LCMS (ESI): m/z [M+H] calc'd for $C_{34}H_{42}N_6O_5$ 615.33; found 615.2.

Step 12.

To a stirred mixture of (6^{3}S,4S)-4-amino-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(5,2)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (180.0 mg, 0.293 mmol) and (2S)-2-[(tert-butoxycarbonyl)(methyl)amino]-3-methylbutanoic acid (135.45 mg, 0.59 mmol) in DMF (2.0 mL) were added HATU (133.60 mg, 0.35 mmol) and DIPEA (756.86 mg, 5.86 mmol) at 0° C. The resulting mixture was stirred for 2 h. Desired product could be detected by LCMS. The mixture was purified by reverse phase column chromatography to afford tert-butyl ((2S)-1-(((6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(5,2)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate (160 mg, 66% yield) as a brown yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{45}H_{61}N_7O_8$ 828.47; found 828.4.

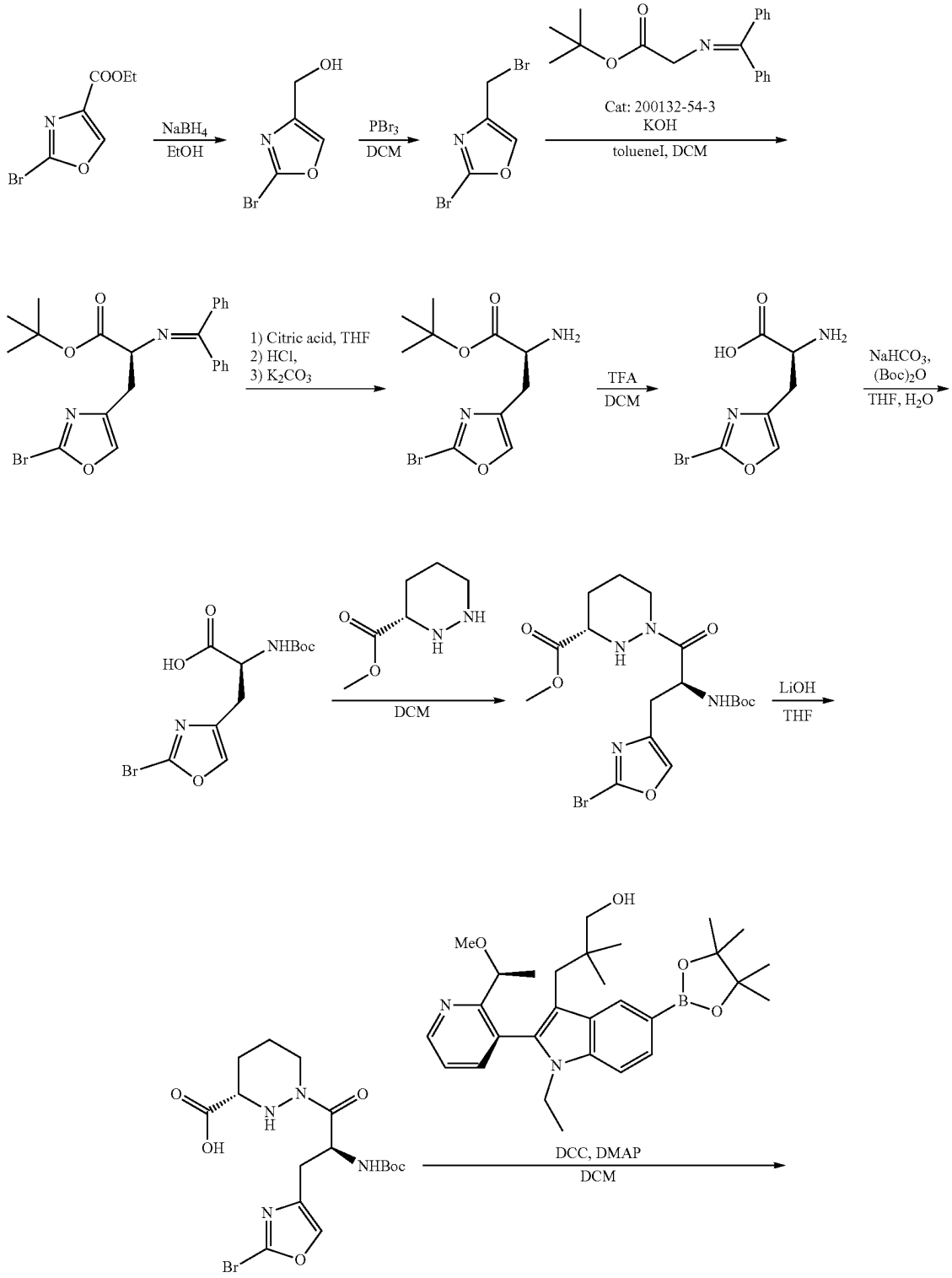
Step 13.

To a stirred mixture of tert-butyl ((2S)-1-(((6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(5,2)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate (160.0 mg, 0.19 mmol) in DCM (2.0 mL) was added TFA (1.0 mL) at 0° C. The resulting mixture was stirred for 2 h. Desired product could be detected by LCMS. The resulting mixture was concentrated under reduced pressure to afford (2S)—N-((6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(5,2)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methyl-2-(methylamino)butanamide (130 mg, crude) as a yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{40}H_{53}N_7O_6$ 728.41; found 728.5.

Step 14.

To a stirred mixture of ((2S)—N-((6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(5,2)-oxazola-1(5,3)-indola-6(1,3)- pyridazinacycloundecaphane-4-yl)-3-methyl-2-(methylamino)butanamide (130.0 mg, 0.18 mmol) and (3S)-1-[4-(dimethylamino)-4-methylpent-2-ynoyl]pyrrolidine-3-carboxylic acid (180.25 mg, 0.72 mmol) in DMF (2.0 mL) were added DIPEA (461.64 mg, 3.57 mmol) and HATU (135.81 mg, 0.36 mmol) at 0° C. The resulting mixture was stirred for 2 h. Desired product could be detected by LCMS. The mixture was purified by reverse phase column chromatography to afford (3S)-1-(4-(dimethylamino)-4-methylpent-2-ynoyl)-N-((2S)-1-(((6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(5,2)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-N-methylpyrrolidine-3-carboxamide (55.3 mg, 31.3% yield) as a solid. ^{1}H NMR (400 MHz, DMSO-d$_6$) δ 8.77 (dd, J=4.8, 1.7 Hz, 1H), 8.09-8.00 (m, 1H), 7.92 (s, 1H), 7.88-7.80 (m, 1H), 7.62 (d, J=8.7 Hz, 1H), 7.59-7.49 (m, 2H), 7.39-7.30 (m, 1H), 5.69 (p, J=8.8 Hz, 1H), 5.44 (d, J=12.1 Hz, 1H), 4.67 (d, J=10.7 Hz, 1H), 4.30-4.15 (m, 3H), 3.99 (dt, J=13.2, 6.4 Hz, 3H), 3.89-3.79 (m, 1H), 3.62 (ddd, J=30.5, 18.6, 11.4 Hz, 5H), 3.39 (dd, J=9.4, 3.8 Hz, 2H), 3.21-3.13 (m, 1H), 3.08 (d, J=15.0 Hz, 3H), 2.99-2.74 (m, 6H), 2.26-2.18 (m, 5H), 2.16 (s, 2H), 2.14-1.94 (m, 3H), 1.86-1.68 (m, 2H), 1.57 (q, J=9.2, 5.8 Hz, 1H), 1.44-1.27 (m, 9H), 0.94 (d, J=6.6 Hz, 4H), 0.89 (dd, J=6.5, 2.5 Hz, 2H), 0.80 (d, J=6.3 Hz, 2H), 0.77-0.69 (m, 4H), 0.58 (d, J=20.4 Hz, 3H). LCMS (ESI): m/z [M+H] calc'd for $C_{53}H_{71}N_9O_8$ 962.55; found 962.5.

Example A675. Synthesis of (2R)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-N-((6^{3}S,4S,Z)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(2,4)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methylbutanamide



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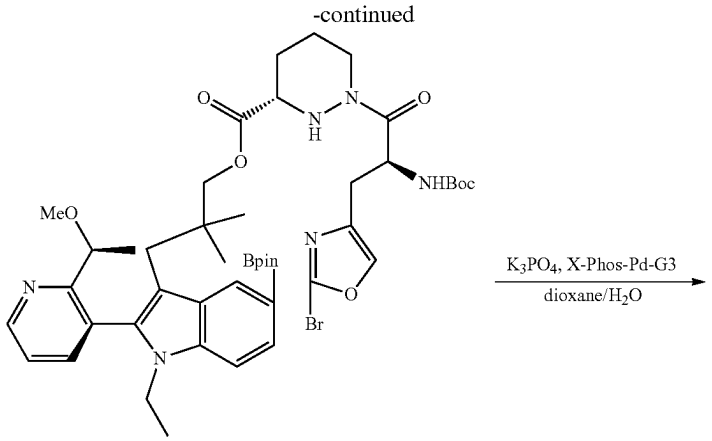
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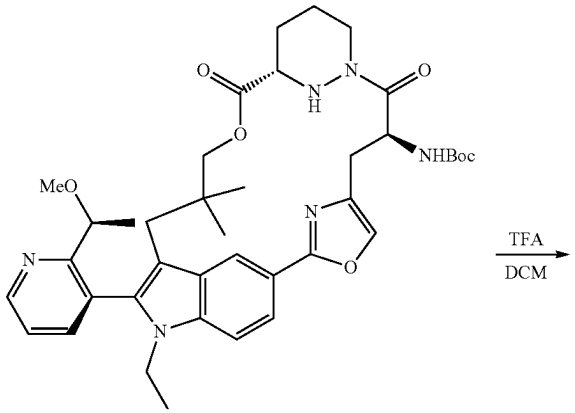
Br
NHBoc
K3PO4, X-Phos-Pd-G3
dioxane/H2O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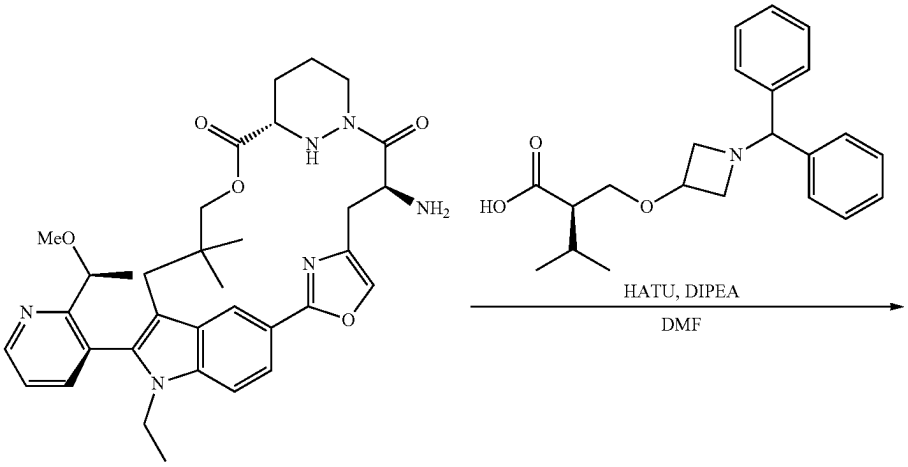
NHBoc
TFA
DCM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NH2
HO
HATU, DIPEA
DMF

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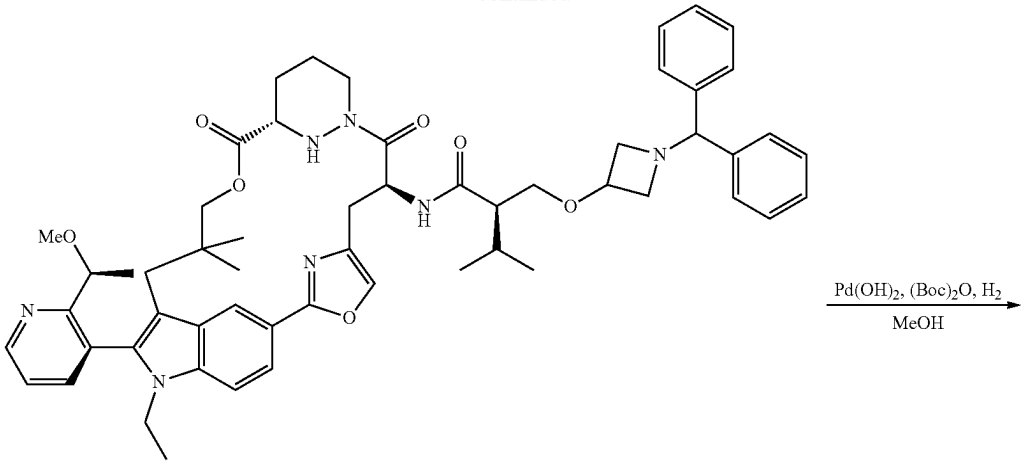
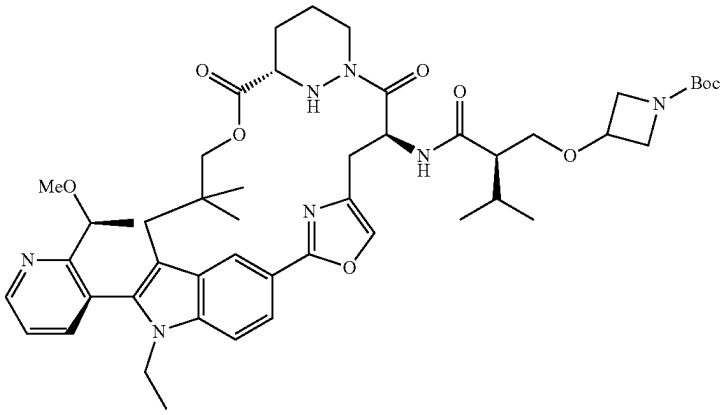
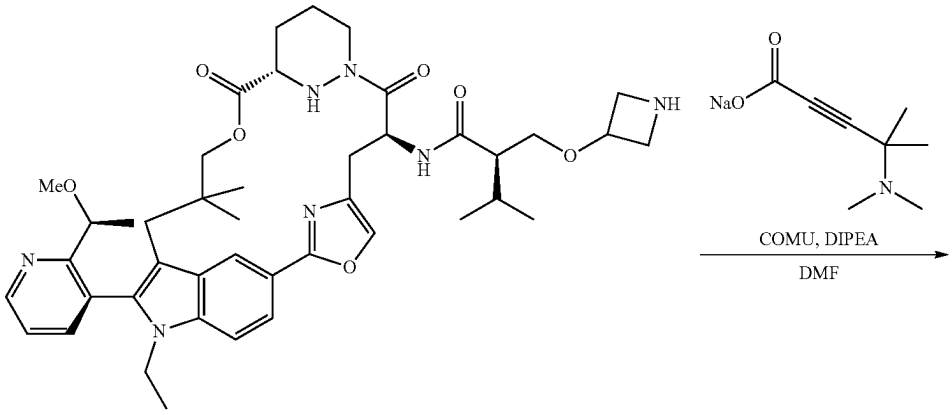
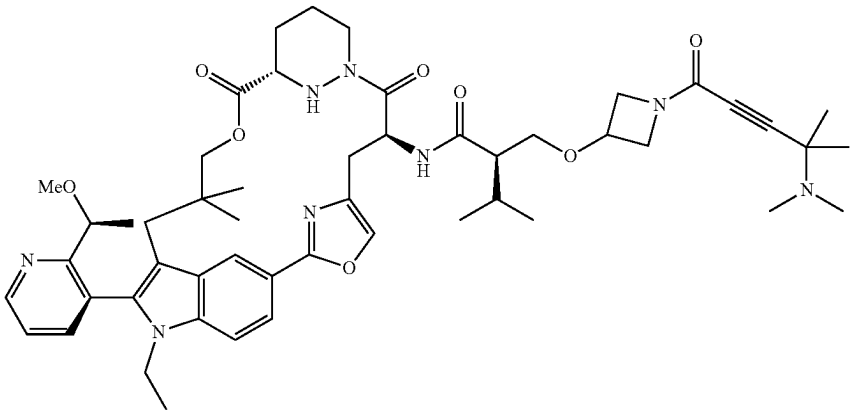








Step 1.

A mixture of 2-bromo-4-(ethoxycarbonyl)-1,3-oxazol-5-ylium (6.83 g, 31.19 mmol), EtOH (100.0 mL) and $NaBH_4$ (4.72 g, 124.76 mmol) at 0° C. was stirred for 6 h at 0° C. under an air atmosphere. The reaction was quenched with H_2O at 0° C. The resulting mixture was extracted with EtOAc (3×100 mL). The combined organic layers were dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure to afford (2-bromo-1,3-oxazol-4-yl)methanol (4.122 g, 74.3% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_4H_4BrNO_2$ 177.95; found 178.0.

Step 2.

A mixture of (2-bromo-1,3-oxazol-4-yl)methanol (4.30 g, 24.16 mmol), DCM (50 mL) and phosphorus tribromide (9809.39 mg, 36.24 mmol) at 0° C. was stirred overnight at 0° C. under an air atmosphere. The reaction was quenched by the addition of $NaHCO_3$ (aq.) at 0° C. The resulting mixture was extracted with EtOAc (3×100 mL). The combined organic layers were dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure to afford 2-bromo-4-(bromomethyl)-1,3-oxazole (3.28 g, 56.4% yield) as a liquid. LCMS (ESI): m/z [M+H] calc'd for $C_4H_3Br_2NO$ 239.87; found 239.9.

Step 3.

A mixture of 2-bromo-4-(bromomethyl)-1,3-oxazole (3280.0 mg, 13.62 mmol), KOH (9M, 10 mL), 30 mL mixture of toluene/DCM (7/3) and tert-butyl 2-[(diphenylmethylidene)amino]acetate (5228.74 mg, 17.70 mmol) at −16° C. was stirred overnight under an air atmosphere. The resulting mixture was extracted with EtOAc (3×50 mL). The combined organic layers were dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure to afford tert-butyl (2S)-3-(2-bromo-1,3-oxazol-4-yl)-2-[(diphenylmethylidene)amino]propanoate (8.33 g, 80.6% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{23}H_{23}BrN_2O_3$ 455.10; found 455.1.

Step 4.

A mixture of tert-butyl (2S)-3-(2-bromo-1,3-oxazol-4-yl)-2-[(diphenylmethylidene)amino]propanoate (4100.0 mg, 9.0 mmol) and citric acid (1 N) (40.0 mL, 0.21 mmol), in THF (40 mL) at room temperature was stirred overnight under an air atmosphere. The reaction was quenched by the addition of HCl (aq.) (100 mL) at 0° C. The aqueous layer was extracted with EtOAc (3×100 mL). K_2CO_3 (aq.) (200 mL) was added to the resulting mixture and extracted with EtOAc (3×100 mL). The organic layer was concentrated under reduced pressure to afford tert-butyl (2S)-2-amino-3-(2-bromo-1,3-oxazol-4-yl)propanoate (1730 mg, 66% yield) as a dark yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{10}H_{15}BrN_2O_3$ 291.03; found 291.0.

Step 5.

A mixture of tert-butyl (2S)-2-amino-3-(2-bromo-1,3-oxazol-4-yl)propanoate (1780.0 mg, 6.11 mmol), TFA (10.0 mL) and DCM (10.0 mL) at 0° C. was stirred for overnight under an air atmosphere. The resulting mixture was concentrated under reduced pressure to afford (2S)-2-amino-3-(2-bromo-1,3-oxazol-4-yl)propanoic acid (1250 mg, 87% yield) as a dark yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_6H_7BrN_2O_3$ 234.97; found 234.9.

Step 6.

A mixture of di-tert-butyl dicarbonate (4178.58 mg, 19.15 mmol), THF (10 mL), H_2O (10 mL),(2S)-2-amino-3-(2-bromo-1,3-oxazol-4-yl)propanoic acid (1500.0 mg, 6.38 mmol) and $NaHCO_3$ (3216.74 mg, 38.29 mmol) at room temperature was stirred overnight under an air atmosphere. The reaction was quenched with H_2O at room temperature. The resulting mixture was concentrated under reduced pressure. The resulting mixture was extracted with EtOAc (3×100 mL). The aqueous layer was acidified to pH 6 with 1 M HCl (aq.). The resulting mixture was extracted with EtOAc (3×100 mL). The combined organic layers were dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure to afford (2S)-3-(2-bromo-1,3-oxazol-4-yl)-2-[(tert-butoxycarbonyl)amino]propanoic acid (920 mg, 43.0% yield) as an oil. LCMS (ESI): m/z [M+H] calc'd for $C_{11}H_{15}BrN_2O_5$ 335.02; found 335.0.

Step 7.

A mixture of 3-(2-bromo-1,3-oxazol-4-yl)-2-[(tert-butoxycarbonyl)amino]propanoic acid (850.0 mg, 2.54 mmol), methyl 1,2-diazinane-3-carboxylate (1.88 g, 13.04 mmol), DIPEA (1966.68 mg, 15.22 mmol), DCM (30.0 mL) and HATU (1446.48 mg, 3.80 mmol) at 0° C. was stirred for 3 h under an air atmosphere. The resulting mixture was extracted with DCM (3×50 mL). The combined organic layers were dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The resulting mixture was purified by reverse flash chromatography to afford methyl 1-[3-(2-bromo-1,3-oxazol-4-yl)-2-[(tert-butoxycarbonyl)amino]propanoyl]-1,2-diazinane-3-carboxylate (610 mg, 52.1% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{17}H_{25}BrN_4O$ 461.10; found 461.0.

Step 8.

A mixture of methyl 1-[3-(2-bromo-1,3-oxazol-4-yl)-2-[(tert-butoxycarbonyl)amino]propanoyl]-1,2-diazinane-3-carboxylate (570.0 mg, 1.24 mmol), LiOH (2.0 mL, 1 M aq.) and THF (2.0 mL) at 0° C. was stirred for 3 h under an air atmosphere. The resulting mixture was concentrated under reduced pressure. The resulting mixture was extracted with EtOAc (3×50 mL). The combined aqueous layers were acidified to pH 5 with 1N HCl (aq.). The aqueous phase was extracted with EtOAc (3×50 mL). The combined organic layers were dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure to afford 1-[3-(2-bromo-1,3-oxazol-4-yl)-2-[(tert-butoxycarbonyl)amino]propanoyl]-1,2-diazinane-3-carboxylic acid (500 mg, 90.5% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{16}H_{23}BrN_4O$ 447.09; found 446.8.

Step 9.

A mixture of 1-[3-(2-bromo-1,3-oxazol-4-yl)-2-[(tert-butoxycarbonyl)amino]propanoyl]-1,2-diazinane-3-carboxylic acid (450.0 mg, 1.01 mmol), 3-[(2M)-1-ethyl-2-[2-[(1S)-1-methoxyethyl]pyridin-3-yl]-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)indol-3-yl]-2,2-dimethylpropan-1-ol (743.19 mg, 1.51 mmol), DMAP (24.58 mg, 0.20 mmol), DCM (15.0 mL) and DCC (311.37 mg, 1.51 mmol) at 0° C. was stirred for 3 h under an air atmosphere. The resulting mixture was extracted with EtOAc (3×20 mL). The combined organic layers were dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by Prep-TLC to afford 3-(1-ethyl-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-indol-3-yl)-2,2-dimethylpropyl(S)-1-((S)-3-(2-bromooxazol-4-yl)-2-((tert-butoxycarbonyl)amino)propanoyl)hexahydropyridazine-3-carboxylate (330 mg, 35.6% yield) as a white solid. LCMS (ESI): m/z [M+H] calc'd for $C_{45}H_{62}BBrN_6O_9$ 921.39; found 921.4.

Step 10.

A mixture of 3-(1-ethyl-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-indol-3-yl)-2,2-dimethylpropyl(S)-1-((S)-3-(2-bromooxazol-4-yl)-2-((tert-butoxycarbonyl)amino)propanoyl)

hexahydropyridazine-3-carboxylate (290.0 mg, 0.33 mmol), K_3PO_4 (206.64 mg, 0.97 mmol), X-Phos (30.94 mg, 0.07 mmol), XPhos Pd G3 (54.93 mg, 0.07 mmol), dioxane (5. mL) and H_2O (1.0 mL) at 70° C. was stirred for 4 h under an argon atmosphere. The resulting mixture was extracted with EtOAc (3×20 mL). The combined organic layers were dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by Prep-TLC to afford tert-butyl ((6^{3}S,4S,Z)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(2,4)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (130 mg, 56.0% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{39}H_{50}N_6O_7$ 715.38; found 715.3.

Step 11.

A mixture of tert-butyl ((6^{3}S,4S,Z)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(2,4)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (120.0 mg), DCM (2.0 mL) and TFA (0.2 mL) at room temperature was stirred for 6 h under an air atmosphere. The resulting mixture was concentrated under reduced pressure. to afford (6^{3}S,4S,2)-4-amino-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(2,4)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (90 mg, 87.2% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{34}H_{42}N_6O_5$ 615.33; found 615.3.

Step 12.

