

US Patent & Trademark Office

Patent Public Search | Text View

United States Patent Application Publication	20250263491
Kind Code	A1
Publication Date	August 21, 2025
Inventor(s)	Boghaert; Erwin R. et al.

ANTI-EGFR ANTIBODY DRUG CONJUGATES

Abstract

The invention relates to anti-Epidermal Growth Factor Receptor (EGFR) antibody drug conjugates (ADCs) which inhibit Bcl-xL, including compositions and methods of using said ADCs.

Inventors: Boghaert; Erwin R. (Pleasant Prairie, WI), Bruncko; Milan (Green Oaks, IL), Judd; Andrew S. (Grayslake, IL), Phillips; Andrew C. (Libertyville, IL), Souers; Andrew J. (Libertyville, IL)

Applicant: AbbVie Inc. (North Chicago, IL)

Family ID: 1000008577985

Appl. No.: 18/823442

Filed: September 03, 2024

Related U.S. Application Data

parent US continuation 18393417 20231221 ABANDONED child US 18823442
parent US continuation 17688908 20220308 ABANDONED child US 18393417
parent US continuation 16308575 20181210 ABANDONED US continuation
PCT/US2017/036399 20170607 child US 17688908
us-provisional-application US 62347258 20160608
us-provisional-application US 62347528 20160608

Publication Classification

Int. Cl.: C07K16/28 (20060101); A61K31/5377 (20060101); A61K39/00 (20060101);
A61K45/06 (20060101); A61K47/65 (20170101); A61K47/68 (20170101); A61P35/00
(20060101)

U.S. Cl.:

CPC C07K16/2863 (20130101); A61K31/5377 (20130101); A61K47/65 (20170801); A61K47/6803 (20170801); A61K47/6845 (20170801); A61K47/6855 (20170801); A61K47/6857 (20170801); A61P35/00 (20180101); A61K2039/505 (20130101); A61K45/06 (20130101); C07K2317/73 (20130101)

Background/Summary

RELATED APPLICATIONS [0001] This application is a continuation of U.S. patent application Ser. No. 18/393,417, filed Dec. 21, 2023, which is a continuation of U.S