A mixture of (6^{3}S,4S,2)-4-amino-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(2,4)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (200.0 mg, 0.33 mmol), (2R)-2-([[1-(diphenylmethyl)azetidin-3-yl]oxy]methyl)-3-methylbutanoic acid (172.49 mg, 0.49 mmol), DIPEA (420.48 mg, 3.25 mmol), DMF (3.0 mL) and HATU (148.44 mg, 0.39 mmol) at 0° C. was stirred for 3 h under an air atmosphere. The resulting mixture was extracted with EtOAc (3×20 mL). The combined organic layers were dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by Prep-TLC to afford (2R)-2-(((1-benzhydrylazetidin-3-yl)oxy)methyl)-N-((6^{3}S,4S,Z)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(2,4)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methylbutanamide (154 mg, 77.0% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{56}H_{67}N_7O_7$ 950.52; found 950.6.

Step 13.

A mixture of (2R)-2-(((1-benzhydrylazetidin-3-yl)oxy)methyl)-N-((6^{3}S,4S,Z)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(2,4)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methylbutanamide (240.0 mg, 0.25 mmol), $(Boc)_2O$ (165.37 mg, 0.76 mmol), MeOH (5.0 mL) and $Pd(OH)_2$ (72.0 mg, 0.51 mmol) at room temperature was stirred overnight under an H_2 atmosphere. The resulting mixture was filtered, the filter cake was washed with MeOH (3×5 mL). The filtrate was concentrated under reduced pressure. The residue was purified by Prep-TLC to afford tert-butyl 3-((2R)-2-(((6^{3}S,4S,Z)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(2,4)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamoyl)-3-methylbutoxy)azetidine-1-carboxylate (150 mg, 67.2% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{48}H_{65}N_7O_9$ 884.49; found 884.2.

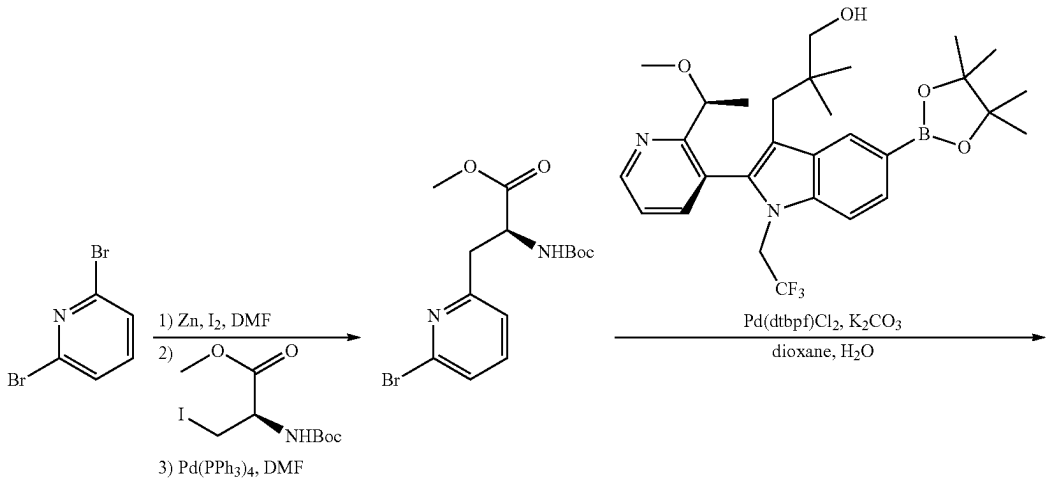
Step 14.

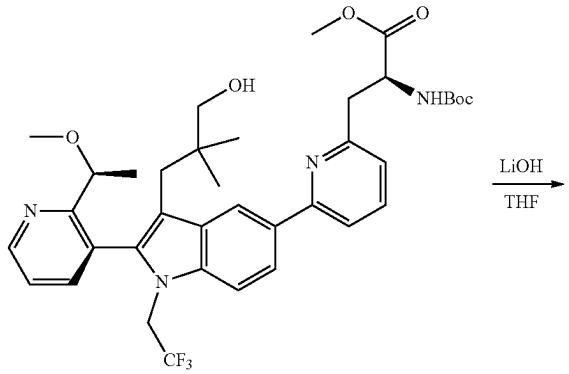
A mixture of tert-butyl 3-((2R)-2-(((6^{3}S,4S,Z)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(2,4)-oxazola-1(5,3)-indola-6(1,3)- pyridazinacycloundecaphane-4-yl)carbamoyl)-3-methylbutoxy)azetidine-1-carboxylate (150.0 mg), DCM (2.0 mL) and TFA (0.40 mL) at 0° C. was stirred for 3 h under an air atmosphere. The resulting mixture was concentrated under reduced pressure to afford (2R)-2-((azetidin-3-yloxy)methyl)-N-((6^{3}S,4S,Z)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(2,4)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methylbutanamide (120 mg, 90.2% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{43}H_{57}N_7O_7$ 784.44; found 784.2.

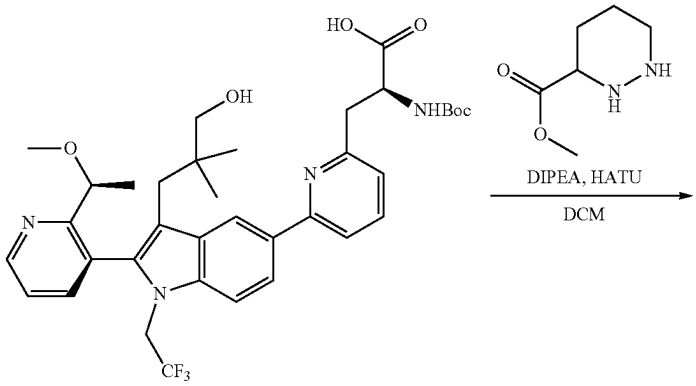
Step 15.

A mixture of (2R)-2-((azetidin-3-yloxy)methyl)-N-((6^{3}S,4S,Z)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(2,4)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methylbutanamide (130.0 mg, 0.17 mmol), sodium 4-(dimethylamino)-4-methylpent-2-ynoate (44.07 mg, 0.25 mmol), DMF (3.0 mL), DIPEA (64.29 mg, 0.50 mmol) and COMU (106.46 mg, 0.25 mmol) at 0° C. was stirred for 3 h under an air atmosphere. The resulting mixture was extracted with EtOAc (3×20 mL). The combined organic layers were dried over anhydrous Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The crude product was purified by reverse phase chromatography to afford (2R)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-N-((6^{3}S,4S,Z)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(2,4)-oxazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methylbutanamide (25 mg, 16.4% yield) as a solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.77 (dd, J=4.8, 1.8 Hz, 1H), 8.55 (s, 1H), 8.14 (dd, J=8.3, 2.9 Hz, 1H), 7.82 (d, J=7.6 Hz, 1H), 7.76-7.65 (m, 3H), 7.54 (dd, J=7.7, 4.7 Hz, 1H), 7.54-7.02 (m, 1H), 5.72 (td, J=7.4, 3.4 Hz, 1H), 4.97 (d, J=11.9 Hz, 1H), 4.46-4.24 (m, 6H), 4.18-4.04 (m, 2H), 3.94 (dd, J=32.9, 7.8 Hz, 1H), 3.77-3.63 (m, 2H), 3.57 (s, 1H), 3.49 (s, 2H), 3.21 (s, 3H), 2.90 (d, J=14.6 Hz, 1H), 2.87-2.79 (m, 1H), 2.72 (td, J=15.5, 14.6, 3.1 Hz, 2H), 2.46 (s, 1H), 2.43-2.26 (m, 6H), 2.11-1.99 (m, 1H), 1.82-1.66 (m, 2H), 1.56-1.37 (m, 11H), 0.89 (dt, J=12.3, 7.7 Hz, 12H), 0.35 (s, 3H). LCMS (ESI): m/z [M+H] calc'd for $C_{51}H_{68}N_8O_8$ 921.52; found 921.5.

Example A607. The synthesis of (2R)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-N-((2^{3}S,6^{3}S,4S)-1^1-ethyl-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(1,3)-piperidinacycloundecaphane-4-yl)-3-methylbutanamide







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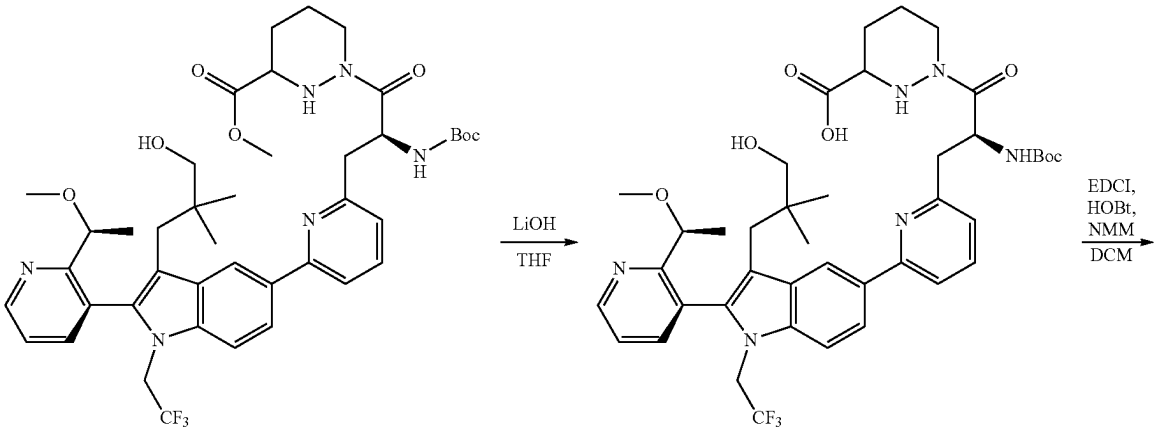

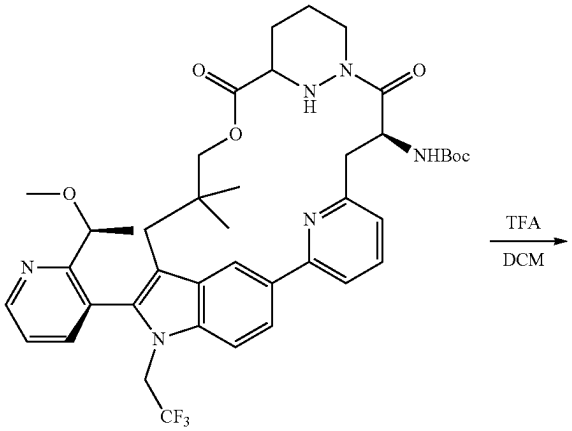

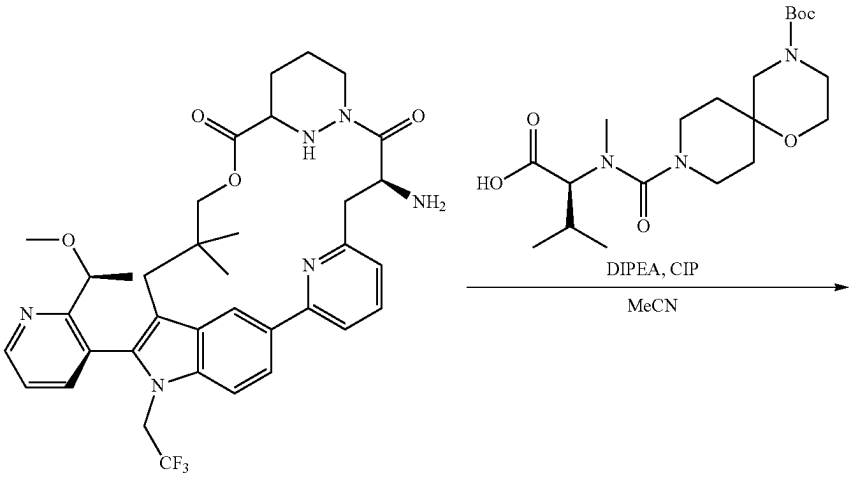

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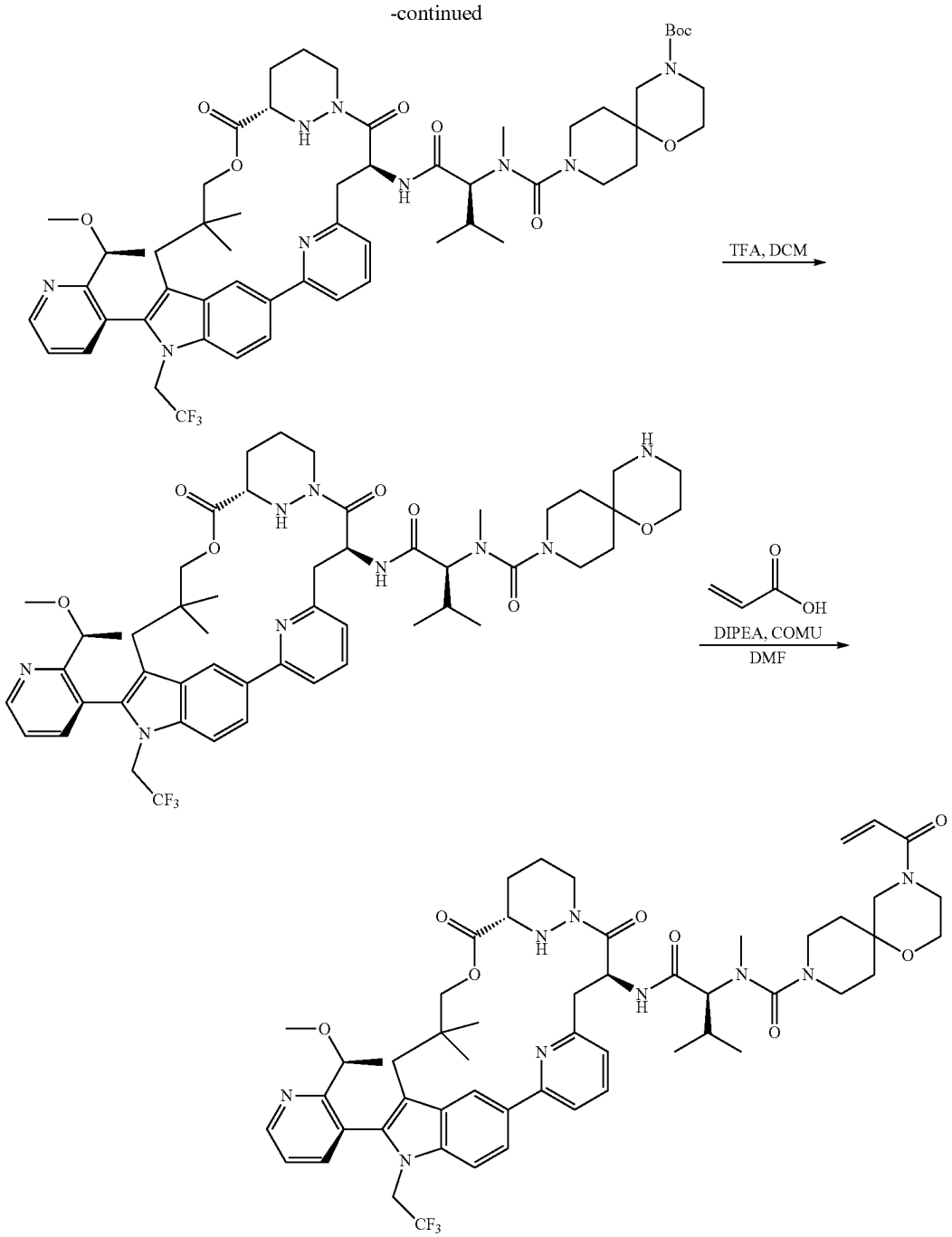


Step 1.

A mixture of Zn (44.18 g, 675.41 mmol) and I_2 (8.58 g, 33.77 mmol) in DMF (120 mL) was stirred for 30 min at 50° C. under an argon atmosphere, followed by the addition of methyl (2R)-2-[(tert-butoxycarbonyl) amino]-3-iodopropanoate (72.24 g, 219.51 mmol) in DMF (200 mL). The reaction mixture was stirred at 50° C. for 2 h under an argon atmosphere. Then a mixture of 2,6-dibromo-pyridine (40 g, 168.85 mmol) and $Pd(PPh_3)_4$ (39.02 g, 33.77 mmol) in DMF (200 mL) was added. The resulting mixture was stirred at 75° C. for 2 h, then cooled down to room temperature and extracted with EtOAc (1 L×3). The combined organic layers were washed with H_2O (1 L×3), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford methyl (2S)-3-(6-bromopyridin-2-yl)-2-[(tert-butoxycarbonyl) amino] propanoate (41 g, 67% yield) as oil. LCMS (ESI): m/z [M+H] calc'd for $C_{14}H_{19}BrN_2O_4$ 358.1; found 359.1.

Step 2.

To a solution of 3-[(2M)-2-[2-[(1 S)-1-methoxyethyl] pyridin-3-yl]-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-(2,2,2-trifluoroethyl) indol-3-yl]-2,2-dimethylpropan-1-ol (45.0 g, 82.35 mmol) in dioxane (400 mL) and H_2O (80 mL), were added potassium carbonate (28.45 g, 205.88 mmol), methyl (2S)-3-(6-bromopyridin-2-yl)-2-[(tert-bu-

toxycarbonyl) amino] propanoate (35.5 g, 98.8 mmol), Pd(dtbpf)Cl_2 (5.37 g, 8.24 mmol) at room temperature. The reaction mixture was stirred at 70° C. for 2 h under a nitrogen atmosphere. The resulting mixture was extracted with EtOAc (500 mL×3). The combined organic layers were washed with H_2O (300 mL×3), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography to afford methyl (S)-2-((tert-butoxycarbonyl)amino)-3-(6-(3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl) pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1H-indol-5-yl)pyridin-2-yl)propanoate (48 g, 83% yield) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{37}H_{45}F_3N_4O_6$ 698.3; found 699.4.

Step 3.

A solution of methyl (S)-2-((tert-butoxycarbonyl)amino)-3-(6-(3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1H-indol-5-yl)pyridin-2-yl)propanoate (52 g, 74.42 mmol) in THF (520 mL), was added LiOH (74.41 mL, 223.23 mmol) at 0° C.

The reaction mixture was stirred at room temperature for 3 h. The resulting mixture was acidified to pH 5 with HCl (aq.) and extracted with EtOAc (1 L×3). The combined organic layers were washed with H_2O (1 L×3), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to afford (S)-2-((tert-butoxycarbonyl) amino)-3-(6-(3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1H-indol-5-yl)pyridin-2-yl)propanoic acid (50 g, 98% yield) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{36}H_{43}F_3N_4O_6$ 684.3; found 685.1.

Step 4.

To a solution of (S)-2-((tert-butoxycarbonyl)amino)-3-(6-(3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1H-indol-5-yl) pyridin-2-yl)propanoic acid (55 g, 80.32 mmol) in DCM (600 mL), were added DIPEA (415.23 g, 3212.82 mmol), and HATU (45.81 g, 120.48 mmol) at 0° C. The reaction mixture was stirred at room temperature for 12 h and then quenched with H_2O. The resulting mixture was extracted with EtOAc (1 L×3). The combined organic layers were washed with H_2O (1 L), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford methyl 1-((S)-2-((tert-butoxycarbonyl) amino)-3-(6-(3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1H-indol-5-yl)pyridin-2-yl)propanoyl)hexahydropyridazine-3-carboxylate (63 g, 96% yield) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{42}H_{53}F_3N_6O_7$ 810.4; found 811.3.

Step 5.

A solution of methyl 1-((S)-2-((tert-butoxycarbonyl) amino)-3-(6-(3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1H-indol-5-yl)pyridin-2-yl)propanoyl)hexahydropyridazine-3-carboxylate (50 g, 61.66 mmol) in THF (500 mL) and 3M LiOH (61.66 mL, 184.980 mmol) at 0° C. was stirred at room temperature for 3 h, then acidified to pH 5 with HCl (aq.). The resulting mixture was extracted with EtOAc (800 mL×3). The combined organic layers were washed with H_2O (800 mL), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to afford 1-((S)-2-((tert-butoxycarbonyl)amino)-3-(6-(3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1H-indol-5-yl)pyridin-2-yl)propanoyl) hexahydropyridazine-3-carboxylic acid (48 g, 97% yield) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{41}H_{51}F_3N_6O_7$ 796.3; found 797.1.

Step 6.

To a solution of 1-((S)-2-((tert-butoxycarbonyl)amino)-3-(6-(3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1H-indol-5-yl)pyridin-2-yl)propanoyl)hexahydropyridazine-3-carboxylic acid (50 g, 62.74 mmol) in DCM (10 L) at 0° C., were added DIPEA (243.28 g, 1882.32 mmol), EDCI (360.84 g, 1882.32 mmol) and HOBT (84.78 g, 627.44 mmol). The reaction mixture was stirred at room temperature for 3 h, quenched with H_2O and concentrated under reduced pressure. The residue was extracted with EtOAc (2 L×3). The combined organic layers were washed with H_2O (2 L×3), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford tert-butyl ((4S)-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(2,6)-pyridinacycloundecaphane-4-yl)carbamate (43.6 g, 89% yield) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{41}H_{49}F_3N_6O_6$ 778.3; found 779.3.

Step 7.

To a solution of tert-butyl ((4S)-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(2,6)-pyridinacycloundecaphane-4-yl)carbamate (300 mg) in DCM (10 mL), was added TFA (3 mL) at 0° C. The reaction mixture was stirred at room temperature for 1 h. The resulting mixture was diluted with toluene (10 mL) and concentrated under reduced pressure three times to afford (4S)-4-amino-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(2,6)-pyridinacycloundecaphane-5,7-dione (280 mg, crude) as oil. LCMS (ESI): m/z [M+H] calc'd for $C_{36}H_{41}F_3N_6O_4$ 679.2; found 678.3.

Step 8.

To a solution of (4S)-4-amino-1^2-(2-((S)-1-methoxyethyl) pyridin-3-yl)-10,10-dimethyl-1'-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(2,6)-pyridinacycloundecaphane- 5,7-dione (140 mg, 0.21 mmol) in MeCN (2 mL), were added DIPEA (266.58 mg, 2.06 mmol), N-(4-(tert-butoxycarbonyl)-1-oxa-4,9-diazaspiro[5.5]undecane-9-carbonyl)-N-methyl-L-valine (127.94 mg, 0.31 mmol) and CIP (114.68 mg, 0.41 mmol) at 0° C. The reaction mixture was stirred at room temperature for 1 h. The resulting mixture was concentrated under reduced pressure. The residue was extracted with EtOAc (10 mL×3). The combined organic layers were washed with H_2O (10 mL×3), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography to afford tert-butyl 9-(((2S)-1-(((6^3S,4S)-1^2-(2-((S)-1-methoxyethyl) pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(2,6)-pyridinacycloundecaphane-4-yl) amino)-3-methyl-1-oxobutan-2-yl) (methyl)carbamoyl)-1-oxa-4,9-diazaspiro [5.5] undecane-4-carboxylate (170 mg, 76% yield) as solid. LCMS (ESI): m/z [M+H] calc'd for $C_{56}H_{74}F_3N_9O_9$ 1073.5; found 1074.6.

Step 9.

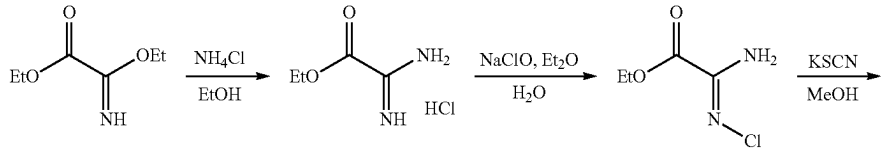
To a solution of tert-butyl 9-(((2S)-1-(((6^3S,4S)-1^2-(2-((S)-1-methoxyethyl) pyridin-3-yl)-10,10-dimethyl-5,7-di-

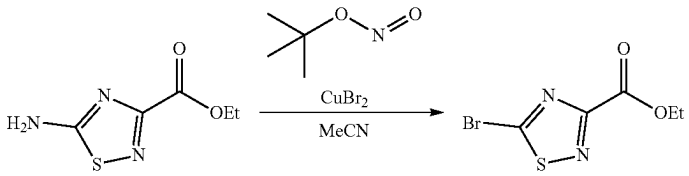
oxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(2,6)-pyridinacycloundecaphane-4-yl) amino)-3-methyl-1-oxobutan-2-yl) (methyl)carbamoyl)-1-oxa-4,9-diazaspiro [5.5] undecane-4-carboxylate (160 mg, 0.15 mmol) in DCM (5 mL) at 0° C., was dropwise added TFA (1.5 mL). The reaction mixture was stirred at 0° C. for 1 h. The resulting mixture was diluted with toluene (10 mL) and concentrated under reduced pressure three times to afford N-((2S)-1-(((6^{3}S,4S)-1^2-(2-((S)-1-methoxyethyl) pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(2,6)-pyridinacycloundecaphane-4-yl) amino)-3-methyl-1-oxobutan-2-yl)-N-methyl-1-oxa-4,9-diazaspiro [5.5] undecane-9-carboxamide (150 mg, crude) as oil. LCMS (ESI): m/z [M+H] calc'd for $C_{51}H_{65}F_3N_9O_7$ 973.5; found 974.4.

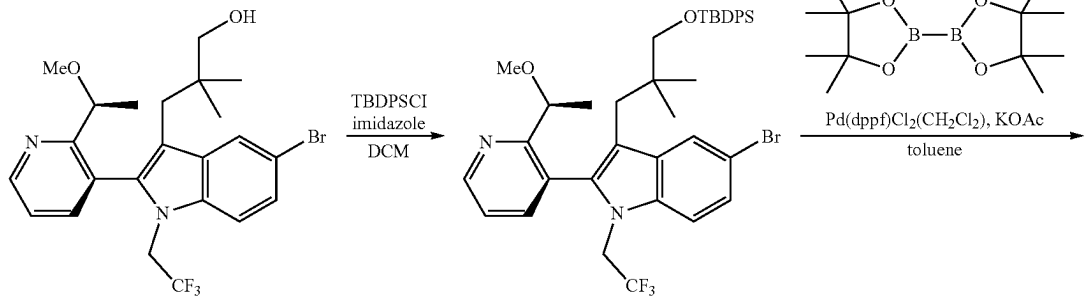
Step 10.

To a solution of N-((2S)-1-(((6^{3}S,4S)-1^2-(2-((S)-1-methoxyethyl) pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(2,6)-pyridinacycloundecaphane-4-yl) amino)-3-methyl-1-oxobutan-2-yl)-N-methyl-1-oxa-4,9-diazaspiro [5.5] undecane-9-carboxamide (150 mg, 0.15 mmol) in DMF (3 mL), were added DIPEA (199.01 mg, 1.54 mmol), acrylic acid (16.64 mg, 0.23 mmol) and COMU (98.39 mg, 0.23 mmol) at 0° C. The reaction mixture was stirred at room temperature for 1 h and concentrated under reduced pressure. The residue was extracted with EtOAc (10 mL×3). The combined organic layers were washed with H_2O (10 mL×3), dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography and reverse phase chromatography to afford 4-acryloyl-N-((2S)-1-(((6^{3}S,4S)-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-1(5,3)-indola-6(1,3)-pyridazina-2(2,6)-pyridinacycloundecaphane-4-yl)amino)-3-methyl-1-oxobutan-2-yl)-N-methyl-1-oxa-4,9-diazaspiro [5.5]undecane-9-carboxamide (53 mg, 33% yield) as solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.79 (dd, J=4.8, 1.9 Hz, 2H), 8.09 (d, J=31.4 Hz, 1H), 7.96 (d, J=8.9 Hz, 1H), 7.90-7.72 (m, 3H), 7.64 (t, J=7.8 Hz, 1H), 7.56 (dd, J=7.8, 4.7 Hz, 1H), 7.03 (s, 1H), 6.86 (dd, J=16.6, 10.4 Hz, 1H), 6.20 (d, J=14.0 Hz, 1H), 5.73 (d, J=12.2 Hz, 2H), 5.47 (s, 1H), 5.35 (d, J=11.8 Hz, 1H), 4.68 (s, 1H), 4.28 (d, J=12.5 Hz, 1H), 4.13 (d, J=6.4 Hz, 1H), 3.92 (s, 1H), 3.84-3.64 (m, 6H), 3.59 (d, J=14.0 Hz, 3H), 3.50 (s, 3H), 3.10 (s, 5H), 3.02 (d, J=13.3 Hz, 2H), 2.85 (d, J=12.1 Hz, 3H), 2.08-1.89 (m, 2H), 1.81 (s, 1H), 1.75-1.51 (m, 5H), 1.39 (d, J=6.1 Hz, 4H), 1.24 (s, OH), 0.91-0.66 (m, 10H), 0.53 (s, 3H). LCMS (ESI): m/z [M+H] calc'd for $C_{54}H_{68}F_3N_9O_8$ 1027.5; found 1028.1.

Example A590. The synthesis of (2R)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-N-((6^{3}S,4S,Z)-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1'-(2,2,2-trifluoroethyl)-6^1, 6^2,6^3,6^4,6^5,6^6-hexahydro-1^1H-8-oxa-2(5,3)-thiadiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methylbutanamide







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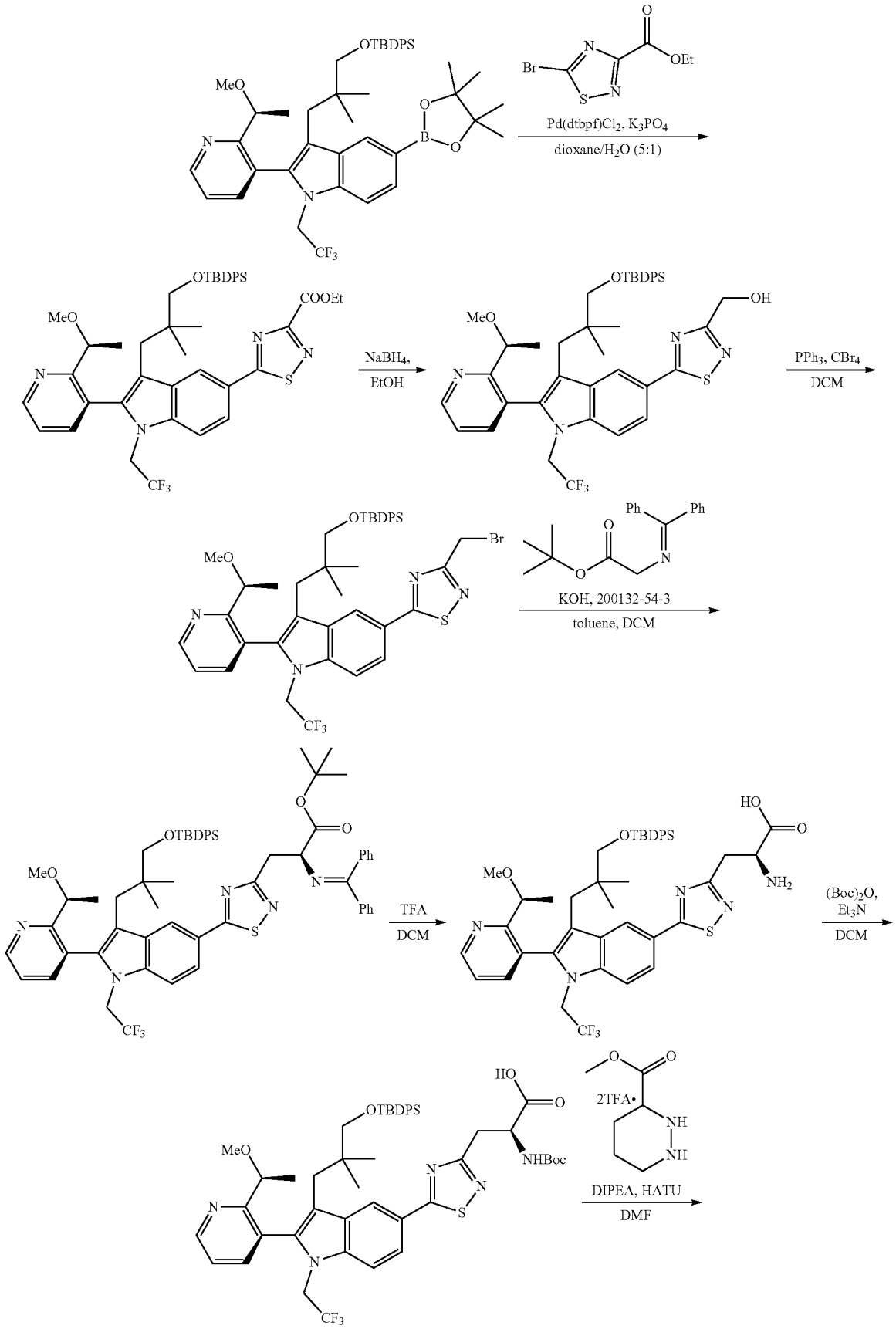
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1109 **1110**

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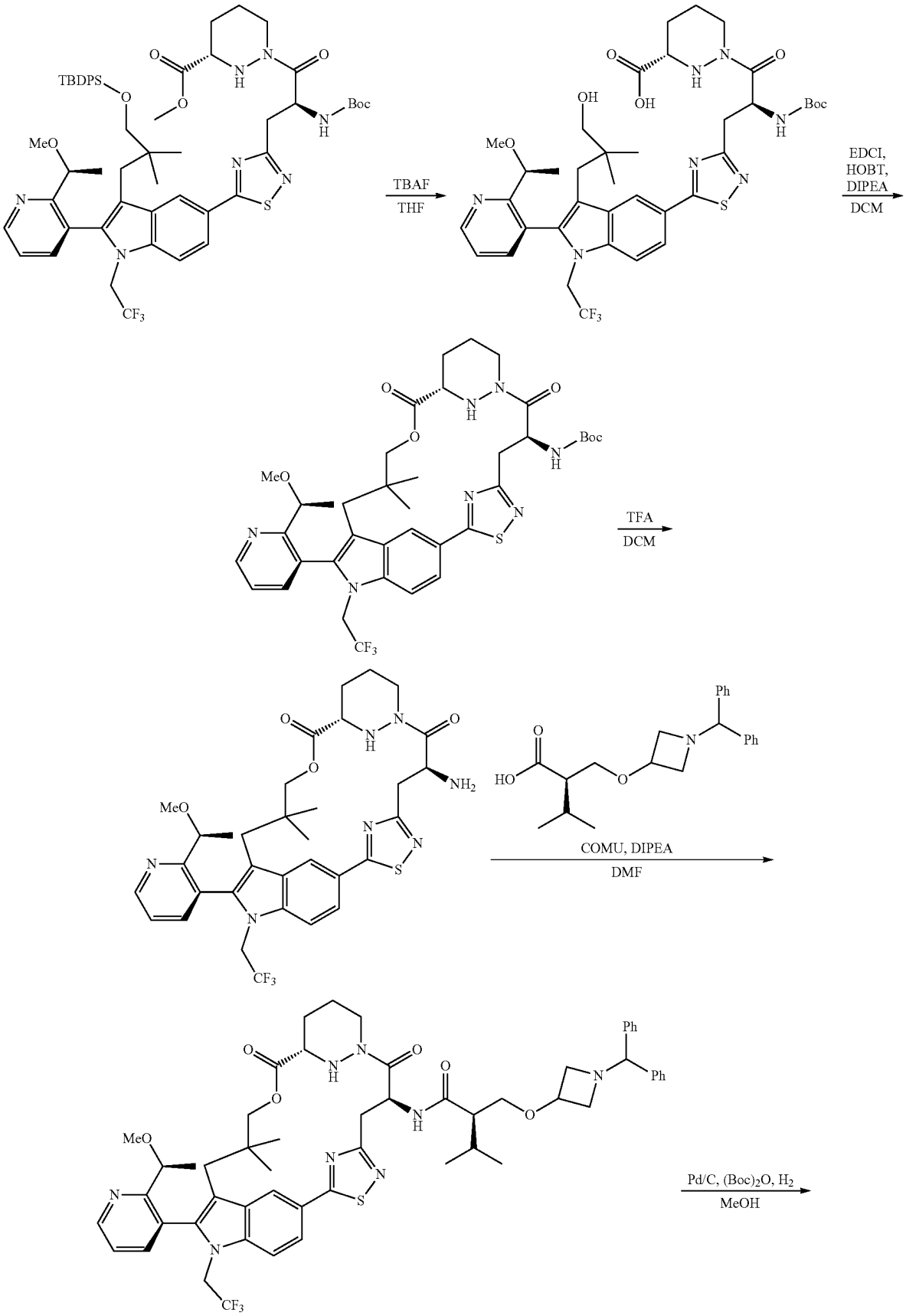

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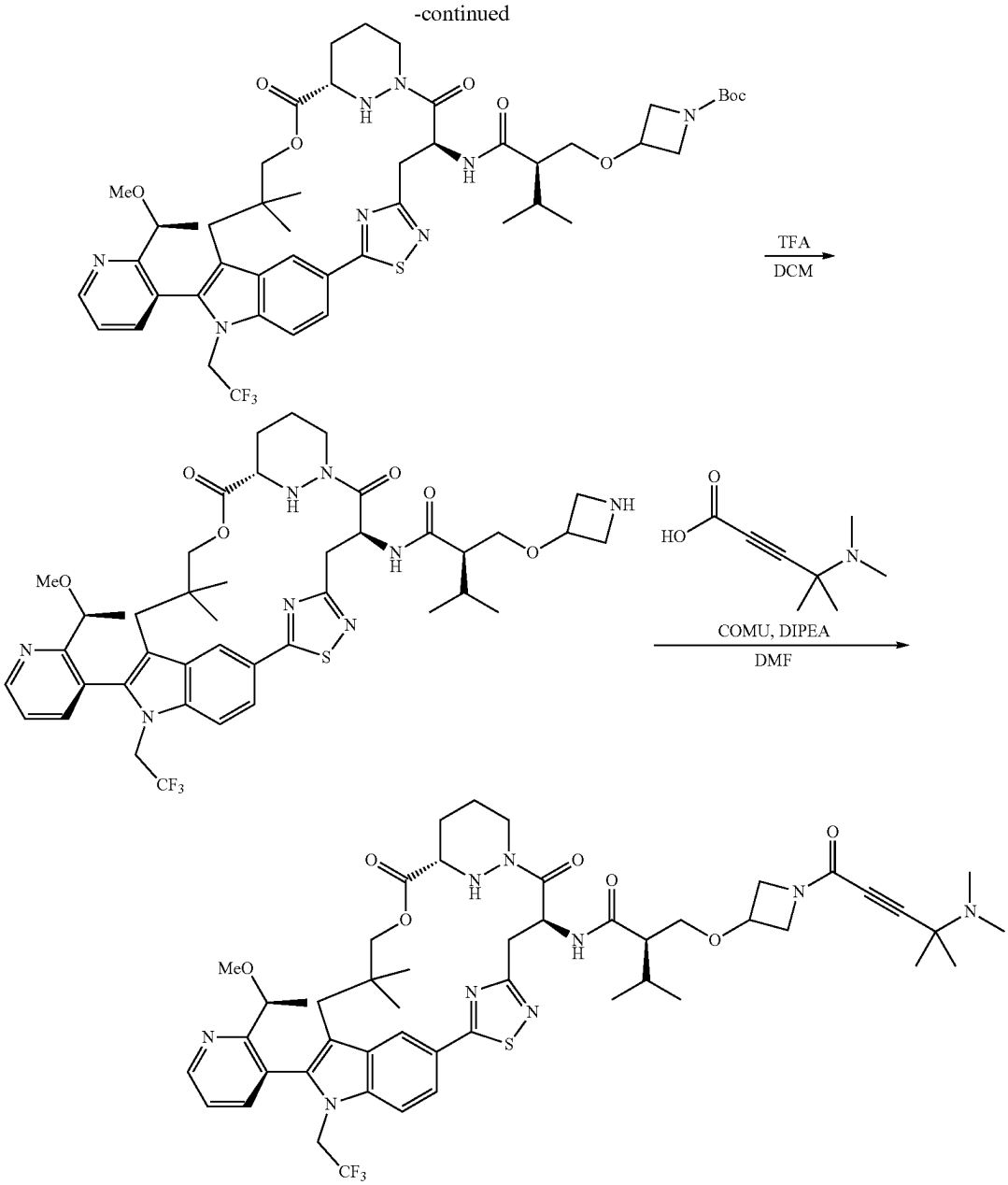

Step 1.

To a mixture of ethyl 2-ethoxy-2-iminoacetate (25.0 g, 172.23 mmol) and EtOH (250.0 mL) at 0° C. was added ammonium chloride (9.21 g, 172.23 mmol) in portions then stirred for 4 h at room temperature under an argon atmosphere. The resulting mixture was concentrated under reduced pressure and washed with Et_2O (3×200 mL). The organic layers were combined and concentrated under reduced pressure. This resulted in ethyl 2-amino-2-iminoacetate hydrochloride (20 g, crude) as a light yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_4H_8N_2O_2$ 117.07; found 116.9.

Step 2.

To a mixture of ethyl 2-amino-2-iminoacetate hydrochloride (13.30 g, 87.17 mmol), H_2O (50.0 mL) and Et_2O (100.0 mL) at 0° C. was added sodium hypochlorite pentahydrate (7.79 g, 104.60 mmol) dropwise. The resulting mixture was stirred for 3 h under an argon atmosphere. The mixture was extracted with Et_2O (3×200 mL). The resulting solution was washed with brine (3×100 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to afford ethyl (Z)-2-amino-2-(chloroimino) acetate (7 g, crude) as a light yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_4H_7ClN_2O_2$ 151.03; found 150.8.

Step 3.

To a solution of ethyl (Z)-2-amino-2-(chloroimino) acetate (8.40 g, 55.792 mmol) and MeOH (130.0 mL) at 0° C. was added potassium thiocyanate (5.42 g, 55.79 mmol) in portions. The resulting mixture was stirred for 4 h at room temperature under an argon atmosphere. The reaction was

quenched with H_2O/Ice. The mixture was extracted with EtOAc (5×100 mL). The resulting organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford ethyl 5-amino-1,2,4-thiadiazole-3-carboxylate (2.3 g, 23.8% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_5H_7N_3O_2S$ 174.03; found 173.8.

Step 4.

To a solution of ethyl 5-amino-1,2,4-thiadiazole-3-carboxylate (5.80 g, 33.49 mmol), MeCN (90.0 mL) and $CuBr_2$ (11.22 g, 50.23 mmol) at 0° C. was added 2-methyl-2-propylnitrit (6.91 g, 66.98 mmol) dropwise under an argon atmosphere. The mixture was stirred for 30 min. The mixture was then stirred for 4 h at 50° C. The mixture was cooled to 0° C. and quenched with H_2O/Ice. The mixture was extracted with EtOAc (3×100 mL). The resulting organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel chromatography to afford ethyl 5-bromo-1,2,4-thiadiazole-3-carboxylate (6.2 g, 78.1% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_5H_5BrN_2O_2S$ 236.93; found 237.1.

Step 5.

To a solution of (S)-3-(5-bromo-2-(2-(1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1H-indol-3-yl)-2,2-dimethylpropan-1-ol (16.60 g, 33.24 mmol), DCM (170.0 mL) and imidazole (5.66 g, 83.10 mmol) at 0° C. was added tert-butyl-chlorodiphenylsilane (11.88 g, 43.21 mmol) dropwise. The resulting mixture was stirred for 3 h at room temperature under an argon atmosphere. The reaction was quenched with H_2O/Ice. The mixture was extracted with EtOAc (3×200 mL). The organic layer was washed with brine (3×100 mL), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford (S)-5-bromo-3-(3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-(1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1H-indole (22 g, 89.7% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{39}H_{44}BrF_3N_2O_2Si$ 737.24; found 737.0.

Step 6.

To a solution of (S)-5-bromo-3-(3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-(1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1H-indole (28.0 g, 37.95 mmol), toluene (270.0 mL), KOAc (9.31 g, 94.88 mmol) and bis(pinacolato)diboron (19.27 g, 75.90 mmol) at 0° C. was added $Pd(dppf)Cl_2 \cdot CH_2Cl_2$ (6.18 g, 7.59 mmol) in portions. The resulting mixture was stirred for 3 h at 90° C. under an argon atmosphere. The mixture was cooled to room temperature and quenched with H_2O/Ice. The mixture was extracted with EtOAc (3×200 mL). The resulting organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford (S)-3-(3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-(1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-(2,2,2-trifluoroethyl)-1H-indole (28.2 g, 94.7% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{45}H_{56}BF_3N_2O_4Si$ 785.41; found 785.4.

Step 7.

To a solution of (S)-3-(3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-(1-methoxyethyl)pyridin-3-yl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-(2,2,2-trifluoroethyl)-1H-indole (19.60 g, 24.97 mmol), 1,4-dioxane (200 mL), H_2O (40 mL), ethyl 5-bromo-1,2,4-thiadiazole-3-carboxylate (5.92 g, 24.97 mmol) and K_3PO_4 (13.25 g, 62.43 mmol) at 0° C. was added $Pd(dtbpf)Cl_2$ (1.63 g, 2.50 mmol) in portions. The resulting mixture was stirred for 1.5 h at 75° C. under an argon atmosphere. The mixture was cooled to 0° C. and quenched with H_2O/Ice and extracted with EtOAc (3×200 mL). The resulting organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure.

The residue was purified by silica gel column chromatography to afford ethyl (S)-5-(3-(3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-(1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1H-indol-5-yl)-1,2,4-thiadiazole-3-carboxylate (14 g, 68.8% yield) as a yellow solid. LCMS (ESI): m/z [M+H] calc'd for $C_{44}H_{49}F_3N_4O_4SSi$ 815.33; found 815.2.

Step 8.

To a solution of ethyl (S)-5-(3-(3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-(1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1H-indol-5-yl)-1,2,4-thiadiazole-3-carboxylate (13.60 g, 16.69 mmol) and EtOH (140.0 mL) at 0° C. was added $NaBH_4$ (3.16 g, 83.43 mmol) in portions. The resulting mixture was stirred for 3 h then quenched with H_2O/Ice. The resulting mixture was washed with brine (3×100 mL) and extracted with EtOAc (3×200 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford (S)-5-(3-(3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-(1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1H-indol-5-yl)-1,2,4-thiadiazol-3-ol (9.7 g, 75.2% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{42}H_{47}F_3N_4O_3SSi$ 773.32; found 773.3.

Step 9.

To a solution of (S)-5-(3-(3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-(1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1H-indol-5-yl)-1,2,4-thiadiazol-3-ol (9.70 g, 12.55 mmol), DCM (100.0 mL) and CBr_4 (8.32 g, 25.10 mmol) at 0° C. was added PPh_3 (6.58 g, 25.10 mmol) in DCM (20.0 mL) dropwise. The resulting mixture was stirred for 2 h under an argon atmosphere then quenched with H_2O/Ice. The mixture was extracted with DCM (3×200 mL). The resulting organic phase was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by reverse flash chromatography to afford (S)-3-(bromomethyl)-5-(3-(3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-(1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1H-indol-5-yl)-1,2,4-thiadiazole (9.5 g, 90.6% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{42}H_{46}BrF_3N_4O_2SSi$ 835.23; found 834.9.

Step 10.

To a stirred solution of (S)-3-(bromomethyl)-5-(3-(3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-(1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1H-indol-5-yl)-1,2,4-thiadiazole (9.40 g, 11.25 mmol), toluene (84.0 mL), DCM (36.0 mL), tert-butyl 2-[(diphenylmethylidene)amino]acetate (3.32 g, 11.25 mmol) and O-Allyl-N-(9-anthracenylmethyl)cinchonidinium bromide (0.68 g, 1.13 mmol) at 0° C. was added 9M KOH aqueous (94.0 mL) dropwise. The resulting mixture was stirred overnight under an argon atmosphere. The mixture was extracted with EtOAc (3×200 mL) and the organic phase was concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford tert-butyl (S)-3-(5-(3-(3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1H-indol-5-yl)-1,2,4-thiadiazol-3-yl)-2-((diphenylmethylene)amino)propanoate (9 g, 76.2% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{61}H_{66}F_3N_5O_4SSi$ 1050.46; found 1050.8.

Step 11.

To a solution of tert-butyl (S)-3-(5-(3-(3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1H-indol-5-yl)-1,2,4-thiadiazol-3-yl)-2-((diphenylmethylene)amino) propanoate (8.0 g, 7.62 mmol) and DCM (40.0 mL) at 0° C. solution was added TFA (40.0 mL) dropwise. The resulting mixture was stirred overnight at room temperature then concentrated under reduced pressure. The residue was basified to pH 8 with $NaHCO_3$. The mixture was extracted with EtOAc (3×200 mL). The organic phase was concentrated under reduced pressure. The residue was purified by reverse flash chromatography to afford (S)-2-amino-3-(5-(3-(3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1H-indol-5-yl)-1,2,4-thiadiazol-3-yl)propanoic acid (5 g, 79.1% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{44}H_{50}F_3N_5O_4SSi$ 830.34; found 830.2.

Step 12.

To a solution of (S)-2-amino-3-(5-(3-(3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1H-indol-5-yl)-1,2,4-thiadiazol-3-yl)propanoic acid (4.70 g, 5.66 mmol), DCM (50.0 mL) and Et_3N (2.86 g, 28.31 mmol) at 0° C. was added $(Boc)_2O$ (1.36 g, 6.23 mmol) dropwise. The resulting mixture was stirred for 3 h at room temperature under an argon atmosphere then concentrated under reduced pressure and purified by reverse flash chromatography to afford (S)-2-((tert-butoxycarbonyl)amino)-3-(5-(3-(3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1H-indol-5-yl)-1,2,4-thiadiazol-3-yl)propanoic acid (5 g, 94.9% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{49}H_{58}F_3N_5O_6SSi$ 930.39; found 930.3.

Step 13.

To a mixture of (S)-2-((tert-butoxycarbonyl)amino)-3-(5-(3-(3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1H-indol-5-yl)-1,2,4-thiadiazol-3-yl)propanoic acid (5.30 g, 5.70 mmol), DMF (60.0 mL), methyl 1,2-diazinane-3-carboxylate (1.64 g, 11.40 mmol) and DIPEA (22.09 g, 170.94 mmol) at 0° C. was added HATU (2.82 g, 7.41 mmol) in DMF (5 mL) dropwise. The resulting mixture was stirred for 3 h at room temperature under an argon atmosphere. The reaction was then quenched with H_2O/Ice. The mixture was extracted with EtOAc (3×100 mL) and the organic phase was washed with brine (3×100 mL). The resulting mixture was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford methyl (S)-1-((S)-2-((tert-butoxycarbonyl)amino)-3-(5-(3-(3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1H-indol-5-yl)-1,2,4-thiadiazol-3-yl)propanoyl)hexahydropyridazine-3-carboxylate (5.6 g) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{55}H_{68}F_3N_7O_7SSi$ 1056.47; found 1056.2.

Step 14.

A mixture of methyl (S)-1-((S)-2-((tert-butoxycarbonyl) amino)-3-(5-(3-(3-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1H-indol-5-yl)-1,2,4-thiadiazol-3-yl) propanoyl)hexahydropyridazine-3-carboxylate (5.60 g, 5.30 mmol) and TBAF in THF (56.0 mL) was stirred overnight at 40° C. under an argon atmosphere. The reaction was quenched with sat. NH_4Cl (aq.). The mixture was extracted with EtOAc (3×100 mL) and the organic phase was concentrated under reduced pressure. The residue was purified by reverse flash chromatography to afford (S)-1-((S)-2-((tert-butoxycarbonyl)amino)-3-(5-(3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1H-indol-5-yl)-1,2,4-thiadiazol-3-yl) propanoyl)hexahydropyridazine-3-carboxylic acid (4.1 g, 96.2% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{38}H_{48}F_3N_7O_7S$ 804.34; found 804.3.

Step 15.

To a solution of (S)-1-((S)-2-((tert-butoxycarbonyl) amino)-3-(5-(3-(3-hydroxy-2,2-dimethylpropyl)-2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-1-(2,2,2-trifluoroethyl)-1H-indol-5-yl)-1,2,4-thiadiazol-3-yl)propanoyl)hexahydropyridazine-3-carboxylic acid (4.0 g, 5.0 mmol) and DCM (450.0 mL) at 0° C. were added DIPEA (51.45 g, 398.08 mmol), HOBt (6.72 g, 49.76 mmol) and EDCI (57.23 g, 298.55 mmol) in portions. The resulting mixture was stirred for 16 h at room temperature under an argon atmosphere. The reaction was quenched with H_2O/Ice and extracted with EtOAc (3×30 mL). The organic phase was concentrated under reduced pressure. The residue was purified by silica gel column chromatography to afford tert-butyl ((6^{3}S,4S,Z)-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(5,3)-thiadiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (1.7 g, 43.5% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{38}H_{46}F_3N_7O_6S$ 786.33; found 786.3.

Step 16.

To a solution of ((6^{3}S,4S,Z)-1^2-(2-((S)-1-methoxyethyl) pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(5,3)-thiadiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamate (300.0 mg, 0.38 mmol) and DCM (2.0 mL) at 0° C. was added TFA (1.0 mL) dropwise. The resulting mixture was stirred for 2 h at room temperature then concentrated under reduced pressure. The residue was basified to pH 8 with saturated $NaHCO_3$ (aq.). The mixture was extracted with EtOAc (3×20 mL). The organic phase was concentrated under reduced pressure to afford (6^{3}S,4S,Z)-4-amino-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(5,3)-thiadiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (270 mg, crude) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{33}H_{38}F_3N_7O_4S$ 686.27; found 686.1.

Step 17.

To a solution of (6^{3}S,4S,Z)-4-amino-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(5,3)-thiadiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-5,7-dione (160.0 mg, 0.23 mmol), (R)-2-(((1-benzhydrylazetidin-3-yl)oxy)methyl)-3-methylbutanoic acid (123.70 mg, 0.35 mmol) and DMF (2.0 mL) at 0° C. were added DIPEA (603.09 mg, 4.660 mmol) and COMU (119.91 mg, 0.28 mmol) in DMF (0.5 mL). The resulting mixture was stirred for 2 h at room temperature under an argon atmosphere. The reaction was quenched with H_2O/Ice and extracted with EtOAc (3×20 mL). The organic phase was washed with brine (3×10 mL) and concentrated under reduced pressure. The residue was purified by Prep-TLC to afford (2R)-2-(((1-benzhydrylazetidin-3-yl)oxy)methyl)-N-((6^{3}S,4S,Z)-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(5,3)-thiadiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methylbutan-

amide (160 mg, 67.2% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{55}H_{63}F_3N_8O_6S$ 1021.46; found 1021.4.

Step 18.

To a solution of (2R)-2-(((1-benzhydrylazetidin-3-yl)oxy)methyl)-N-((6^{3}S,4S,Z)-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(5,3)-thiadiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methylbutanamide (160.0 mg, 0.16 mmol) and MeOH (5.0 mL) at 0° C. was added $(Boc)_2O$ (85.49 mg, 0.39 mmol) dropwise followed by Pd/C (320.0 mg) in portions. The resulting mixture was stirred overnight at room temperature under a hydrogen atmosphere. The resulting mixture was filtered and the filter cake was washed with EtOAc (3×20 mL). The filtrate was concentrated under reduced pressure. The residue was purified by Prep-TLC to afford tert-butyl 3-((2R)-2-(((6^{3}S,4S,Z)-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(5,3)-thiadiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamoyl)-3-methylbutoxy)azetidine-1-carboxylate (80 mg, 53.5% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{47}H_{61}F_3N_8O_8S$ 955.44; found 955.2.

Step 19.

To a solution of tert-butyl 3-((2R)-2-(((6^{3}S,4S,Z)-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1'-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(5,3)-thiadiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)carbamoyl)-3-methylbutoxy)azetidine-1-carboxylate (120.0 mg, 0.13 mmol) and DCM (0.80 mL) at 0° C. was added TFA (0.4 mL) dropwise and the resulting mixture was stirred for 2 h at room temperature. The mixture was basified to pH 8 with saturated $NaHCO_3$ (aq.). The mixture was extracted with EtOAc (3×10 mL) and concentrated under reduced pressure. The residue was purified by reverse flash chromatography to afford (2R)-2-((azetidin-3-yloxy)methyl)-N-((6^{3}S,4S,2)-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(5,3)-thiadiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methylbutanamide (40 mg, 37.2% yield) as a solid. LCMS (ESI): m/z [M+H] calc'd for $C_{42}H_{53}F_3N_8O_6S$ 855.38; found 855.3.

Step 20.

To a solution of (2R)-2-((azetidin-3-yloxy)methyl)-N-((6^{3}S,4S,Z)-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1'-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(5,3)-thiadiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methylbutanamide (32.0 mg, 0.037 mmol),4-(dimethylamino)-4-methylpent-2-ynoic acid (11.62 mg, 0.074 mmol) and DMF (0.50 mL) at 0° C. were added DIPEA (193.49 mg, 1.48 mmol) and COMU (19.23 mg, 0.044 mmol) in DMF (0.1 mL) dropwise. The resulting mixture was stirred for 2 h at room temperature under an argon atmosphere. The reaction was quenched with H_2O/Ice and extracted with EtOAc (3×20 mL). The organic phase was washed with brine (3×10 mL) and concentrated under reduced pressure. The crude product (60 mg) was purified by reverse phase chromatography to afford (2R)-2-(((1-(4-(dimethylamino)-4-methylpent-2-ynoyl)azetidin-3-yl)oxy)methyl)-N-((6^{3}S,4S,Z)-1^2-(2-((S)-1-methoxyethyl)pyridin-3-yl)-10,10-dimethyl-5,7-dioxo-1^1-(2,2,2-trifluoroethyl)-6^1,6^2,6^3,6^4,6^5,6^6-hexahydro-1^{1}H-8-oxa-2(5,3)-thiadiazola-1(5,3)-indola-6(1,3)-pyridazinacycloundecaphane-4-yl)-3-methylbutanamide (11.7 mg, 30.9% yield) as a solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.80 (dd, J=4.7, 1.8 Hz, 1H), 8.58 (s, 1H), 8.30 (d, J=8.9 Hz, 1H), 7.92 (d, J=8.6 Hz, 1H), 7.84-7.69 (m, 2H), 7.56 (dd, J=7.8, 4.8 Hz, 1H), 5.78 (t, J=8.6 Hz, 2H), 5.10 (d, J=12.1 Hz, 1H), 4.91 (dd, J=16.9, 8.8 Hz, 1H), 4.31 (d, J=6.6 Hz, 6H), 4.05 (dd, J=16.3, 6.6 Hz, 2H), 3.87 (d, J=6.3 Hz, 1H), 3.75 (d, J=11.3 Hz, 1H), 3.61 (d, J=11.0 Hz, 1H), 3.53 (d, J=9.8 Hz, 1H), 3.45 (s, 3H), 3.25 (s, 1H), 3.09 (d, J=10.4 Hz, 1H), 3.01 (d, J=14.5 Hz, 1H), 2.79 (s, 1H), 2.45-2.35 (m, 2H), 2.20 (d, J=5.4 Hz, 6H), 2.15 (d, J=12.0 Hz, 1H), 1.81 (d, 2H), 1.74-1.65 (m, 1H), 1.54 (s, 1H), 1.41-1.30 (m, 9H), 1.24 (s, 1H), 0.94 (s, 3H), 0.90-0.81 (m, 5H), 0.29 (s, 3H). LCMS (ESI): m/z [M+H] calc'd for $C_{50}H_{64}F_3N_9O_7S$ 992.47; found 992.5.

The following table of compounds (Table 3) were prepared using the aforementioned methods or variations thereof, as is known to those of skill in the art.

TABLE 3

Exemplary Compounds Prepared by Methods of the Present Invention

Ex#	LCMS (ESI): m/z [M + H] Found
A1	944.5
A2	888.6
A3	903.2
A4	876.3
A5	903.4
A6	945.8
A7	1058.1
A8	960.8
A9	921.7
A10	928.8
A11	936.4
A12	942.5
A13	932.5
A14	917.5
A15	961.5
A16	890.6
A17	875.4
A18	918.5
A19	975.6
A20	934.5
A21	903.3
A22	903.6
A23	844.6
A24	887.6
A25	921.4
A26	930.4
A27	915.6
A28	890.6
A29	903.5
A30	902.7
A31	901.4
A32	1003.4
A33	917.4
A34	942.7
A35	956.1
A36	1005.5
A37	917.5
A38	959.5
A39	942.9
A40	915.7
A41	917.5
A42	888.6
A43	942.5
A44	904.35
A45	846.35
A46	982.5
A47	960.1
A48	905.3
A49	981.8
A50	916.9
A51	946.7
A52	916.5
A53	921.6
A54	947.7

TABLE 3-continued

Exemplary Compounds Prepared by Methods of the Present Invention

Ex#	LCMS (ESI): m/z [M + H] Found
A55	916.5
A56	904.5
A57	930.5
A58	895.5
A59	944.7
A60	931.5
A61	847.7
A62	871.5
A63	930.4
A64	893.7
A65	874.7
A66	876.5
A67	970.5
A68	915.4
A69	927.3
A70	876.4
A71	933.5
A72	1003.5
A73	903.6
A74	885.5
A75	904.7
A76	904.5
A77	860.6
A78	1018.5
A79	947.5
A80	933.3
A81	917.8
A82	908.7
A83	890.6
A84	890.6
A85	891.5
A86	885.5
A87	947.7
A88	945.5
A89	944.8
A90	898
A91	890.5
A92	903.4
A93	901.4
A94	881.5
A95	987.8
A96	945.7
A97	931.8
A98	943.8
A99	849.7
A100	942.8
A101	893.4
A102	904.4
A103	947.5
A104	931.7
A105	915.6
A106	902.5
A107	945.7
A108	902.3
A109	906.5
A110	793.4
A111	946.7
A112	905.14
A113	849.6
A114	888.4
A115	888.4
A116	986.5
A117	879.5
A118	881.6
A119	874.5
A120	929.7
A121	943.6
A122	888.8
A123	959.3
A124	924.6
A125	962.5
A126	986.5
A127	946.6
A128	903.4

TABLE 3-continued

Exemplary Compounds Prepared by Methods of the Present Invention

Ex#	LCMS (ESI): m/z [M + H] Found
A129	1001.2
A130	930.5
A131	959.5
A132	940.5
A133	969.7
A134	990.5
A135	933.5
A136	875.4
A137	889.5
A138	929.4
A139	930.4
A140	943.7
A141	943.8
A142	901.7
A143	852.3
A144	886.7
A145	916.7
A146	963.4
A147	864.4
A148	885.5
A149	947.5
A150	902.7
A151	904.5
A152	885.5
A153	949.6
A154	907.5
A155	944.5
A156	943.7
A157	943.5
A158	904.5
A159	904.5
A160	858.5
A161	947.4
A162	973.5
A163	989.4
A164	947.5
A165	960.3
A166	907.7
A167	918.5
A168	907.4
A169	859.4
A170	874.3
A171	867.5
A172	883.6
A173	900.5
A174	848.4
A175	959.5
A176	944.5
A177	935.4
A178	930.4
A179	849.5
A180	849.5
A181	918.8
A182	1002.2
A183	961.5
A184	1003.5
A185	928.5
A186	992.5
A187	945.5
A188	960.4
A189	1000.6
A190	934.3
A191	985.5
A192	971.5
A193	871.6
A194	918.5
A195	927.4
A196	972.3
A197	896.4
A198	916.6
A199	916.6
A200	987.4
A201	993.7
A202	896.4

TABLE 3-continued

Exemplary Compounds Prepared by Methods of the Present Invention

Ex#	LCMS (ESI): m/z [M + H] Found
A203	987.6
A204	987.6
A205	933.5
A206	930.5
A207	930.5
A208	958.8
A209	1015.7
A210	906.5
A211	905.5
A212	901.3
A213	969.5
A214	933.6
A215	890.3
A216	916.4
A217	930.5
A218	983.5
A219	917.4
A220	996.5
A221	915.4
A222	930.05
A223	920.01
A224	983.5
A225	982.7
A226	873.7
A227	887.7
A228	911
A229	896.7
A230	905.5
A231	912.4
A232	849.4
A233	905.4
A234	1053.3
A235	835.5
A236	882.3
A237	1015.4
A238	971.5
A239	869.4
A240	884.4
A241	987.4
A242	877.4
A243	863.5
A244	1029.6
A245	1029.5
A246	501.4
A247	985.5
A248	896.4
A249	984.5
A250	927.4
A251	985.4
A252	927.3
A253	990.4
A254	933.3
A255	930.7
A256	890.4
A257	947.4
A258	990.3
A259	933.3
A260	918.4
A261	975.5
A262	919.4
A263	976.5
A264	990.5
A265	933.5
A266	945.5
A267	987.5
A268	973.5
A269	487.3
A270	959.4
A271	861.3
A272	847.6
A273	861.6
A274	930.5
A275	916.3
A276	892.8

TABLE 3-continued

Exemplary Compounds Prepared by Methods of the Present Invention

Ex#	LCMS (ESI): m/z [M + H] Found
A277	895.7
A278	879.5
A279	895.4
A280	954.6
A281	916.6
A282	952.5
A283	931.4
A284	889.5
A285	839.4
A286	877.4
A287	894.7
A288	879.3
A289	908.7
A290	971.6
A291	978.5
A292	987.5
A293	921.4
A294	902.8
A295	916.4
A296	930.8
A297	938.4
A298	952.6
A299	966.7
A300	919.6
A301	907.7
A302	895.8
A303	895.4
A304	897.5
A305	968.6
A306	968.7
A307	952.6
A308	952.7
A309	933.6
A310	926.8
A311	944.7
A312	897.5
A313	976.5
A314	893.3
A316	930.5
A317	982.6
A318	883.5
A319	916.5
A320	886.6
A321	900.6
A322	1004.4
A323	976.5
A324	969.5
A325	1033.7
A326	997.5
A327	906.6
A328	986.7
A329	890.5
A330	895.6
A331	879.5
A332	942.5
A333	919.6
A334	930.7
A335	924.5
A336	927.4
A337	501.4
A338	919.4
A339	971.6
A340	895.7
A341	938.5
A342	906.8
A343	992.4
A344	901.40
A345	897.40
A346	942.50
A347	896.30
A348	1,002.30
A349	924.6
A350	968.2
A351	997.6

TABLE 3-continued

Exemplary Compounds Prepared by Methods of the Present Invention

Ex#	LCMS (ESI): m/z [M + H] Found
A352	952.7
A353	966.3
A354	966.2
A355	855.6
A356	910.4
A357	1091.0
A358	902.5
A359	904.7
A360	924.5
A361	967.5
A362	936.6
A363	961.4
A364	946.5
A365	995.6
A366	921.5
A367	950.6
A368	924.5
A369	924.5
A370	1087.3
A371	902.5
A372	992.1
A373	902.6
A374	1,034.40
A375	993.50
A376	984.40
A377	939.50
A378	897.50
A379	1005.6
A380	949.4
A381	921.5
A382	938.5
A383	938.5
A384	924.5
A385	938.3
A386	938.4
A387	938.5
A388	938.5
A389	936.5
A390	924.5
A391	1011.6
A392	958.0
A393	922.4
A394	950.3
A395	936.5
A396	924.4
A397	924.7
A398	914.6
A399	902.5
A400	956.6
A401	955.9
A402	940.8
A403	948.40
A404	980.40
A405	911.50
A406	938.7
A407	922.5
A408	922.5
A409	922.6
A410	1046.9
A411	990.7
A412	912.7
A413	914.7
A414	983.5
A415	950.5
A416	924.6
A417	936.7
A418	938.5
A419	922.3
A420	936.7
A421	924.5
A422	910.5
A423	1028.9
A424	934.6
A425	1012.5

TABLE 3-continued

Exemplary Compounds Prepared by Methods of the Present Invention

Ex#	LCMS (ESI): m/z [M + H] Found
A426	912.5
A427	895.30
A428	951.30
A429	911.50
A430	938.5
A431	952.3
A432	924.2
A433	924.2
A434	924.7
A435	910.5
A436	924.6
A437	912.5
A438	898.5
A439	1016.6
A440	990.5
A441	939.6
A442	889.2
A443	913.5
A444	913.5
A445	890.1
A446	957.6
A447	880.5
A448	888.4
A449	920.5
A450	897.2
A451	902.6
A452	888.4
A453	901.3
A454	1044.6
A455	949.2
A456	1059.5
A457	916.7
A458	916.2
A459	902.3
A460	874.5
A461	957.9
A462	854.3
A463	910.7
A464	964.5
A465	964.5
A466	1033.7
A467	874.5
A468	879.6
A469	902.6
A470	900.6
A471	900.6
A472	964.6
A473	854.3
A474	914.4
A475	914.40
A476	983.6
A477	907.5
A478	900.5
A479	993.6
A480	902.2
A481	930.6
A482	974.6
A483	847.5
A484	847.4
A485	938.4
A486	893.5
A487	900.6
A488	888.5
A489	962.6
A490	896.5
A491	910.2
A492	910.5
A493	910.5
A494	886.3
A495	907.5
A496	900.4
A497	902.7
A498	902.6
A499	998.7

TABLE 3-continued

Exemplary Compounds Prepared by Methods of the Present Invention

Ex#	LCMS (ESI): m/z [M + H] Found
A500	897.5
A501	906.3
A502	949.2
A503	920.6
A504	920.8
A505	920.0
A506	879.3
A507	897.4
A508	942.4
A509	942.4
A510	899.3
A511	919.5
A512	900.5
A513	983.7
A514	983.7
A515	985.7
A516	985.7
A517	892.6
A518	920.6
A519	920.4
A520	990.6
A521	990.6
A522	945.3
A523	945.4
A524	914.5
A525	914.4
A526	1005.4
A527	972.4
A528	985.4
A529	987.4
A530	898.5
A531	912.3
A532	1013.6
A533	999.3
A534	983.4
A535	919.5
A536	961.7
A537	961.6
A538	999.7
A539	960.4
A540	1050.2
A541	1078.7
A542	1155.7
A543	952.6
A544	952.6
A545	981.2
A546	922.3
A547	922.5
A548	950.5
A549	997.4
A550	1025.6
A551	997.5
A552	950.4
A553	974.6
A554	957.5
A555	1054.4
A556	972.5
A557	952.4
A558	959.6
A559	1011.7
A560	1011.6
A561	922.5
A562	954.3
A563	978.5
A564	982.5
A565	1039.3
A566	1039.4
A567	1039.4
A568	1039.3
A569	997.4
A570	974.7
A571	1038.5
A572	1056.5
A573	1063.5

TABLE 3-continued

Exemplary Compounds Prepared by Methods of the Present Invention

Ex#	LCMS (ESI): m/z [M + H] Found
A574	990.6
A575	1004.5
A576	991.5
A577	1062.3
A578	1048.3
A579	963.3
A580	966.6
A581	966.6
A582	953.5
A583	938.5
A584	944.7
A585	944.6
A586	950.6
A587	962.6
A588	938.5
A589	1016.6
A590	992.5
A591	1008.7
A592	997.5
A593	1020.5
A594	1020.6
A595	1000.6
A596	966.7
A597	946.6
A598	979.6
A599	958.7
A600	887.4
A601	985.5
A602	1036.5
A603	989.9
A604	1006.1
A605	1059.7
A606	1032.6
A607	1028.1
A608	1027.1
A609	966.6
A610	945.6
A611	1021.5
A612	920.6
A613	956.1
A614	992.5
A615	1010.5
A616	1010.5
A617	990.5
A618	950.7
A619	950.4
A620	938.1
A621	966.1
A622	982.6
A623	999.6
A624	1019.5
A625	1019.5
A626	913.5
A627	913.4
A628	1046.5
A629	952.2
A630	967.6
A631	942.5
A632	942.6
A633	938.6
A634	938.6
A635	978.6
A636	916.6
A637	909.5
A638	909.5
A639	928.3
A640	942.0
A641	942.6
A642	1047.5
A643	966.6
A644	944.4
A645	986.5
A646	937.5
A647	1012.5

TABLE 3-continued

Exemplary Compounds Prepared by Methods of the Present Invention	
Ex#	LCMS (ESI): m/z [M + H] Found
A648	944.5
A649	921.6
A650	1007.6
A651	964.2
A652	1903.1
A653	938.6
A654	938.6
A655	934.4
A656	1005.5
A657	960.7
A658	1003.3
A659	974.6
A660	925.5
A661	936.6
A662	936.6
A663	935.5
A664	1047.5
A665	1035.5
A666	1035.2
A667	936.3
A668	965.6
A669	1049.7
A670	1047.7
A671	964.6
A672	997.9
A673	1015.3
A674	975.5
A675	921.5
A676	966.5
A677	966.4
A678	950.5
A679	990.2
A680	943.6
A681	980.6
A682	968.6
A683	1000.6
A684	915.0
A685	977.6
A686	922.5
A687	936.4
A688	936.5
A689	1036.9
A690	979.6
A691	923.5
A692	962.5
A693	906.5
A694	896.1
A695	980.7
A696	978.4
A697	978.3
A698	964.3
A699	1003.3
A700	927.2
A701	993.7
A702	950.6
A703	985.5
A704	938.6
A705	925.5
A706	952.5
A707	1016.5
A708	903.2
A709	981.5
A710	967.5
A711	964.5
A712	937.9
A713	939.9
A714	952.5
A715	937.4
A716	893.5
A717	924.6
A718	936.6
A719	950.5
A720	935.3
A721	937.3

TABLE 3-continued

Exemplary Compounds Prepared by Methods of the Present Invention	
Ex#	LCMS (ESI): m/z [M + H] Found
A722	929.4
A723	910.4
A724	985.4
A725	919.3
A726	1010.4
A727	1025.5
A728	924.6
A729	993.5
A730	993.2
A731	922.7
A732	928.6
A733	997.6
A734	936.5
A735	952.5
A736	922.2
A737	922.1
A738	924.5
A739	924.5
A740	895.5
A741	895.4

Blank = not determined

Matched Pair Analysis

FIGS. **1**A-**1**B compare the potency in two different cell-based assays of compounds of Formula BB of the present invention (points on the right) and corresponding compounds of Formula AA (points on the left) wherein a H is replaced with (S)Me. The y axes represent pERK EC50 (FIG. **1**A) or CTG IC50 (FIG. **1**B) as measured in an H358 cell line. Assay protocols are below. The linked points represent a matched pair that differs only between H and (S)Me substitution. Each compound of Formula BB demonstrated reduced potency in cell assays compared to the corresponding compound of Formula AA.

Biological Assays

Potency Assay: pERK

The purpose of this assay is to measure the ability of test compounds to inhibit K-Ras in cells. Activated K-Ras induces increased phosphorylation of ERK at Threonine 202 and Tyrosine 204 (pERK). This procedure measures a decrease in cellular pERK in response to test compounds. The procedure described below in NCI-H358 cells is applicable to K-Ras G12C.

Note: This protocol may be executed substituting other cell lines to characterize inhibitors of other RAS variants, including, for example, AsPC-1 (K-Ras G12D), Capan-1 (K-Ras G12V), or NCI-H1355 (K-Ras G13C).

NCI-H358 cells were grown and maintained using media and procedures recommended by the ATCC. On the day prior to compound addition, cells were plated in 384-well cell culture plates (40 μl/well) and grown overnight in a 37° C., 5% CO_2 incubator. Test compounds were prepared in 10, 3-fold dilutions in DMSO, with a high concentration of 10 mM. On the day of assay, 40 nL of test compound was added to each well of cell culture plate using an Echo550 liquid handler (LabCyte®). Concentrations of test compound were tested in duplicate. After compound addition, cells were incubated 4 hours at 37° C., 5% CO_2. Following incubation, culture medium was removed and cells were washed once with phosphate buffered saline.

In some experiments, cellular pERK level was determined using the AlphaLISA SureFire Ultra p-ERK1/2 Assay Kit (PerkinElmer). Cells were lysed in 25 μL lysis buffer, with shaking at 600 RPM at room temperature. Lysate (10 μL)

was transferred to a 384-well Opti-plate (PerkinElmer) and 5 μL acceptor mix was added. After a 2-hour incubation in the dark, 5 μL donor mix was added, plate was sealed, and incubated 2 hours at room temperature. Signal was read on an Envision plate reader (PerkinElmer) using standard AlphaLISA settings. Analysis of raw data was carried out in Excel (Microsoft) and Prism (GraphPad). Signal was plotted vs. the decadal logarithm of compound concentration, and IC_{50} was determined by fitting a 4-parameter sigmoidal concentration response model.

In other experiments, cellular pERK was determined by In-Cell Western. Following compound treatment, cells were washed twice with 200 μL tris buffered saline (TBS) and fixed for 15 minutes with 150 μL 4% paraformaldehyde in TBS. Fixed cells were washed 4 times for 5 minutes with TBS containing 0.1% Triton X-100 (TBST) and then blocked with 100 μL Odyssey blocking buffer (LI-COR) for 60 minutes at room temperature. Primary antibody (pERK, CST-4370, Cell Signaling Technology) was diluted 1:200 in blocking buffer, and 50 μL was added to each well and incubated overnight at 4° C. Cells were washed 4 times for 5 minutes with TBST. Secondary antibody (IR-800CW rabbit, LI-COR, diluted 1:800) and DNA stain DRAQ5 (LI-COR, diluted 1:2000) were added and incubated 1-2 hours at room temperature. Cells were washed 4 times for 5 minutes with TBST. Plates were scanned on a LI-COR Odyssey CLx Imager. Analysis of raw data was carried out in Excel (Microsoft) and Prism (GraphPad). Signal was plotted vs. the decadal logarithm of compound concentration, and IC_{50} was determined by fitting a 4-parameter sigmoidal concentration response model.

The following compounds exhibited a pERK EC50 of under 5 uM (H358 KRAS G12C): A48, A15, A272, A174, A163, A453, A447, A279, A240, A214, A225, A136, A226, A219, A228, A21, A12, A78, A424, A219, A378, A224, A4, A53, A187, A218, A213, A314, A220, A208, A24, A9, A126, A345, A46, A203, A210, A184, A 469, A366, A113, A328, A693, A639, A364, A100, A249, A486, A307, A347, A33, A210, A192, A285, A468, A185, A 612, A109, A284, A200, A2, A6, A606, A325, A139, A496, A393, A561, A125, A494, A547, A215, A258, A195, A259, A212, A637, A53, A63, A68, A178, A189, A205, A78, A254, A690, A563, A14, A19, A92, A576, A278, A331, A42, A6 7, A209, A350, A562, A652, A703, A623, A191, A241, A199, A193, A478, A251, A177, A222, A23, A59, A26, A211, A106, A279, A120, A7, A134, A521, A116, A467, A694, A729, A151, A110, A277, A340, A221, A723, A13, A442, A6 11, A50, A190, A553, A696, A211, A303, A613, A37, A146, A666, A688, A216, A390, A548, A238, A160, A183, A16 4, A451, A481, A524, A1, A186, A37, A635, A71, A269, A289, A489, A400, A731, A497, A568, A274, A253, A471, A 720, A241, A179, A180, A426, A117, A363, A716, A423, A217, A708, A227, A3, A12, A8, A381, A84, A408, A85, A1 71, A263, A473, A258, A564, A118, A103, A565, A641, A655, A47, A11, A392, A169, A487, A640, A206, A449, A35 8, A192, A148, A4, A41, A5, A18, A301, A10, A65, A554, A159, A264, A99, A79, A142, A143, A25, A98, A80, A101, A 730, A212, A359, A61, A441, A283, A413, A717, A145, A182, A62, A181, A233, A232, A634, A495, A34, A251, A53 9, A632, A54, A327, A37, A196, A607, A645, A35, A214, A225, A638, A40, A52, A268, A448, A575, A176, A593, A1 5, A17, A94, A170, A713, A93, A402, A64, A261, A399, A422, A214, A225, A625, A31, A119, A135, A281, A676, A7 09, A81, A32, A633, A39, A646, A662, A124, A732, A320, A81, A187, A354, A45, A570, A165, A66, A20, A455, A43 1, A270, A250, A457, A153, A404, A710, A541, A127, A373, A369, A557, A349, A598, A618, A60, A636, A499, A87, A156, A680, A477, A406, A330, A202, A535, A617, A737, A201, A302, A722, A209, A374, A631, A29, A555, A420, A380, A111, A306, A173, A628, A672, A51, A167, A588, A512, A194, A282, A412, A701, A583, A396, A678, A649, A27, A204, A626, A257, A614, A409, A172, A372, A353, A58, A728, A74, A619, A144, A183, A538, A445, A531, A3 60, A361, A459, A536, A344, A267, A574, A677, A530, A415, A30, A73, A152, A490, A702, A714, A483, A567, A43, A310, A319, A86, A321, A656, A739, A115, A130, A155, A608, A648, A168, A485, A738, A129, A650, A715, A488, A147, A121, A470, A115, A133, A510, A421, A309, A335, A387, A386, A734, A95, A430, A604, A458, A592, A384, A664, A197, A725, A89, A83, A586, A622, A305, A498, A668, A427, A630, A158, A644, A735, A70, A683, A352, A3 41, A719, A674, A70, A44, A501, A438, A698, A377, A417, A154, A433, A104, A184, A603, A280, A712, A237, A10 5, A394, A605, A517, A704, A566, A77, A356, A454, A600, A643, A112, A569, A529, A247, A463, A437, A718, A47 2, A461, A558, A48, A671, A395, A670, A681, A687, A382, A82, A686, A342, A436, A296, A16, A545, A533, A416, A149, A207, A371, A596, A675, A132, A419, A56, A579, A733, A573, A707, A597, A697, A75, A653, A362, A615, A 332, A69, A162, A128, A432, A654, A22, A397, A526, A582, A418, A91, A260, A97, A191, A55, A581, A375, A522, A108, A367, A610, A552, A571, A57, A543, A661, A138, A196, A246, A337, A446, A265, A96, A509, A123, A627, A 651, A682, A157, A572, A624, A691, A532, A462, A580, A695, A186, A316, A540, A590, A665, A244, A166, A587, A629, A595, A518, A519, A131, A502, A726, A452, A141, A181, A262, A338, A155, A389, A124, A275, A414, A546, A679, A425, A669, A28, A520, A88, A131, A589, A621, A182, A297, A594, A283, A194, A250, A336, A706, A252, A440, A107, A724, A525, A388, A175, A300, A333, A659, A346, A150, A476, A368, A528, A503, A504, A505, A684, A76, A736, A551, A383, A491, A492, A493, A410, A316, A295, A559, A511, A38, A140, A663, A334, A700, A692, A348, A584, A513, A657, A328, A515, A317, A135, A660, A351, A544, A281, A685, A602, A556, A385, A326, A464, A465, A403, A133, A299, A667, A255, A334, A256, A585, A642, A133, A443, A435, A560, A444, A439, A324, A12 0, A407, A527, A245, A370, A537, A247, A474, A475, A705, A323, A112, A298, A609, A673, A292, A599, A132, A1 45, A266, A601, A466, A549, A379, A727, A167, A711, A75, A76, A121, A357, A620, A316, A479, A290, A339, A32 2, A376, A456, A391, A291, A550, A343, A721, A689, A411, A578, A616, A534, A365, A658, A699, A577, A647, A5 91, A542, A279, A294.

Determination of Cell Viability in RAS Mutant Cancer Cell Lines

Protocol: CellTiter-Glo® Cell Viability Assay

Note—The following protocol describes a procedure for monitoring cell viability of K-Ras mutant cancer cell lines in response to a compound of the invention. Other RAS isoforms may be employed, though the number of cells to be seeded will vary based on cell line used.

The purpose of this cellular assay was to determine the effects of test compounds on the proliferation of three human cancer cell lines (NCI-H358 (K-Ras G12C), AsPC-1 (K-Ras G12D), and Capan-1 (K-Ras G12V)) over a 5-day treatment period by quantifying the amount of ATP present at endpoint using the CellTiter-Glo® 2.0 Reagent (Promega).

Cells were seeded at 250 cells/well in 40 μL of growth medium in 384-well assay plates and incubated overnight in a humidified atmosphere of 5% CO_2 at 37° C. On the day of the assay, 10 mM stock solutions of test compounds were first diluted into 3 mM solutions with 100% DMSO. Well-

mixed compound solutions (15 μL) were transferred to the next wells containing 30 μL of 100% DMSO, and repeated until a 9-concentration 3-fold serial dilution was made (starting assay concentration of 10 μM). Test compounds (132.5 nL) were directly dispensed into the assay plates containing cells. The plates were shaken for 15 seconds at 300 rpm, centrifuged, and incubated in a humidified atmosphere of 5% CO_2 at 37° C. for 5 days. On day 5, assay plates and their contents were equilibrated to room temperature for approximately 30 minutes. CellTiter-Glo® 2.0 Reagent (25 μL) was added, and plate contents were mixed for 2 minutes on an orbital shaker before incubation at room temperature for 10 minutes. Luminescence was measured using the PerkinElmer Enspire. Data were normalized by the following: (Sample signal/Avg. DMSO)*100. The data were fit using a four-parameter logistic fit.

Disruption of B-Raf Ras-Binding Domain ($BRAF^{RBD}$) Interaction with K-Ras by Compounds of the Invention (Also Called a FRET Assay or an MOA Assay)

Note—The following protocol describes a procedure for monitoring disruption of K-Ras G12C (GMP-PNP) binding to $BRAF^{RBD}$ by a compound of the invention. This protocol may also be executed substituting other Ras proteins or nucleotides.

The purpose of this biochemical assay was to measure the ability of test compounds to facilitate ternary complex formation between a nucleotide-loaded K-Ras isoform and Cyclophilin A; the resulting ternary complex disrupts binding to a $BRAF^{RBD}$ construct, inhibiting K-Ras signaling through a RAF effector. Data is reported as IC50 values.

In assay buffer containing 25 mM HEPES pH 7.3, 0.002% Tween20, 0.1% BSA, 100 mM NaCl and 5 mM $MgCl_2$, tagless Cyclophilin A, His6-K-Ras-GMPPNP, and GST-$BRAF^{RBD}$ were combined in a 384-well assay plate at final concentrations of 25 μM, 12.5 nM and 50 nM, respectively. Compound was present in plate wells as a 10-point 3-fold dilution series starting at a final concentration of 30 μM. After incubation at 25° C. for 3 hours, a mixture of Anti-His Eu-W1024 and anti-GST allophycocyanin was then added to assay sample wells at final concentrations of 10 nM and 50 nM, respectively, and the reaction incubated for an additional 1.5 hours. TR-FRET signal was read on a microplate reader (Ex 320 nm, Em 665/615 nm). Compounds that facilitate disruption of a K-Ras:RAF complex were identified as those eliciting a decrease in the TR-FRET ratio relative to DMSO control wells.

TABLE 4

Biological Assay Data for Representative Compounds of the Present Invention

Ex#	H358 pERK (K-Ras G12C) EC50, uM	H358 Cell Viability (K-Ras G12C) IC50, uM	FRET (K-Ras G12C) IC50, uM	FRET (K-Ras G13C) IC50, uM
A208	0.013	0.004	0.023	7.48
A193	0.067	0.054	0.295	10.2
A186	0.013	0.005	0.038	0.94
A89a	0.003	0.0008	0.009	0.82
A89b	0.028	0.011	0.034	0.58

TABLE 5

Additional H358 Cell Viability assay data (K-Ras G12C, IC50, uM):

IC50*	Examples
+	A104, A107, A108, A112, A120, A121, A123, A124, A128, A131, A131, A132, A133, A133, A135, A138, A140, A141, A145, A150, A155, A157, A162, A166, A167, A175, A181, A182, A183, A186, A191, A194, A196, A237, A244, A245, A246, A247, A250, A255, A256, A260, A266, A275, A28, A281, A283, A290, A291, A292, A295, A296, A298, A299, A300, A309, A316, A316, A316, A317, A322, A323, A324, A326, A328, A332, A333, A334, A334, A336, A337, A339, A343, A344, A346, A348, A351, A352, A356, A357, A365, A368, A370, A371, A375, A376, A377, A379, A38, A380, A382, A383, A384, A385, A388, A389, A391, A394, A395, A396, A397, A403, A407, A410, A411, A414, A416, A419, A425, A427, A435, A439, A440, A443, A444, A445, A446, A452, A454, A456, A461, A462, A463, A464, A465, A466, A472, A474, A475, A476, A479, A483, A485, A488, A491, A492, A493, A502, A503, A504, A505, A509, A511, A513, A515, A517, A518, A519, A520, A522, A525, A526, A527, A528, A532, A533, A534, A537, A540, A542, A543, A544, A546, A549, A55, A550, A551, A552, A556, A559, A56, A560, A566, A567, A57, A571, A572, A577, A578, A579, A581, A584, A585, A587, A589, A590, A591, A592, A594, A595, A596, A597, A599, A600, A601, A602, A604, A609, A610, A615, A616, A620, A621, A624, A627, A629, A631, A636, A642, A643, A644, A647, A657, A658, A659, A660, A661, A663, A665, A667, A673, A674, A675, A677, A684, A685, A686, A689, A691, A692, A699, A700, A704, A705, A706, A707, A711, A712, A714, A718, A721, A724, A726, A727, A733, A735, A736, A738, A75, A76, A76, A88, A91, A96
++	A10, A105, A111, A112, A115, A115, A119, A12, A121, A124, A129, A130, A132, A133, A135, A143, A144, A145, A147, A149, A15, A152, A154, A155, A156, A158, A16, A165, A167, A168, A172, A173, A176, A181, A182, A184, A187, A194, A197, A20, A201, A202, A204, A207, A209, A212, A214, A214, A22, A225, A225, A232, A233, A238, A241, A247, A250, A251, A252, A257, A261, A262, A264, A265, A267, A268, A269, A27, A270, A280, A281, A282, A283, A297, A30, A302, A305, A306, A31, A310, A319, A320, A321, A327, A330, A335, A338, A34, A341, A342, A349, A35, A353, A354, A358, A359, A360, A361, A362, A363, A367, A369, A37, A372, A373, A374, A381, A386, A387, A39, A392, A393, A399, A40, A400, A402, A404, A406, A408, A409, A41, A412, A413, A415, A417, A418, A420, A421, A422, A423, A426, A43, A430, A431, A432, A433, A436, A437, A438, A44, A441, A442, A448, A449, A45, A451, A455, A457, A458, A459, A467, A468, A47, A470, A471, A473, A477, A478, A48, A481, A487, A489, A490, A495, A497, A498, A499, A5, A501, A51, A510, A512, A52, A524, A529, A530, A531, A535, A536, A538, A54, A541, A545, A555, A558, A564, A565, A568, A569, A573, A574, A575, A58, A580, A582, A583, A586, A588, A598, A60, A603, A605, A607, A608, A61, A611, A613, A614, A617, A618, A619, A62, A622, A623, A625, A626, A628, A630, A633, A634, A637, A638, A64, A640, A641, A645, A646, A648, A649, A650, A651, A653, A654, A655, A656, A66, A662, A664, A666, A668, A669, A670, A671, A672, A676, A678, A679, A680, A681, A682, A683, A687, A69, A694, A695, A697, A698, A70, A70, A701, A702, A703, A708, A709, A710, A713, A715, A716, A717, A719, A720, A722, A723, A725, A728, A73, A730, A732, A734, A737, A739, A74, A75, A77, A79, A81, A82, A83, A86, A87, A89, A93, A94, A95, A97, A98, A99
+++	A1, A100, A101, A103, A106, A109, A11, A110, A113, A114, A116, A117, A118, A120, A127, A13, A134, A139, A14, A142, A144, A146, A148, A151, A153, A159, A160, A164, A169, A17, A170, A171, A177, A18, A183, A185, A186, A19, A190, A191, A192, A192, A193, A195, A196, A199, A200, A205,

TABLE 5-continued

Additional H358 Cell Viability assay data (K-Ras G12C, IC50, uM):	
IC50*	Examples
	A206, A209, A210, A211, A211, A212, A215, A216, A217, A219, A219, A221, A222, A226, A227, A23, A241, A249, A25, A251, A253, A254, A258, A258, A259, A26, A263, A274, A277, A278, A279, A284, A289, A29, A3, A301, A303, A304, A304, A304, A304, A308, A314, A32, A325, A328, A329, A33, A331, A340, A345, A347, A350, A355, A364, A366, A37, A37, A378, A390, A398, A4, A401, A42, A424, A447, A453, A469, A482, A484, A486, A494, A496, A50, A506, A507, A508, A514, A521, A523, A53, A53, A539, A547, A548, A553, A554, A557, A561, A562, A563, A570, A576, A59, A593, A6, A606, A612, A63, A632, A635, A639, A65, A652, A67, A68, A688, A690, A693, A696, A7, A71, A729, A731, A78, A8, A80, A81, A84, A85, A9, A92
++++	A102, A12, A122, A125, A126, A136, A137, A148, A15, A161, A163, A174, A178, A179, A180, A184, A187, A188, A189, A198, A2, A203, A208, A21, A210, A210, A210, A211, A211, A213, A213, A214, A214, A215, A216, A217, A218, A220, A220, A221, A222, A223, A223, A224, A224, A225, A225, A228, A229, A230, A231, A231, A231, A234, A235, A236, A239, A239, A24, A240, A242, A243, A248, A261, A271, A272, A273, A276, A279, A279, A283, A285, A286, A287, A288, A293, A307, A311, A312, A313, A318, A318, A32, A4, A405, A428, A429, A450, A46, A460, A48, A480, A49, A500, A516, A72, A78, A90

*Key:
++++: IC50 ≥ 1 uM
+++: 1 uM > IC50 ≥ 0.1 uM
++: 0.1 uM > IC50 ≥ 0.01 uM
+: IC50 < 0.01 uM

Additional Ras-Raf Disruption/FRET/MOA Assay Data (IC50, uM):

TABLE 6

KRAS G12S FRET data	
IC50*	Examples
+	none
++	A028, A075, A076, A076, A087, A112, A145, A155, A167, A181, A183, A184, A194, A233, A255, A256, A260, A262, A264, A265, A266, A268, A270, A275, A292, A294, A295, A298, A299, A300, A319, A333, A334, A334, A338, A367, A382, A383, A385, A388, A389, A418, A433, A464, A465, A479, A491, A492, A493, A527, A537, A542, A577, A596, A598, A602, A609, A621, A629, A664, A665, A679, A712, A734, A736
+++	A004, A005, A012, A015, A016, A022, A027, A029, A032, A034, A037, A037, A041, A043, A044, A047, A048, A051, A052, A054, A055, A056, A057, A058, A064, A070, A070, A072, A074, A077, A079, A082, A083, A086, A088, A091, A093, A095, A096, A097, A101, A103, A105, A107, A123, A127, A128, A129, A131, A132, A135, A141, A147, A148, A149, A150, A153, A154, A158, A164, A166, A178, A182, A189, A193, A194, A196, A206, A207, A237, A241, A244, A245, A246, A247, A250, A251, A252, A257, A261, A261, A263, A267, A269, A280, A281, A281, A296, A297, A305, A306, A328, A336, A337, A349, A350, A351, A353, A354, A357, A360, A361, A368, A369, A379, A384, A386, A387, A393, A394, A395, A397, A406, A407, A408, A409, A410, A415, A416, A417, A419, A420, A421, A430, A431, A432, A435, A436, A437, A438, A443, A455, A463, A470, A490, A502, A515, A517, A518, A519, A529, A536, A538, A539, A541, A543, A544, A545, A548, A554, A555, A557, A558, A560, A564, A570, A571, A572, A573, A575,

TABLE 6-continued

KRAS G12S FRET data	
IC50*	Examples
	A578, A580, A581, A582, A583, A586, A591, A594, A603, A604, A605, A606, A608, A611, A612, A618, A620, A622, A624, A625, A630, A631, A632, A633, A634, A635, A636, A650, A651, A653, A654, A655, A656, A659, A661, A662, A667, A668, A669, A670, A671, A672, A677, A678, A680, A681, A682, A683, A684, A685, A686, A687, A689, A690, A695, A696, A697, A698, A699, A700, A701, A702, A704, A709, A710, A711, A713, A714, A717, A718, A719, A728, A729, A730, A731, A732, A737
++++	A001, A003, A006, A007, A008, A010, A013, A014, A017, A018, A019, A020, A025, A030, A031, A033, A035, A036, A038, A039, A040, A045, A050, A053, A060, A061, A062, A063, A066, A067, A068, A069, A071, A073, A075, A081, A081, A089, A092, A098, A099, A104, A108, A109, A110, A111, A112, A113, A115, A115, A117, A119, A120, A121, A124, A125, A131, A132, A133, A133, A134, A135, A137, A140, A143, A145, A146, A151, A155, A159, A161, A162, A167, A168, A169, A172, A173, A175, A176, A179, A180, A181, A182, A183, A184, A186, A187, A197, A199, A202, A204, A209, A210, A211, A212, A213, A214, A214, A215, A216, A217, A221, A223, A225, A225, A226, A227, A238, A241, A247, A249, A250, A251, A253, A254, A258, A258, A259, A277, A278, A282, A283, A290, A291, A301, A302, A309, A310, A314, A317, A320, A321, A324, A325, A326, A327, A330, A331, A332, A335, A339, A340, A341, A342, A343, A344, A347, A348, A356, A358, A359, A363, A364, A365, A366, A370, A371, A372, A373, A376, A378, A380, A381, A390, A391, A392, A396, A399, A401, A402, A411, A412, A413, A414, A422, A423, A424, A425, A426, A427, A439, A440, A442, A444, A445, A446, A447, A449, A451, A452, A454, A456, A457, A458, A459, A461, A462, A466, A468, A469, A471, A472, A473, A474, A475, A476, A481, A485, A487, A488, A489, A495, A498, A499, A501, A503, A504, A505, A506, A507, A509, A510, A511, A512, A513, A520, A521, A522, A525, A526, A528, A530, A531, A532, A533, A534, A535, A540, A546, A547, A549, A550, A551, A552, A553, A559, A561, A562, A563, A565, A566, A567, A568, A569, A574, A579, A585, A588, A590, A592, A593, A595, A597, A600, A601, A607, A610, A616, A619, A623, A626, A628, A637, A638, A639, A640, A641, A642, A645, A647, A652, A658, A660, A673, A676, A688, A691, A692, A693, A694, A705, A706, A707, A716, A720, A722, A723, A725, A726, A727, A733, A738, A739
+++++	A002, A004, A009, A011, A012, A015, A021, A023, A024, A026, A032, A036, A037, A038, A042, A046, A048, A049, A053, A059, A065, A078, A078, A080, A084, A085, A090, A094, A100, A102, A106, A114, A116, A118, A120, A121, A122, A124, A126, A130, A133, A136, A138, A139, A142, A144, A144, A148, A152, A156, A157, A160, A163, A165, A170, A171, A174, A177, A185, A186, A187, A188, A190, A191, A191, A192, A192, A195, A196, A198, A200, A201, A203, A205, A208, A209, A210, A210, A210, A211, A211, A211, A212, A213, A214, A214, A215, A216, A217, A218, A218, A219, A219, A219, A219, A220, A220, A221, A222, A222, A223, A224, A224, A225, A225, A228, A229, A230, A231, A231, A231, A232, A234, A235, A236, A239, A239, A240, A242, A243, A248, A271, A272, A273, A274, A276, A279, A279, A279, A283, A283, A284, A285, A286, A287, A288, A289, A293, A303, A304, A304, A304, A304, A307, A308, A311, A312, A313, A316, A316, A316, A318, A318, A322, A323, A328, A329, A345, A346, A352, A355, A362, A374, A375, A377, A398, A400, A403, A404, A405, A428, A429, A441, A448, A450, A453, A460, A467, A477, A478, A480, A482, A483, A484, A486, A494, A496, A497, A500, A508, A514, A516, A523, A524, A556, A576, A584, A587, A589, A599,

TABLE 6-continued

KRAS G12S FRET data	
IC50*	Examples
	A613, A614, A615, A617, A627, A643, A644, A646, A648, A649, A657, A663, A666, A674, A675, A703, A708, A715, A721, A724, A735

+++++: IC50 ≥ 10 uM
++++: 10 uM > IC50 ≥ 1 uM
+++: 1 uM > IC50 ≥ 0.1 uM
++: 0.1 uM > IC50 ≥ 0.01 uM
+: IC50 < 0.01 uM

TABLE 7

KRAS G12D FRET data	
IC50*	Examples
+	none
++	A183, A264, A265, A268, A319, A388, A464, A465, A664
+++	A028, A054, A075, A076, A076, A087, A107, A112, A123, A131, A145, A153, A155, A167, A184, A189, A194, A233, A247, A255, A256, A257, A260, A261, A262, A263, A266, A269, A270, A275, A292, A294, A295, A298, A300, A333, A334, A334, A338, A349, A353, A354, A367, A368, A382, A383, A384, A385, A386, A387, A389, A394, A407, A409, A417, A418, A432, A433, A436, A479, A491, A492, A493, A527, A529, A537, A542, A555, A577, A578, A594, A596, A598, A602, A603, A605, A608, A609, A612, A621, A622, A629, A633, A650, A651, A653, A654, A662, A665, A667, A669, A671, A678, A679, A681, A682, A683, A685, A696, A697, A698, A700, A701, A704, A709, A710, A711, A712, A719, A734, A736, A737
++++	A005, A007, A012, A015, A016, A022, A029, A034, A037, A037, A038, A038, A039, A043, A044, A046, A047, A048, A051, A052, A055, A056, A057, A058, A067, A070, A070, A071, A072, A074, A077, A079, A082, A086, A088, A090, A091, A093, A096, A097, A101, A103, A105, A108, A110, A116, A120, A120, A121, A121, A127, A128, A129, A130, A131, A132, A133, A133, A133, A134, A135, A138, A139, A140, A141, A142, A144, A144, A147, A148, A149, A150, A152, A154, A155, A156, A157, A162, A163, A164, A166, A175, A178, A179, A180, A181, A182, A183, A194, A196, A207, A213, A218, A218, A220, A224, A224, A237, A241, A244, A245, A246, A247, A250, A251, A252, A253, A254, A258, A261, A267, A280, A281, A281, A282, A296, A297, A299, A305, A306, A314, A326, A328, A330, A332, A336, A337, A341, A342, A350, A351, A356, A357, A358, A360, A361, A365, A366, A369, A370, A373, A379, A380, A381, A390, A393, A395, A396, A397, A406, A408, A410, A411, A412, A415, A416, A419, A420, A421, A422, A430, A431, A435, A437, A438, A440, A443, A444, A451, A454, A455, A456, A459, A463, A470, A476, A487, A488, A490, A499, A501, A502, A503, A504, A505, A510, A513, A515, A517, A518, A519, A520, A525, A534, A536, A538, A539, A541, A543, A544, A545, A546, A547, A548, A550, A551, A552, A553, A554, A557, A558, A559, A560, A561, A562, A564, A566, A567, A570, A571, A572, A573, A575, A580, A581, A582, A583, A586, A588, A591, A593, A595, A600, A604, A606, A607, A610, A611, A618, A619, A620, A623, A624, A625, A626, A630, A631, A632, A634, A635, A636, A640, A645, A647, A655, A656, A658, A659, A660, A661, A668, A670, A672, A673, A676, A677, A680, A684, A686, A687, A688, A689, A690, A691, A694, A695, A699, A702, A707, A713, A714, A717, A718, A722, A727, A728, A729, A730, A731, A732, A738, A739
+++++	A001, A002, A003, A004, A004, A006, A008, A009, A010, A011, A012, A013, A014, A015, A017, A018, A019, A020, A021, A023, A024, A025, A026, A027, A030, A031, A032, A032, A033, A035, A036, A036,

TABLE 7-continued

KRAS G12D FRET data	
IC50*	Examples
	A037, A040, A041, A042, A045, A048, A049, A050, A053, A053, A059, A060, A061, A062, A063, A064, A065, A066, A068, A069, A073, A075, A078, A078, A080, A081, A081, A083, A084, A085, A089, A092, A094, A095, A098, A099, A100, A102, A104, A106, A109, A111, A112, A113, A114, A115, A115, A117, A118, A119, A122, A124, A124, A125, A126, A132, A135, A136, A137, A143, A145, A146, A148, A151, A158, A159, A160, A161, A165, A167, A168, A169, A170, A171, A172, A173, A174, A176, A177, A181, A182, A184, A185, A186, A186, A187, A187, A188, A190, A191, A191, A192, A192, A193, A195, A196, A197, A198, A199, A200, A201, A202, A203, A204, A205, A206, A208, A209, A209, A210, A210, A210, A210, A211, A211, A211, A211, A212, A212, A213, A214, A214, A214, A214, A215, A215, A216, A216, A217, A217, A219, A219, A219, A219, A220, A221, A221, A222, A222, A223, A223, A225, A225, A225, A225, A226, A227, A228, A229, A230, A231, A231, A231, A232, A234, A235, A236, A238, A239, A239, A240, A241, A242, A243, A248, A249, A250, A251, A258, A259, A271, A272, A273, A274, A276, A277, A278, A279, A279, A279, A283, A283, A283, A284, A285, A286, A287, A288, A289, A290, A291, A293, A301, A302, A303, A304, A304, A304, A304, A307, A308, A309, A310, A311, A312, A313, A316, A316, A316, A317, A318, A318, A320, A321, A322, A323, A324, A325, A327, A328, A329, A331, A335, A339, A340, A343, A344, A345, A346, A347, A348, A352, A355, A359, A362, A363, A364, A371, A372, A374, A375, A376, A377, A378, A391, A392, A398, A399, A400, A401, A402, A403, A404, A405, A413, A414, A423, A424, A425, A426, A427, A428, A429, A439, A441, A442, A445, A446, A447, A448, A449, A450, A452, A453, A457, A458, A460, A461, A462, A466, A467, A468, A469, A471, A472, A473, A474, A475, A477, A478, A480, A481, A482, A483, A484, A485, A486, A489, A494, A495, A496, A497, A498, A500, A506, A507, A508, A509, A511, A512, A514, A516, A521, A522, A523, A524, A526, A528, A530, A531, A532, A533, A535, A540, A549, A556, A563, A565, A568, A569, A574, A576, A579, A584, A585, A587, A589, A590, A592, A597, A599, A601, A613, A614, A615, A616, A617, A627, A628, A637, A638, A639, A641, A642, A643, A644, A646, A648, A649, A652, A657, A663, A666, A674, A675, A692, A693, A703, A705, A706, A708, A715, A716, A720, A721, A723, A724, A725, A726, A733, A735

TABLE 8

KRAS G13C FRET data	
IC50*	Examples
+	A088, A096, A097, A107, A131, A132, A155, A175, A247, A250, A251, A252, A253, A255, A258, A262, A270, A292, A294, A297, A298, A299, A334, A336, A338, A474, A475, A529, A542, A683
++	A031, A037, A038, A046, A067, A069, A071, A075, A076, A076, A090, A108, A112, A116, A121, A123, A124, A131, A132, A133, A133, A135, A140, A141, A145, A150, A155, A163, A167, A172, A178, A182, A183, A194, A196, A218, A224, A233, A244, A245, A246, A247, A249, A250, A251, A254, A256, A257, A258, A259, A260, A261, A263, A264, A265, A266, A267, A268, A275, A280, A281, A283, A295, A296, A300, A305, A306, A319, A333, A334, A337, A379, A383, A385, A388, A389, A394, A396, A397, A417, A418, A421, A432, A433, A438, A452, A464, A465, A479, A491, A492, A493, A517, A525, A527, A537, A538, A539, A540, A541, A545, A546, A552, A553, A554, A555, A564, A570, A571, A572, A573, A574,

TABLE 8-continued

KRAS G13C FRET data	
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	A575, A577, A578, A591, A594, A595, A597, A600, A602, A603, A604, A605, A606, A607, A608, A609, A610, A621, A622, A629, A630, A632, A650, A664, A668, A679, A680, A695, A698, A699, A706, A711, A714, A718, A719, A734, A736, A737
+++	A005, A012, A013, A016, A018, A022, A027, A028, A029, A033, A036, A037, A038, A043, A044, A047, A048, A049, A052, A054, A055, A056, A057, A070, A070, A072, A074, A077, A082, A086, A087, A091, A093, A095, A101, A105, A110, A117, A120, A125, A126, A127, A128, A129, A130, A135, A138, A142, A146, A147, A149, A153, A157, A158, A162, A164, A165, A166, A179, A180, A181, A184, A189, A196, A199, A207, A209, A213, A216, A218, A220, A220, A222, A224, A232, A237, A238, A241, A241, A261, A269, A277, A281, A302, A314, A322, A323, A324, A328, A332, A340, A341, A342, A344, A349, A350, A351, A353, A354, A356, A357, A358, A360, A361, A365, A366, A367, A368, A369, A370, A371, A372, A373, A380, A382, A384, A386, A387, A390, A393, A395, A401, A406, A407, A408, A409, A410, A411, A412, A415, A416, A419, A420, A422, A423, A425, A430, A431, A435, A436, A437, A439, A440, A443, A444, A445, A449, A451, A454, A455, A457, A458, A459, A463, A466, A470, A471, A476, A485, A487, A488, A490, A497, A501, A502, A503, A504, A505, A509, A510, A513, A515, A518, A519, A524, A534, A536, A543, A544, A547, A549, A550, A551, A557, A558, A559, A560, A561, A562, A563, A566, A567, A576, A580, A581, A582, A583, A586, A593, A596, A598, A601, A611, A612, A618, A619, A620, A624, A625, A631, A633, A634, A635, A636, A640, A642, A645, A647, A651, A652, A653, A654, A655, A658, A659, A660, A661, A662, A665, A667, A669, A670, A671, A672, A673, A678, A681, A682, A684, A685, A686, A687, A688, A689, A690, A692, A694, A696, A697, A700, A701, A702, A704, A707, A709, A710, A712, A717, A722, A725, A726, A727, A728, A729, A730, A731, A732, A733, A738, A739
++++	A001, A003, A004, A006, A007, A008, A010, A014, A015, A017, A019, A020, A025, A030, A032, A034, A035, A039, A040, A041, A045, A051, A058, A060, A061, A063, A064, A068, A073, A075, A078, A078, A079, A081, A081, A083, A089, A092, A098, A099, A103, A104, A111, A112, A115, A115, A119, A120, A121, A124, A133, A134, A139, A144, A144, A145, A148, A152, A154, A156, A161, A167, A168, A169, A170, A171, A174, A176, A181, A182, A183, A186, A187, A191, A193, A194, A197, A200, A201, A204, A206, A212, A213, A214, A214, A214, A217, A221, A225, A225, A225, A227, A235, A242, A278, A279, A282, A283, A283, A284, A289, A290, A291, A301, A307, A308, A309, A310, A316, A316, A317, A320, A321, A325, A326, A327, A330, A331, A335, A339, A343, A345, A346, A347, A348, A359, A362, A363, A364, A375, A376, A377, A378, A381, A391, A392, A398, A399, A400, A402, A403, A413, A414, A424, A426, A427, A441, A442, A446, A447, A456, A460, A461, A462, A467, A468, A469, A472, A473, A477, A480, A481, A483, A484, A486, A489, A494, A495, A496, A498, A499, A506, A507, A508, A511, A512, A514, A516, A520, A521, A522, A526, A528, A530, A531, A532, A533, A535, A548, A556, A565, A568, A569, A579, A584, A585, A588, A589, A590, A592, A599, A614, A616, A623, A626, A628, A637, A638, A639, A641, A643, A644, A648, A656, A657, A663, A674, A676, A677, A691, A693, A703, A705, A708, A713, A716, A720, A721, A723, A735
+++++	A210, A173, A109, A042, A062, A137, A303, A228, A666, A214, A225, A627, A675, A229, A015, A066, A184, A209, A328, A288, A011, A587, A448, A613, A617, A226, A240, A316, A113, A059, A002, A374, A223, A313, A190, A212, A219, A065, A032, A615, A143, A215, A080, A026, A050, A191, A219, A216, A217, A106, A649, A151, A053, A478, A222, A219, A037, A211, A404, A724, A085, A248, A202, A122,

TABLE 8-continued

KRAS G13C FRET data	
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	A159, A094, A004, A009, A012, A021, A023, A024, A036, A048, A053, A084, A100, A102, A114, A118, A136, A148, A160, A177, A185, A186, A187, A188, A192, A192, A195, A198, A203, A205, A208, A210, A210, A210, A211, A211, A211, A215, A219, A221, A223, A230, A231, A231, A231, A234, A236, A239, A239, A243, A271, A272, A273, A274, A276, A279, A279, A285, A286, A287, A293, A304, A304, A304, A304, A311, A312, A318, A318, A329, A352, A355, A405, A428, A429, A450, A453, A482, A500, A523, A646, A715

TABLE 9

KRAS G12V FRET data	
IC50*	Examples
+	None
++	A028, A075, A076, A076, A087, A112, A132, A145, A155, A167, A183, A184, A194, A233, A245, A262, A264, A265, A266, A268, A275, A292, A295, A298, A300, A305, A319, A333, A334, A338, A368, A383, A388, A389, A418, A464, A465, A479, A491, A492, A493, A527, A537, A542, A602, A609, A621, A629, A665, A679, A734
+++	A004, A005, A012, A015, A016, A022, A027, A029, A034, A037, A037, A041, A043, A044, A047, A048, A051, A052, A054, A055, A056, A057, A058, A069, A070, A072, A074, A077, A079, A082, A086, A088, A091, A093, A095, A096, A097, A101, A103, A105, A107, A123, A127, A128, A129, A131, A135, A141, A147, A148, A149, A150, A153, A154, A155, A162, A164, A166, A178, A180, A181, A182, A183, A189, A193, A194, A196, A206, A207, A209, A237, A238, A241, A244, A246, A247, A250, A251, A252, A253, A255, A256, A257, A258, A260, A261, A263, A267, A269, A270, A280, A281, A281, A294, A296, A297, A299, A306, A334, A336, A337, A349, A350, A353, A354, A357, A360, A361, A366, A367, A369, A373, A379, A380, A382, A384, A385, A386, A387, A394, A395, A397, A406, A407, A408, A409, A410, A415, A416, A417, A419, A420, A421, A431, A432, A433, A435, A436, A437, A438, A443, A444, A463, A490, A517, A518, A519, A529, A536, A538, A539, A543, A544, A545, A546, A548, A550, A554, A555, A564, A570, A571, A572, A577, A578, A580, A581, A582, A586, A591, A594, A596, A598, A603, A604, A605, A606, A608, A611, A612, A618, A620, A622, A624, A625, A630, A631, A632, A633, A634, A635, A650, A651, A653, A654, A655, A659, A660, A662, A664, A667, A668, A669, A670, A671, A672, A673, A678, A680, A681, A682, A683, A684, A685, A686, A687, A695, A696, A697, A698, A699, A700, A701, A702, A704, A709, A710, A711, A712, A714, A717, A718, A719, A728, A736, A737
++++	A001, A003, A006, A007, A008, A010, A013, A017, A018, A025, A030, A031, A032, A035, A036, A038, A039, A040, A045, A046, A050, A060, A061, A062, A063, A064, A066, A067, A068, A070, A071, A073, A075, A080, A081, A081, A083, A089, A092, A098, A099, A104, A108, A109, A110, A111, A112, A113, A115, A115, A117, A120, A121, A124, A125, A131, A132, A133, A133, A134, A135, A137, A138, A140, A143, A145, A146, A151, A152, A158, A159, A161, A167, A168, A169, A172, A173, A175, A176, A179, A181, A182, A184, A186, A187, A191, A197, A199, A204, A210, A211, A212, A213, A215, A216, A217, A218, A221, A223, A224, A226, A227, A232, A241, A242, A247, A249, A251, A254, A258, A259, A261, A277, A278, A282, A283, A289, A290, A291, A301, A302, A307, A309, A310, A314, A316, A317, A320, A321, A324, A325, A326, A328, A330, A331, A332, A335, A339, A340, A341, A342, A343, A344, A346, A348, A351, A356, A358, A359, A362, A363, A364, A365, A370, A372, A375, A376, A378, A381, A390, A391, A392, A393, A396, A398, A399, A401, A402, A403, A411, A412, A413, A414, A422, A423, A424, A426, A427, A430, A439, A440, A445, A446, A447, A449, A451, A452, A454, A455,

TABLE 9-continued

KRAS G12V FRET data	
IC50*	Examples
	A456, A457, A458, A459, A461, A462, A466, A468, A469, A470, A472, A473, A474, A475, A476, A477, A481, A483, A485, A487, A488, A489, A495, A498, A499, A501, A502, A503, A504, A505, A506, A507, A509, A510, A511, A512, A513, A515, A520, A521, A522, A525, A526, A528, A530, A531, A532, A533, A534, A540, A541, A547, A549, A551, A552, A553, A556, A557, A558, A559, A560, A561, A562, A563, A565, A566, A567, A573, A574, A575, A579, A583, A584, A585, A588, A590, A592, A593, A595, A597, A599, A600, A601, A607, A610, A616, A619, A623, A626, A628, A636, A637, A638, A639, A640, A641, A642, A645, A647, A652, A656, A658, A661, A676, A677, A688, A689, A690, A691, A692, A693, A694, A703, A705, A706, A707, A708, A713, A716, A720, A721, A722, A723, A725, A726, A727, A729, A730, A731, A732, A733, A738, A739
+++++	A002, A004, A009, A011, A012, A014, A015, A019, A020, A021, A023, A024, A026, A032, A033, A036, A037, A038, A042, A048, A049, A053, A053, A059, A065, A078, A078, A084, A085, A090, A094, A100, A102, A106, A114, A116, A118, A119, A120, A121, A122, A124, A126, A130, A133, A136, A139, A142, A144, A144, A148, A156, A157, A160, A163, A165, A170, A171, A174, A177, A185, A186, A187, A188, A190, A191, A192, A192, A195, A196, A198, A200, A201, A202, A203, A205, A208, A209, A210, A210, A210, A211, A211, A211, A212, A213, A214, A214, A214, A214, A215, A216, A217, A218, A219, A219, A219, A219, A220, A220, A221, A222, A222, A223, A224, A225, A225, A225, A225, A228, A229, A230, A231, A231, A231, A234, A235, A236, A239, A239, A240, A243, A248, A250, A271, A272, A273, A274, A276, A279, A279, A283, A283, A284, A285, A286, A287, A288, A293, A303, A304, A304, A304, A304, A308, A311, A312, A313, A316, A316, A318, A318, A322, A323, A327, A328, A329, A345, A347, A352, A355, A371, A374, A377, A400, A404, A405, A425, A428, A429, A441, A442, A448, A450, A453, A460, A467, A471, A478, A480, A482, A484, A486, A494, A496, A497, A500, A508, A514, A516, A523, A524, A535, A568, A569, A576, A587, A589, A613, A614, A615, A617, A627, A643, A644, A646, A648, A649, A657, A663, A666, A674, A675, A715, A724, A735

TABLE 10

KRAS WT FRET data	
IC50*	Examples
+	A388
++	A075, A076, A076, A112, A132, A155, A167, A183, A194, A233, A256, A260, A261, A262, A263, A264, A265, A268, A270, A280, A294, A295, A296, A297, A298, A299, A300, A319, A333, A334, A338, A367, A382, A383, A385, A386, A387, A389, A418, A432, A433, A436, A464, A465, A479, A491, A492, A493, A527, A537, A542, A569, A594, A598, A602, A603, A605, A609, A621, A629, A633, A664, A665, A667, A679, A704, A711, A712, A734, A736, A737
+++	A005, A012, A016, A022, A028, A029, A037, A037, A043, A052, A054, A056, A069, A070, A070, A072, A074, A077, A082, A087, A088, A091, A093, A096, A097, A101, A105, A107, A110, A123, A128, A131, A141, A145, A147, A148, A149, A150, A153, A158, A164, A166, A180, A181, A182, A184, A189, A207, A244, A245, A246, A247, A250, A251, A252, A253, A254, A255, A257, A258, A259, A266, A267, A269, A275, A281, A281, A292, A305, A306, A334, A336, A337, A349, A350, A351, A353, A354, A356, A357, A358, A360, A361, A368, A369, A379, A384, A393, A394, A395, A397, A406, A407, A408, A409, A410, A415, A416, A417, A419, A420, A421, A430, A431, A435, A437, A438, A452, A454, A455, A457, A463, A490, A501, A517, A518, A519, A529, A536, A538, A539, A541, A543, A544, A545, A547, A548, A550, A552, A553, A554, A555, A557, A558, A564, A570, A571, A572, A573, A575, A577, A578, A580, A581, A582, A583, A586, A591, A595, A600, A604, A606, A607,

TABLE 10-continued

KRAS WT FRET data	
IC50*	Examples
	A608, A610, A611, A612, A618, A620, A622, A624, A625, A630, A631, A632, A634, A635, A636, A647, A650, A651, A653, A654, A655, A656, A659, A660, A661, A662, A668, A669, A670, A671, A672, A676, A677, A678, A680, A681, A682, A683, A684, A685, A686, A687, A688, A690, A695, A696, A697, A698, A699, A700, A701, A702, A709, A710, A713, A714, A717, A718, A719, A722, A728, A729, A730, A731, A732, A738, A739
++++	A004, A006, A007, A008, A010, A013, A014, A015, A017, A018, A019, A020, A025, A027, A030, A031, A032, A033, A034, A035, A036, A038, A039, A040, A041, A044, A045, A047, A048, A051, A055, A057, A058, A061, A063, A064, A067, A068, A071, A073, A075, A079, A081, A081, A083, A086, A089, A092, A095, A098, A103, A104, A108, A111, A112, A115, A115, A116, A117, A120, A121, A124, A125, A127, A129, A131, A132, A133, A133, A134, A135, A137, A138, A140, A145, A146, A152, A154, A155, A159, A161, A162, A163, A167, A168, A169, A172, A175, A178, A179, A181, A182, A183, A187, A193, A194, A196, A197, A199, A204, A206, A209, A212, A213, A214, A214, A214, A214, A216, A217, A222, A225, A225, A225, A225, A227, A232, A237, A238, A241, A241, A247, A249, A250, A251, A258, A261, A277, A278, A282, A283, A290, A291, A302, A309, A310, A314, A317, A320, A321, A324, A326, A328, A330, A331, A332, A335, A339, A340, A341, A342, A343, A344, A347, A348, A352, A359, A363, A364, A365, A366, A370, A371, A372, A373, A375, A376, A378, A380, A381, A390, A391, A392, A396, A398, A399, A401, A402, A411, A412, A413, A422, A423, A424, A426, A427, A439, A440, A443, A444, A445, A446, A447, A449, A451, A456, A458, A459, A461, A462, A466, A468, A469, A470, A471, A472, A473, A474, A475, A476, A477, A481, A483, A485, A487, A488, A489, A494, A495, A497, A498, A499, A502, A503, A504, A505, A506, A509, A510, A511, A512, A513, A515, A520, A521, A522, A525, A526, A528, A530, A531, A532, A533, A534, A540, A546, A549, A551, A556, A559, A560, A561, A562, A563, A566, A567, A574, A576, A579, A585, A588, A589, A590, A593, A597, A601, A616, A619, A623, A626, A637, A638, A639, A640, A641, A642, A645, A648, A652, A658, A673, A689, A691, A692, A693, A694, A703, A705, A706, A707, A708, A716, A720, A723, A725, A726, A727, A735
+++++	A001, A002, A003, A004, A009, A011, A012, A015, A021, A023, A024, A026, A032, A036, A037, A038, A042, A046, A048, A049, A050, A053, A053, A059, A060, A062, A065, A066, A078, A078, A080, A084, A085, A090, A094, A099, A100, A102, A106, A109, A113, A114, A118, Al19, A120, A121, A122, A124, A126, A130, A133, A135, A136, A139, A142, A143, A144, A144, A148, A151, A156, A157, A160, A165, A170, A171, A173, A174, A176, A177, A184, A185, A186, A186, A187, A188, A190, A191, A191, A192, A192, A195, A196, A198, A200, A201, A202, A203, A205, A208, A209, A210, A210, A210, A210, A211, A211, A211, A211, A212, A213, A215, A215, A216, A217, A218, A218, A219, A219, A219, A219, A220, A220, A221, A221, A222, A223, A223, A224, A224, A226, A228, A229, A230, A231, A231, A231, A234, A235, A236, A239, A239, A240, A242, A243, A248, A271, A272, A273, A274, A276, A279, A279, A279, A283, A283, A284, A285, A286, A287, A288, A289, A293, A301, A303, A304, A304, A304, A304, A307, A308, A311, A312, A313, A316, A316, A316, A318, A318, A322, A323, A325, A327, A328, A329, A345, A346, A355, A362, A374, A377, A400, A403, A404, A405, A414, A425, A428, A429, A441, A442, A448, A450, A453, A460, A467, A478, A480, A482, A484, A486, A496, A500, A507, A508, A514, A516, A523, A524, A535, A565, A568, A584, A587, A592, A596, A599, A613, A614, A615, A617, A627, A628, A643, A644, A646, A649, A657, A663, A666, A674, A675, A715, A721, A724, A733

TABLE 11

KRAS G12C FRET data	
IC50*	Examples
+	A038, A075, A076, A088, A095, A096, A097, A107, A108, A112, A116, A120, A121, A129, A130, A131, A131, A132, A133, A133, A135, A140, A141, A145, A157, A162, A167, A175, A176, A182, A183, A184, A194, A196, A209, A244, A245, A246, A247, A247, A255, A256, A257, A266, A268, A270, A290, A291, A292, A294, A298, A299, A300, A316, A316, A316, A317, A322, A323, A324, A326, A333, A334, A337, A339, A343, A346, A348, A351, A365, A375, A376, A377, A379, A380, A381, A388, A391, A403, A407, A411, A414, A425, A427, A474, A475, A477, A479, A509, A527, A529, A534, A542, A543, A544, A549, A550, A551, A556, A577, A578, A579, A591, A599, A600, A601, A609, A620, A626, A627, A628, A638, A642, A647, A658, A660, A663, A667, A673, A684, A689, A691, A692, A699, A705, A707, A711, A721, A726, A727, A735
++	A004, A005, A012, A015, A016, A020, A022, A027, A028, A029, A031, A032, A037, A037, A038, A043, A044, A047, A048, A051, A052, A054, A055, A056, A057, A058, A064, A067, A069, A070, A070, A071, A072, A074, A076, A077, A079, A082, A083, A086, A087, A091, A093, A103, A104, A105, A115, A121, A123, A124, A126, A127, A128, A132, A133, A134, A135, A138, A139, A142, A144, A148, A149, A150, A152, A153, A154, A155, A155, A156, A158, A164, A165, A166, A172, A178, A179, A181, A182, A183, A186, A187, A189, A191, A193, A196, A200, A201, A204, A207, A209, A214, A214, A225, A225, A233, A237, A238, A241, A241, A250, A250, A251, A251, A252, A253, A260, A261, A262, A263, A264, A265, A267, A269, A275, A280, A281, A283, A295, A296, A297, A305, A319, A328, A332, A334, A336, A338, A342, A344, A349, A350, A352, A353, A359, A361, A362, A363, A366, A367, A368, A370, A371, A382, A383, A384, A385, A386, A387, A389, A394, A396, A397, A400, A410, A416, A417, A418, A421, A423, A432, A433, A435, A436, A437, A438, A439, A440, A443, A444, A445, A448, A452, A454, A456, A458, A459, A463, A464, A465, A466, A476, A478, A485, A489, A490, A491, A492, A493, A499, A501, A503, A504, A505, A510, A513, A515, A517, A518, A519, A525, A526, A528, A532, A533, A536, A537, A538, A545, A552, A558, A559, A560, A566, A567, A568, A569, A572, A573, A575, A580, A581, A584, A585, A587, A589, A590, A592, A594, A595, A596, A597, A598, A602, A603, A610, A612, A614, A615, A616, A617, A621, A622, A624, A629, A632, A634, A636, A637, A643, A644, A645, A646, A649, A650, A651, A653, A657, A659, A661, A662, A664, A665, A668, A669, A671, A672, A674, A675, A678, A679, A681, A682, A683, A685, A686, A697, A698, A700, A701, A703, A704, A706, A709, A710, A712, A715, A719, A720, A722, A724, A730, A733, A734, A736, A737
+++	A001, A003, A006, A007, A008, A010, A011, A013, A014, A017, A018, A019, A023, A025, A026, A030, A033, A034, A035, A039, A040, A041, A042, A045, A046, A050, A053, A053, A060, A061, A062, A063, A065, A066, A068, A073, A075, A078, A078, A080, A081, A081, A089, A092, A094, A098, A099, A101, A109, A110, A111, A112, A113, A115, A117, A118, A119, A120, A125, A137, A143, A144, A145, A146, A147, A151, A159, A160, A161, A167, A168, A169, A173, A174, A177, A180, A181, A184, A185, A188, A190, A191, A192, A194, A195, A197, A199, A205, A206, A208, A210, A211, A212, A213, A215, A216, A217, A218, A220, A221, A222, A223, A224, A226, A227, A229, A232, A249, A254, A258, A258, A259, A261, A277, A278, A281, A282, A284, A301, A302, A306, A310, A314, A320, A321, A327, A330, A331, A335, A340, A341, A347, A354, A356, A357, A358, A360, A364, A369, A372, A373, A374, A378, A390, A392, A393, A395, A399, A402, A404, A406, A408, A409, A412, A413, A415, A419, A420, A422, A424, A426, A430, A431, A442, A446, A447, A449, A451, A455, A457, A461, A462, A468, A469, A470, A471, A472, A473, A481, A483, A484, A486, A487, A488, A494, A495, A497, A498, A502, A506, A507, A508, A511, A512, A520, A522, A524, A530, A531, A539, A540, A541, A546, A547, A548, A553, A554, A555, A557, A561, A562, A563, A564, A565, A570, A571, A574, A576, A582, A583, A586, A588, A593, A604, A605, A606, A607, A608, A611, A613, A618, A619, A623, A625, A630, A631, A633, A635, A639, A640, A641, A648, A652, A654, A655, A656, A670, A676, A677, A680, A687, A688,

TABLE 11-continued

KRAS G12C FRET data	
IC50*	Examples
	A690, A693, A694, A695, A696, A702, A708, A713, A714, A716, A717, A718, A723, A725, A728, A729, A731, A732, A738, A739
++++	A002, A004, A009, A012, A015, A021, A024, A032, A036, A037, A048, A049, A059, A084, A085, A090, A100, A102, A106, A124, A136, A148, A163, A170, A171, A186, A187, A192, A202, A203, A210, A211, A212, A214, A214, A216, A218, A219, A219, A220, A222, A224, A225, A225, A228, A235, A240, A242, A248, A272, A274, A276, A279, A283, A283, A289, A303, A307, A309, A313, A325, A328, A345, A398, A401, A405, A441, A453, A460, A467, A480, A496, A514, A516, A521, A523, A535, A666
+++++	A036, A114, A122, A198, A210, A210, A211, A211, A213, A215, A217, A219, A219, A221, A223, A230, A231, A231, A231, A234, A236, A239, A239, A243, A271, A273, A279, A279, A285, A286, A287, A288, A293, A304, A304, A304, A304, A308, A311, A312, A318, A318, A329, A355, A428, A429, A450, A482, A500

TABLE 12

KRAS G13D FRET data	
IC50*	Examples
+	None
++	A075, A076, A112, A155, A183, A260, A261, A262, A263, A264, A265, A267, A268, A270, A294, A296, A298, A300, A319, A333, A338, A388, A464, A465, A527, A537, A542, A664
+++	A028, A054, A096, A105, A107, A123, A128, A131, A132, A141, A145, A149, A150, A153, A158, A164, A167, A181, A182, A184, A189, A194, A196, A207, A233, A244, A245, A246, A247, A250, A251, A252, A253, A255, A256, A257, A258, A261, A266, A269, A275, A280, A281, A281, A292, A295, A297, A299, A305, A306, A334, A334, A336, A337, A349, A350, A353, A354, A357, A361, A367, A368, A379, A382, A383, A384, A385, A386, A387, A389, A394, A395, A397, A406, A407, A410, A415, A416, A417, A418, A421, A432, A433, A436, A438, A463, A479, A490, A491, A492, A493, A518, A519, A529, A536, A538, A543, A544, A545, A555, A558, A573, A577, A578, A580, A581, A582, A591, A594, A596, A598, A602, A603, A605, A606, A608, A609, A611, A612, A620, A621, A622, A624, A629, A631, A633, A650, A651, A653, A654, A655, A659, A661, A662, A665, A667, A669, A670, A671, A672, A678, A679, A681, A682, A683, A684, A685, A686, A687, A690, A695, A697, A698, A699, A700, A701, A704, A709, A710, A711, A712, A718, A719, A729, A730, A734, A736, A737
++++	A016, A038, A069, A075, A088, A095, A103, A104, A108, A110, A111, A112, A116, A117, A120, A120, A121, A121, A125, A127, A129, A130, A131, A132, A133, A133, A133, A134, A135, A138, A140, A142, A144, A144, A145, A146, A147, A148, A152, A154, A155, A156, A157, A159, A161, A162, A163, A166, A175, A178, A179, A180, A181, A182, A183, A187, A193, A194, A197, A206, A209, A212, A213, A232, A237, A238, A241, A241, A247, A249, A251, A254, A258, A259, A277, A278, A282, A290, A302, A314, A321, A326, A328, A330, A331, A332, A335, A339, A340, A341, A342, A344, A351, A356, A358, A359, A360, A363, A364, A365, A366, A369, A370, A372, A373, A380, A381, A390, A391, A393, A396, A399, A401, A408, A409, A411, A412, A413, A419, A420, A422, A424, A427, A430, A431, A435, A437, A439, A440, A443, A444, A445, A446, A449, A451, A452, A454, A455, A456, A457, A458, A459, A466, A468, A469, A470, A472, A473, A474, A475, A476, A485, A487, A488, A489, A499, A501, A502, A503, A504, A505, A509, A510, A511, A513, A515, A517, A520, A521, A522, A525, A534, A539, A540, A541, A546, A547, A548, A549, A550, A551, A552, A553, A554, A557, A559, A560, A561, A562, A563, A564, A566, A567, A570, A571, A572, A574, A575, A583, A586, A588, A593, A595, A597, A600, A601, A604, A607, A610, A616, A618, A619, A623, A625, A626, A630,

TABLE 12-continued

KRAS G13D FRET data	
IC50*	Examples
	A632, A634, A635, A636, A637, A638, A640, A641, A642, A645, A647, A652, A656, A658, A660, A668, A673, A676, A677, A680, A688, A689, A691, A693, A694, A696, A702, A706, A707, A713, A714, A717, A720, A722, A725, A726, A727, A728, A731, A732, A738, A739
+++++	A024, A065, A094, A102, A106, A109, A113, A114, A115, A115, A118, A119, A122, A124, A124, A126, A135, A136, A137, A139, A143, A148, A151, A160, A165, A167, A168, A169, A170, A171, A172, A173, A174, A176, A177, A184, A185, A186, A186, A187, A188, A190, A191, A191, A192, A192, A195, A196, A198, A199, A200, A201, A202, A203, A204, A205, A208, A209, A210, A210, A210, A210, A211, A211, A211, A211, A212, A213, A214, A214, A214, A225, A225, A225, A226, A227, A228, A229, A230, A231, A231, A231, A234, A235, A236, A239, A239, A240, A242, A243, A248, A250, A271, A272, A273, A274, A276, A279, A279, A279, A283, A283, A283, A284, A285, A286, A287, A288, A289, A291, A293, A301, A303, A304, A304, A304, A304, A307, A308, A309, A310, A311, A312, A313, A316, A316, A316, A317, A318, A318, A320, A322, A323, A324, A325, A327, A328, A329, A343, A345, A346, A347, A348, A352, A355, A362, A371, A374, A375, A376, A377, A378, A392, A398, A400, A402, A403, A404, A405, A414, A423, A425, A426, A428, A429, A441, A442, A447, A448, A450, A453, A460, A461, A462, A467, A471, A477, A478, A480, A481, A482, A483, A484, A486, A494, A495, A496, A497, A498, A500, A506, A507, A508, A512, A514, A516, A523, A524, A526, A528, A530, A531, A532, A533, A535, A556, A565, A568, A569, A576, A579, A584, A585, A587, A589, A590, A592, A599, A613, A614, A615, A617, A627, A628, A639, A643, A644, A646, A648, A649, A657, A663, A666, A674, A675, A692, A703, A705, A708, A715, A716, A721, A723, A724, A733, A735

TABLE 13

KRAS Q61H FRET data	
IC50*	Examples
+	A297, A299, A388, A598, A621
++	A012, A022, A075, A076, A077, A082, A087, A105, A148, A167, A181, A207, A262, A275, A281, A300, A333, A338, A349, A350, A353, A354, A367, A368, A379, A382, A383, A384, A385, A386, A387, A389, A394, A407, A416, A417, A463, A491, A492, A493, A537, A542, A543, A544, A545, A557, A577, A578, A580, A582, A586, A594, A596, A602, A603, A605, A606, A608, A609, A611, A612, A620, A622, A629, A631, A633, A650, A651, A653, A654, A655, A656, A661, A662, A664, A665, A667, A668, A669, A671, A672, A676, A678, A679, A681, A682, A683, A684, A686, A687, A696, A697, A698, A701, A702, A704, A709, A710, A711, A712, A718, A719, A734, A736, A737, A739
+++	A016, A045, A064, A074, A083, A086, A089, A104, A115, A166, A172, A193, A252, A277, A314, A328, A330, A332, A340, A341, A342, A344, A351, A356, A357, A358, A360, A361, A365, A366, A369, A372, A373, A390, A393, A468, A474, A475, A494, A502, A503, A504, A505, A522, A534, A538, A539, A540, A541, A546, A547, A548, A549, A551, A552, A553, A554, A555, A558, A559, A560, A561, A564, A566, A567, A570, A571, A572, A573, A574, A575, A581, A583, A588, A591, A593, A595, A597, A600, A604, A607, A610, A618, A619, A623, A624, A625, A626, A630, A632, A634, A635, A636, A640, A659, A660, A670, A673, A677, A680, A685, A688, A689, A690, A694, A695, A699, A700, A706, A713, A714, A717, A725, A728, A729, A730, A731, A732, A738
++++	A023, A025, A026, A050, A053, A060, A061, A062, A080, A084, A085, A102, A113, A136, A143, A151, A160, A168, A202, A210, A211, A226, A227, A242, A278, A290, A301, A302, A303, A331, A335, A339, A343, A345, A346, A347, A348, A352, A359, A362, A363, A364, A370, A371, A376, A380, A381, A391, A392, A403, A427, A442, A447, A461,

TABLE 13-continued

KRAS Q61H FRET data	
IC50*	Examples
	A472, A481, A495, A496, A498, A531, A550, A562, A563, A565, A568, A569, A576, A579, A584, A585, A589, A590, A592, A601, A616, A627, A637, A638, A639, A641, A642, A645, A647, A652, A658, A666, A674, A691, A692, A693, A703, A705, A707, A708, A716, A720, A721, A722, A723, A726, A727, A733, A735
+++++	A170, A171, A205, A240, A243, A248, A285, A304, A304, A313, A316, A329, A355, A374, A375, A377, A378, A482, A486, A507, A587, A599, A613, A614, A615, A617, A628, A643, A644, A646, A648, A649, A657, A663, A675, A715, A724

TABLE 14

NRAS G12C FRET data	
IC50*	Examples
+	A038, A075, A076, A088, A095, A096, A107, A108, A112, A116, A120, A121, A123, A129, A130, A131, A131, A132, A133, A133, A135, A140, A141, A145, A155, A157, A162, A167, A175, A176, A182, A183, A184, A194, A196, A209, A244, A245, A246, A247, A247, A250, A255, A257, A266, A268, A270, A290, A292, A294, A297, A298, A299, A300, A316, A316, A316, A323, A324, A326, A333, A334, A336, A337, A339, A343, A348, A351, A365, A375, A376, A377, A380, A388, A391, A407, A411, A414, A427, A435, A474, A475, A479, A509, A527, A529, A534, A537, A542, A543, A544, A550, A551, A556, A577, A578, A579, A591, A600, A601, A609, A612, A616, A620, A626, A627, A628, A638, A642, A647, A658, A660, A663, A667, A673, A684, A689, A691, A692, A699, A705, A707, A711, A721, A727, A735
++	A016, A054, A069, A103, A104, A105, A115, A121, A124, A126, A127, A128, A132, A133, A134, A135, A138, A142, A144, A148, A149, A150, A152, A153, A154, A155, A158, A164, A165, A166, A172, A178, A179, A181, A182, A183, A186, A187, A189, A191, A196, A200, A204, A207, A233, A237, A241, A241, A250, A251, A251, A252, A256, A260, A261, A262, A263, A264, A265, A267, A269, A275, A281, A291, A295, A296, A305, A317, A319, A322, A328, A332, A334, A338, A344, A346, A349, A350, A352, A353, A354, A361, A362, A363, A367, A368, A370, A371, A379, A381, A382, A383, A384, A385, A386, A387, A389, A394, A396, A397, A400, A403, A410, A417, A418, A419, A421, A423, A425, A432, A433, A436, A437, A438, A439, A440, A443, A444, A445, A448, A452, A454, A456, A458, A459, A463, A464, A465, A466, A476, A477, A478, A485, A489, A490, A491, A492, A493, A499, A501, A503, A504, A505, A510, A513, A515, A517, A518, A519, A525, A526, A528, A532, A533, A536, A538, A545, A549, A552, A558, A559, A560, A566, A567, A569, A572, A573, A574, A575, A580, A581, A582, A584, A585, A587, A589, A590, A592, A594, A595, A596, A597, A598, A599, A602, A603, A610, A614, A615, A617, A621, A622, A624, A629, A631, A632, A633, A634, A636, A637, A643, A644, A645, A646, A649, A650, A651, A653, A654, A657, A659, A661, A662, A664, A665, A668, A669, A671, A672, A674, A675, A676, A678, A679, A681, A682, A683, A685, A686, A695, A697, A698, A700, A701, A703, A704, A706, A709, A710, A712, A715, A719, A720, A722, A724, A726, A730, A733, A734, A736, A737
+++	A065, A075, A094, A109, A111, A112, A113, A115, A117, A118, A119, A120, A137, A139, A143, A144, A145, A146, A147, A151, A156, A159, A161, A167, A169, A173, A177, A180, A181, A184, A185, A188, A190, A192, A193, A194, A195, A197, A199, A201, A206, A209, A210, A211, A212, A214, A225, A226, A227, A232, A238, A249, A253, A254, A258, A258, A259, A261, A277, A278, A280, A281, A282, A283, A301, A302, A306, A310, A314, A320, A321, A327, A330, A331, A335, A340, A341, A342, A347, A356, A357, A358, A359, A360, A364, A366, A369, A372, A373, A374, A378, A390, A392, A393, A395, A399, A402, A404, A406, A408, A409, A412, A413, A415, A416, A420, A422, A424, A430, A431, A442, A446, A447, A449, A451, A455, A457,

TABLE 14-continued

NRAS G12C FRET data	
IC50*	Examples
	A461, A462, A467, A468, A469, A470, A471, A472, A473, A481, A483, A486, A487, A488, A494, A495, A497, A498, A502, A506, A507, A508, A511, A512, A520, A522, A524, A530, A531, A539, A540, A541, A546, A547, A548, A553, A554, A555, A557, A561, A562, A563, A564, A565, A568, A570, A571, A576, A583, A586, A588, A593, A604, A605, A606, A607, A608, A611, A618, A619, A623, A625, A630, A635, A639, A640, A641, A648, A652, A655, A656, A670, A677, A680, A687, A688, A690, A693, A694, A696, A702, A708, A713, A714, A716, A717, A718, A723, A725, A728, A729, A731, A732, A738, A739
++++	A024, A102, A106, A110, A124, A125, A136, A160, A163, A168, A170, A171, A174, A186, A187, A191, A192, A202, A203, A205, A208, A210, A211, A212, A213, A228, A229, A235, A240, A242, A248, A274, A276, A279, A283, A284, A289, A303, A307, A309, A313, A325, A328, A345, A398, A401, A426, A441, A453, A460, A480, A484, A496, A514, A516, A521, A523, A535, A613, A666
+++++	A114, A122, A148, A198, A210, A210, A211, A211, A213, A214, A214, A225, A225, A230, A231, A231, A231, A234, A236, A239, A239, A243, A271, A272, A273, A279, A279, A283, A285, A286, A287, A288, A293, A304, A304, A304, A304, A308, A311, A312, A318, A318, A329, A355, A405, A428, A429, A450, A482, A500

TABLE 15

NRAS Q61R FRET data	
IC50*	Examples
+	A388
++	A367, A368, A382, A383, A385, A386, A387, A389, A407, A417, A491, A492, A493, A542, A577, A594, A596, A598, A602, A603, A609, A611, A612, A621, A629, A633, A655, A664, A665, A667, A669, A678, A679, A697, A704, A712, A719, A734, A736, A737
+++	A012, A022, A075, A076, A082, A087, A105, A148, A167, A181, A207, A262, A275, A281, A297, A299, A300, A314, A333, A338, A349, A350, A353, A354, A356, A357, A360, A361, A369, A379, A384, A393, A394, A416, A463, A502, A537, A538, A539, A541, A543, A544, A545, A546, A548, A554, A555, A570, A571, A572, A573, A575, A578, A580, A581, A582, A583, A586, A591, A593, A595, A597, A604, A605, A606, A607, A608, A610, A618, A619, A620, A622, A624, A625, A630, A631, A632, A634, A635, A636, A650, A651, A653, A654, A656, A659, A661, A662, A668, A670, A671, A672, A676, A677, A680, A681, A682, A683, A684, A685, A686, A687, A688, A690, A695, A696, A698, A699, A700, A701, A702, A706, A709, A710, A711, A713, A714, A717, A718, A728, A732, A738
++++	A016, A064, A074, A077, A083, A086, A089, A104, A115, A166, A168, A193, A252, A277, A290, A328, A330, A332, A335, A339, A340, A341, A342, A343, A344, A347, A351, A352, A358, A359, A363, A364, A365, A366, A370, A372, A373, A380, A381, A390, A391, A392, A442, A461, A472, A474, A475, A481, A494, A495, A496, A498, A503, A504, A505, A522, A531, A534, A540, A547, A549, A550, A551, A552, A553, A557, A558, A559, A560, A561, A564, A566, A567, A574, A588, A600, A601, A623, A626, A638, A639, A640, A641, A647, A652, A658, A660, A673, A689, A691, A693, A694, A720, A722, A725, A727, A729, A730, A731, A739
+++++	A023, A025, A026, A045, A050, A053, A060, A061, A062, A080, A084, A085, A102, A113, A136, A143, A151, A160, A170, A171, A172, A202, A205, A210, A211, A226, A227, A240, A242, A243, A248, A278, A285, A301, A302, A303, A304, A304, A313, A316, A329, A331, A345, A346, A348, A355, A362, A371, A374, A375, A376, A377, A378, A403, A427, A447, A468, A482, A486, A507, A556, A562, A563, A565, A568, A569, A576, A579, A584, A585, A587, A589, A590, A592, A599, A613, A614, A615, A616, A617, A627, A628, A637, A642, A643, A644, A645, A646, A648, A649,

TABLE 15-continued

NRAS Q61R FRET data	
IC50*	Examples
	A657, A663, A666, A674, A675, A692, A703, A705, A707, A708, A715, A716, A721, A723, A724, A726, A733, A735

TABLE 16

NRAS Q61K FRET data	
IC50*	Examples
+	A388
++	A262, A297, A299, A338, A349, A350, A353, A354, A367, A368, A369, A382, A383, A384, A385, A386, A387, A389, A394, A407, A416, A417, A463, A491, A492, A493, A542, A543, A544, A545, A577, A580, A582, A594, A596, A598, A602, A603, A609, A611, A612, A620, A621, A622, A629, A631, A633, A650, A651, A653, A654, A655, A661, A662, A664, A665, A667, A668, A669, A671, A678, A679, A681, A682, A683, A686, A687, A696, A697, A698, A701, A702, A704, A709, A710, A711, A712, A718, A719, A734, A736, A737, A739
+++	A012, A022, A074, A075, A076, A077, A082, A086, A087, A105, A148, A167, A181, A207, A275, A277, A281, A300, A314, A330, A333, A340, A356, A360, A361, A379, A390, A393, A502, A537, A538, A539, A541, A546, A547, A548, A554, A555, A561, A570, A571, A572, A573, A574, A575, A578, A581, A583, A586, A588, A593, A595, A597, A604, A605, A606, A607, A608, A610, A618, A619, A623, A624, A625, A630, A632, A634, A635, A636, A656, A660, A670, A672, A676, A677, A680, A684, A685, A688, A690, A694, A695, A699, A700, A706, A713, A714, A717, A728, A729, A730, A731, A732, A738
++++	A016, A025, A045, A050, A060, A061, A064, A080, A083, A089, A104, A115, A143, A151, A166, A168, A172, A193, A202, A210, A211, A227, A252, A278, A290, A328, A331, A332, A335, A339, A341, A342, A343, A344, A347, A351, A352, A357, A358, A359, A363, A364, A365, A366, A370, A372, A373, A380, A381, A391, A427, A442, A461, A468, A472, A474, A475, A481, A494, A495, A496, A498, A503, A504, A505, A522, A531, A534, A540, A549, A550, A551, A552, A553, A557, A558, A559, A560, A562, A563, A564, A566, A567, A576, A590, A591, A600, A601, A626, A637, A638, A639, A640, A641, A647, A652, A659, A673, A689, A691, A693, A705, A720, A722, A725, A727, A735
+++++	A023, A026, A053, A062, A084, A085, A102, A113, A136, A160, A170, A171, A205, A226, A240, A242, A243, A248, A285, A301, A302, A303, A304, A304, A313, A316, A329, A345, A346, A348, A355, A362, A371, A374, A375, A376, A377, A378, A392, A403, A447, A482, A486, A507, A556, A565, A568, A569, A579, A584, A585, A587, A589, A592, A599, A613, A614, A615, A616, A617, A627, A628, A642, A643, A644, A645, A646, A648, A649, A657, A658, A663, A666, A674, A675, A692, A703, A707, A708, A715, A716, A721, A723, A724, A726, A733

TABLE 17

NRAS WT FRET data	
IC50*	Examples
+	A388
++	A075, A076, A087, A167, A262, A275, A297, A299, A300, A333, A338, A349, A367, A368, A382, A383, A385, A386, A387, A389, A407, A417, A491, A492, A493, A537, A542, A577, A594, A596, A598, A602, A603, A605, A609, A612, A621, A629, A633, A664, A665, A667, A669, A679, A697, A704, A709, A711, A712, A719, A734, A736, A737
+++	A012, A016, A022, A074, A077, A082, A086, A105, A148, A166, A181, A207, A252, A277, A281, A340, A350, A351, A353, A354, A356, A357, A358, A360, A361, A369, A379, A384, A393, A394, A416, A463, A502, A538, A539, A541,

TABLE 17-continued

NRAS WT FRET data	
IC50*	Examples
	A543, A544, A545, A546, A547, A548, A550, A552, A554, A555, A557, A558, A564, A570, A571, A572, A573, A575, A578, A580, A581, A582, A583, A586, A591, A593, A595, A600, A604, A606, A607, A608, A610, A611, A618, A620, A622, A624, A625, A630, A631, A632, A634, A635, A636, A647, A650, A651, A653, A654, A655, A656, A659, A660, A661, A662, A668, A670, A671, A672, A676, A677, A678, A680, A681, A682, A683, A684, A685, A686, A687, A688, A690, A695, A696, A698, A699, A700, A701, A702, A710, A713, A714, A717, A718, A722, A728, A729, A730, A731, A732, A738, A739
++++	A025, A045, A060, A061, A062, A064, A083, A089, A104, A115, A151, A168, A172, A193, A202, A210, A211, A227, A278, A290, A314, A328, A330, A331, A332, A335, A339, A341, A342, A343, A344, A347, A348, A352, A359, A362, A363, A364, A365, A366, A370, A371, A372, A373, A375, A376, A378, A380, A381, A390, A391, A392, A427, A447, A461, A468, A472, A474, A475, A481, A494, A495, A496, A498, A503, A504, A505, A507, A522, A531, A534, A540, A549, A551, A553, A556, A559, A560, A561, A562, A563, A566, A567, A574, A576, A579, A584, A585, A588, A589, A590, A597, A601, A616, A619, A623, A626, A637, A638, A639, A640, A641, A642, A645, A648, A652, A658, A673, A689, A691, A693, A694, A703, A705, A706, A707, A708, A716, A720, A721, A723, A725, A726, A727, A735
+++++	A023, A026, A050, A053, A080, A084, A085, A102, A113, A136, A143, A160, A170, A171, A205, A226, A240, A242, A243, A248, A285, A302, A303, A304, A304, A313, A316, A329, A345, A346, A355, A374, A377, A403, A442, A482, A486, A565, A568, A569, A587, A592, A599, A613, A614, A615, A617, A627, A628, A643, A644, A646, A649, A657, A663, A666, A674, A675, A692, A715, A724, A733

In Vitro Cell Proliferation Panels

Potency for inhibition of cell growth was assessed at CrownBio using standard methods. Briefly, cell lines were cultured in appropriate medium, and then plated in 3D methylcellulose. Inhibition of cell growth was determined by CellTiter-Glo® after 5 days of culture with increasing concentrations of compounds. Compound potency was reported as the 50% inhibition concentration (absolute IC50). The assay took place over 7 days. On day 1, cells in 2D culture were harvested during logarithmic growth and suspended in culture medium at 1×105 cells/ml. Higher or lower cell densities were used for some cell lines based on prior optimization. 3.5 ml of cell suspension was mixed with 6.5% growth medium with 1% methylcellulose, resulting in a cell suspension in 0.65% methylcellulose. 90 μl of this suspension was distributed in the wells of 2 96-well plates. One plate was used for day 0 reading and 1 plate was used for the end-point experiment. Plates were incubated overnight at 37 C with 5% CO_2. On day 2, one plate (for t0 reading) was removed and 10 μl growth medium plus 100 μl CellTiter-Glo® Reagent was added to each well. After mixing and a 10 minute incubation, luminescence was recorded on an EnVision Multi-Label Reader (Perkin Elmer). Compounds in DMSO were diluted in growth medium such that the final, maximum concentration of compound was 10 μM, and serial 4-fold dilutions were performed to generate a 9-point concentration series. 10 μl of compound solution at 10 times final concentration was added to wells of the second plate. Plate was then incubated for 120 hours at 37 C and 5% CO_2. On day 7 the plates were removed, 100 μl CellTiter-Glo® Reagent was added to each well, and after mixing and a 10 minute incubation, luminescence was recorded on an EnVision Multi-Label Reader (Perkin Elmer). Data was exported to GeneData Screener and modeled with a sigmoidal concentration response model in order to determine the IC50 for compound response.

Not all cell lines with a given RAS mutation may be equally sensitive to a RAS inhibitor targeting that mutation, due to differential expression of efflux transporters, varying dependencies on RAS pathway activation for growth, or other reasons. This has been exemplified by the cell line KYSE-410 which, despite8 having a KRAS G12C mutation, is insensitive to the KRAS G12C (OFF) inhibitor MRTX-849 (Hallin et al., Cancer Discovery 10:54-71 (2020)), and the cell line SW1573, which is insensitive to the KRAS G12C (OFF) inhibitor AMG51 (Canon et al., Nature 575: 217-223 (2019)).

TABLE 18

IC50 values for various cancer cell lines with Compound B			
Cell Line	Histotype	Mutant	IC50*
NCI-H358	Lung	KRAS G12C	very sensitive
MIA PaCa-2	Pancreas	KRAS G12C	very sensitive
SW837	Intestine/Large/ Colorectum	KRAS G12C	very sensitive
KYSE-410	HN/Esophagus	KRAS G12C	moderately sensitive
NCI-H727	Lung	KRAS G12V	moderately sensitive
OVCAR-5	Ovary	KRAS G12V	moderately sensitive
Capan-2	Pancreas	KRAS G12V	moderately sensitive
NCI-H747	Intestine/Large/ Colorectum	other KRAS (G13D)	moderately sensitive
NCI-H441	Lung	KRAS G12V	moderately sensitive
HEC-1-A	Uterus	KRAS G12D	moderately sensitive
NOZ	Liver/Bile duct	KRAS G12V	moderately sensitive
HCT116	Intestine/Large/ Colorectum	other KRAS (G13D)	moderately sensitive
Calu-6	Lung	other KRAS (Q61K)	moderately sensitive
HuCCT1	Liver/Bile duct	KRAS G12D	moderately sensitive
NCI-H2009	Lung	other KRAS (G12A)	low sensitivity
NCI-H1975	Lung	other MAPK (EGFR T790M, L858R)	low sensitivity
SW1573	Lung	KRAS G12C	not sensitive
AGS	Stomach	KRAS G12D	low sensitivity
AsPC-1	Pancreas	KRAS G12D	low sensitivity
SNU-668	Stomach	other KRAS (Q61K)	low sensitivity
HPAC	Pancreas	KRAS G12D	low sensitivity
NCI-H1838	Lung	other MAPK (NF1 mut)	low sensitivity
NCI-H3122	Lung	other MAPK (EML4-ALK(E13, A20))	low sensitivity
NCI-H460	Lung	other KRAS (Q61H)	low sensitivity
SW403	Intestine/Large/ Colorectum	KRAS G12V	low sensitivity
A549	Lung	other KRAS (G12S)	low sensitivity
CAL-62	HN/Thyroid	other KRAS (G12R)	low sensitivity
DV-90	Lung	other KRAS (G13D)	low sensitivity
OZ	Liver/Bile duct	other KRAS (Q61L)	low sensitivity

TABLE 18-continued

IC50 values for various cancer cell lines with Compound B

Cell Line	Histotype	Mutant	IC50*
A-375	Skin	BRAF V600E	low sensitivity
BxPC-3	Pancreas	other MAPK (BRAF V487_P492delinsA)	low sensitivity
SW48	Intestine/Large/ Colorectum	not MAPK (PIK3CA G914R, EGFR G719S)	low sensitivity
HCC1588	Lung	KRAS G12D	low sensitivity
TOV-21G	Ovary	other KRAS (G13C)	low sensitivity
SW948	Intestine/Large/ Colorectum	other KRAS (Q61L)	low sensitivity
LS513	Intestine/Large/ Colorectum	KRAS G12D	low sensitivity
MeWo	Skin	other MAPK (NF1 mut)	low sensitivity

*Key:
low sensitivity: IC50 ≥ 1 uM
moderately sensitive: 1 uM > IC50 ≥ 0.1 uM
very sensitive: IC50 < 0.1 uM

TABLE 19

Summary of IC50 results for various cancer cell lines with several compounds of the present invention (Compounds B and E-M)

Cell Line	Histotype	Mutant	E	F	G	H	I	J	K	L	B	M
SW837	Intestine/Large/ Colorectum	KRAS G12C	V	V	V	V	V	V	V	V	V	V
MIA PaCa-2	Pancreas	KRAS G12C	V	V	V	V	V	V	V	V	V	V
KYSE-410	HN/Esophagus	KRAS G12C	L	L	L	L	L	L	L	L	L	L
H358	Lung	KRAS G12C	V	V	V	V	V	V	V	V	V	V
H2122	Lung	KRAS G12C	V	V	V	V	V	V	V	V	V	V
H1373	Lung	KRAS G12C	V	V	V	V	V	V	V	V	V	V
H23	Lung	KRAS G12C	V	V	V	V	V	V	V	V	V	V
H1792	Lung	KRAS G12C	V	V	V	V	V	V	V	V	V	V
AsPC-1	Pancreas	KRAS G12D	L	L	M	L	L	M	L	L	M	L
A375	Skin	BRAF V600E	L	L	L	L	L	L	L	L	L	L
H1975	Lung	other MAPK (EGFR T790M, L858R)	M	L	M	L	L	M	L	M	M	L
HCC1588	Lung	KRAS G12D	L	L	L	L	L	L	L	L	L	L
H441	Lung	KRAS G12V	M	M	M	L	M	V	L	M	V	L

*Key:
(L) low sensitivity: IC50 ≥ 1 uM
(M) moderately sensitive: 1 uM > IC50 ≥ 0.1 uM
(V) very sensitive: IC50 < 0.1 uM

In Vivo NSCLC K-Ras G12C Xenograft Models

Compound A:

Methods:

The effects of a compound of the present invention, Compound A (H358 pERK K-Ras G12C EC50: 0.001 uM), on tumor cell growth in vivo were evaluated in the human non-small cell lung cancer NCI-H358 KRASG12C xenograft model using female BALB/c nude mice (6-8 weeks old). Mice were implanted with NCI-H358 tumor cells in 50% Matrigel (5×106 cells/mouse) subcutaneously in the flank. At the indicated tumor volume (dotted line, FIG. **2**A), mice were randomized to treatment groups to start the administration of test articles or vehicle. Compound A was administered by oral gavage daily at the dose of 100 mg/kg. Body weight and tumor volume (using calipers) was measured twice weekly until study endpoints. Spaghetti plot (FIG. **2**B) shows the tumor volume change in individual tumors during the course of treatment.

Results:

FIG. **2**A shows Compound A dosed at 100 mg/kg by daily oral gavage led to tumor regression in NCI-H358 KRASG12C xenograft model, which is a sensitive model to KRASG12C inhibition alone. The spaghetti titer plot (FIG. **2**B) displaying individual tumor growth is shown next to the tumor volume plot (FIG. **2**A). Over the treatment course of 28 days, Compound A drove tumor regression in all 10 animals bearing NCI-H358 KRASG12C tumors.

Compound B:

Methods:

The combinatorial effect of a compound of the present invention, Compound B (H358 pERK K-Ras G12C EC50: 0.003 uM), with cobimetinib on tumor cell growth in vivo were evaluated in the human non-small cell lung cancer NCI-H358 KRASG12C xenograft model using female BALB/c nude mice (6-8 weeks old). Mice were implanted with NCI-H358 tumor cells in 50% Matrigel (5×106 cells/mouse) subcutaneously in the flank. At indicated tumor volume (dotted line, FIG. **3**A), mice were randomized to treatment groups to start the administration of test articles or vehicle. Compound B was administered by intermittent (twice weekly) intravenous injection at the dose of 50 mg/kg. Cobimetinib was administered by daily oral gavage at 2.5 mg/kg. The combination of Compound B and cobimetinib at their respective single-agent dose and regimen was also tested. Body weight and tumor volume (using calipers) was measured twice weekly until study endpoints. End of study responses in individual tumors were plotted as a waterfall plot (FIG. **3**B), and the numbers indicate number of tumor regression in each group. Tumor regression is defined as greater than 10% reduction of tumor volume at the end of study relative to initial volume.

Results:

FIG. **3**A shows the combination of intermittent intravenous administration of Compound B at 50 mg/kg plus daily oral administration of cobimetinib at 2.5 mg/kg drove tumor regression, whereas each single agent led to tumor growth inhibition. End of study responses were shown as waterfall plots (FIG. **3**B), which indicate 6 out 10 mice had tumor

regression in the combination group, whereas no tumor regressions recorded in each single agent group.

Compound C:

Methods:

The combinatorial effect of a compound of the present invention, Compound C (H358 pERK K-Ras G12C EC50: 0.007 uM), with a SHP2 inhibitor, RMC-4550, on tumor cell growth in vivo were evaluated in the human non-small cell lung cancer NCI-H358 KRASG12C xenograft model using female BALB/c nude mice (6-8 weeks old). Mice were implanted with NCI-H358 tumor cells in 50% Matrigel (5×106 cells/mouse) subcutaneously in the flank. At indicated tumor volume (dotted line, FIG. **4**A), mice were randomized to treatment groups to start the administration of test articles or vehicle. Compound C was administered by once weekly intravenous injection at the dose of 60 mg/kg. SHP2 inhibitor was administered by daily oral gavage at 30 mg/kg. The combination of Compound C and SHP2 inhibitor at their respective single-agent dose and regimen was also tested. Body weight and tumor volume (using calipers) was measured twice weekly until study endpoints. End of study responses in individual tumors were plotted as a waterfall plot (FIG. **4**B), and the numbers indicate number of tumor regression in each group. Tumor regression is defined as greater than 10% reduction of tumor volume at the end of study relative to initial volume.

Results:

In FIG. **4**A, the combinatorial activity of once weekly intravenous administration of Compound C at 60 mg/kg plus daily oral administration of SHP2 inhibitor at 30 mg/kg is shown. The combination treatment had similar anti-tumor activity as the single agent SHP2 inhibitor, but the combination treatment led to 8 out of 10 mice with tumor regression, whereas single agent SHP2 inhibitor led to 5 out of 10 mice with tumor regressions. Single agent Compound C administered once weekly via intravenous injection led to tumor growth inhibition with one tumor regression.

Cell Proliferation Assay

Methods:

NCI-H358 cells were plated in 12-well tissue culture plates at a density of 100,000 cells/well in RPMI 1640 (10% FBS, 1% PenStrep) and cultured overnight at 37° C., 5% CO_2. The following day, cells were treated with either trametinib (10 nM) or a compound of the present invention, Compound D (H358 pERK K-Ras G12C EC50: 0.024 uM), (17 nM). These concentrations represent the EC50 values from a 72-hour proliferation assay using the CellTiter-Glo® reagent (Promega). Additionally, cells were treated with the combination of trametinib and Compound D at the above indicated concentrations. The plate was placed in the Incucyte S_3 live cell analysis system (37° C., 5% CO_2) and confluence was measured by recording images at 6-hour intervals for a maximum of 40 days, or until wells reached maximal confluence. Media and drug were replaced at 3-4 day intervals. Data are plotted as % confluence over the time course of the experiment for each single agent and respective combination (FIG. **5**).

Results:

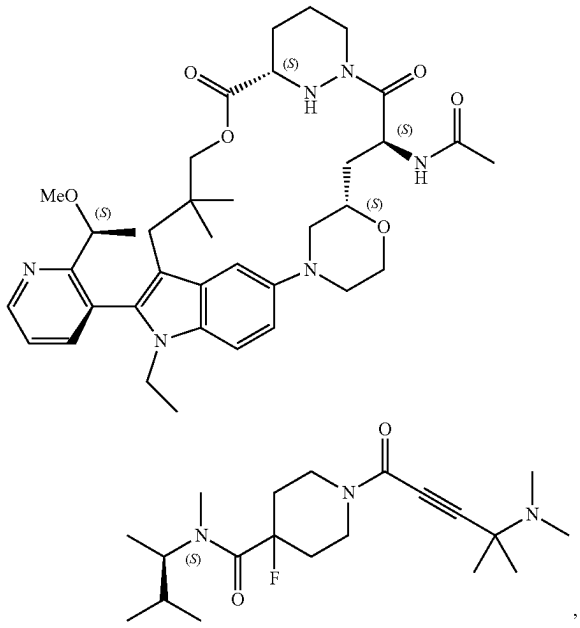
As shown in FIG. **5**, treatment of NCI-H358 cells with submaximal (EC50) concentrations of Compound D or MEK inhibitor results in a short period of growth inhibition, followed by proliferation. Cells reach maximal confluence in ~10 days after the addition of drug. The combination of the MEK inhibitor, trametinib, with Compound D resulted in complete and sustained inhibition of cell growth throughout the duration of the assay.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure come within known or customary practice within the art to which the invention pertains and may be applied to the essential features set forth herein.

All publications, patents and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety.

The invention claimed is:

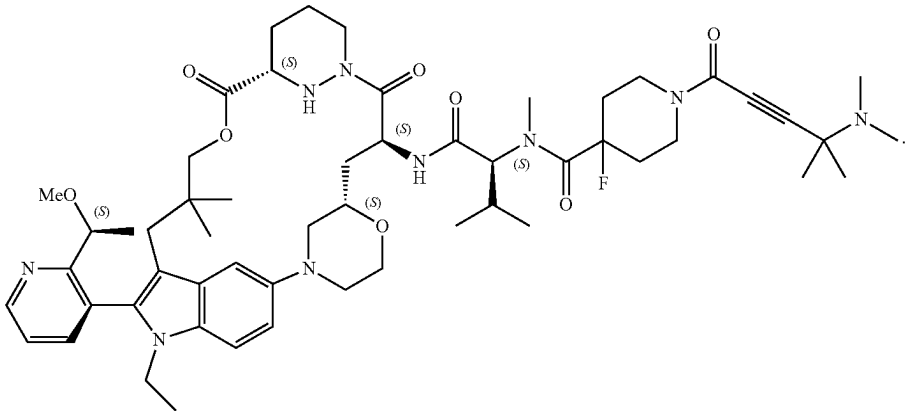
1. A method of treating lung cancer in a subject in need thereof, wherein the lung cancer comprises a K-Ras G12C mutation, the method comprising administering to the subject a therapeutically effective amount of a compound having the following structure:



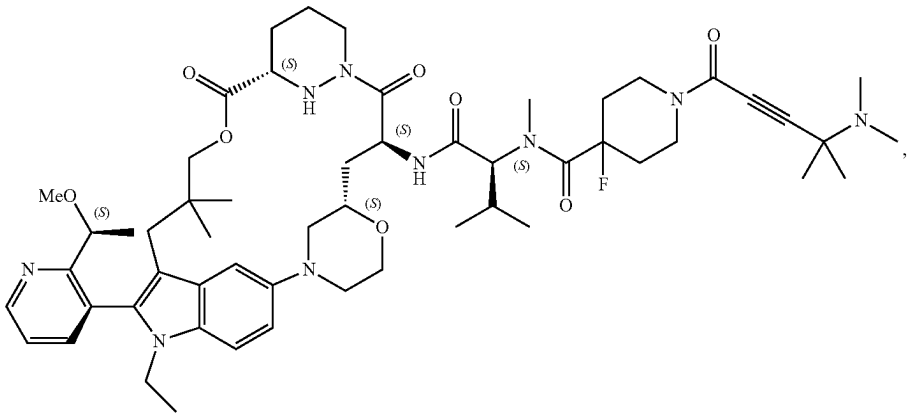
or a pharmaceutically acceptable salt thereof.

2. The method of claim **1**, wherein the lung cancer is non-small cell lung cancer.

3. The method of claim **1**, wherein the method comprises administering to the subject a therapeutically effective amount of the compound having the following structure:

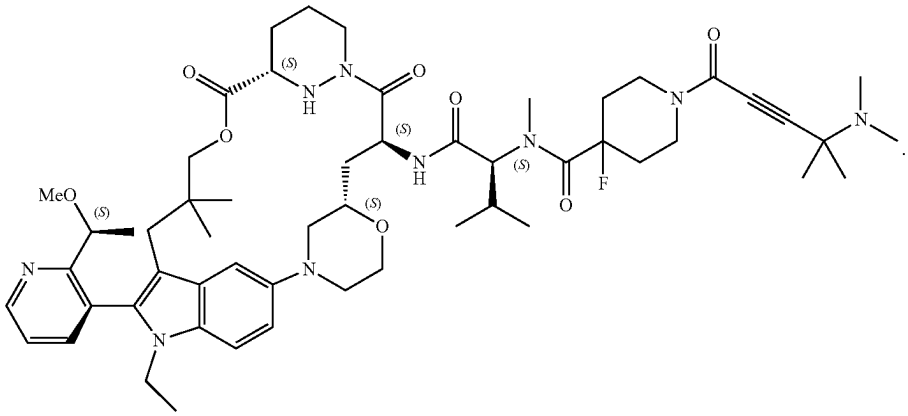


4. A method of treating non-small cell lung cancer in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a compound having the following structure:

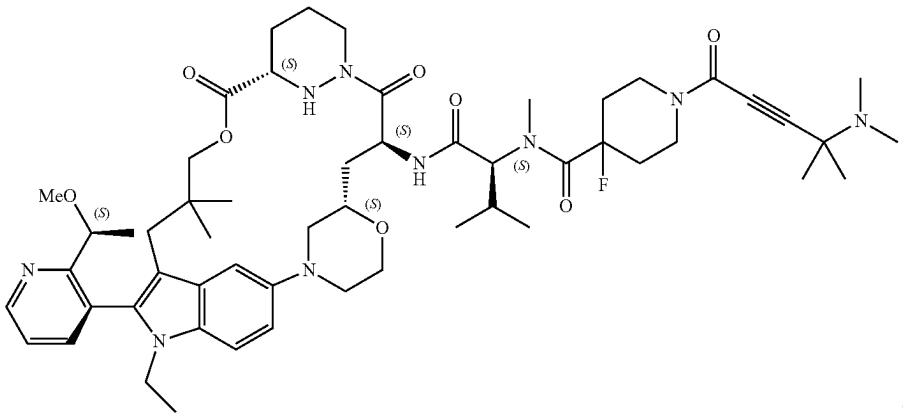


or a pharmaceutically acceptable salt thereof.

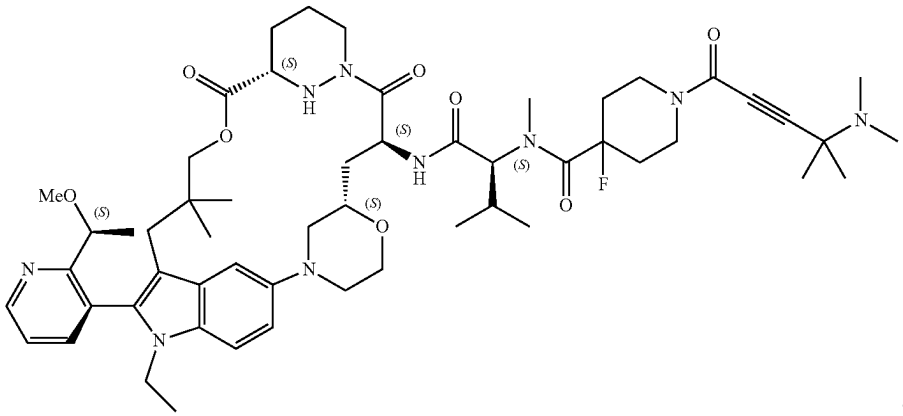
5. The method of claim **4**, wherein the method comprises administering to the subject a therapeutically effective amount of the compound having the following structure:



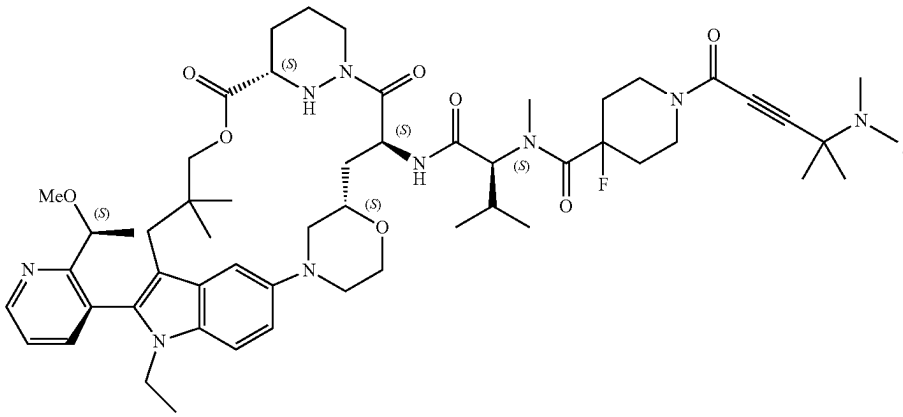
6. The method of claim **2**, wherein the method comprises administering to the subject a therapeutically effective amount of the compound having the following structure:



7. The method of claim **2**, wherein the method comprises administering to the subject a therapeutically effective amount of the pharmaceutically acceptable salt of the compound having the following structure:



8. The method of claim **4**, wherein the method comprises administering to the subject a therapeutically effective amount of the pharmaceutically acceptable salt of the compound having the following structure:



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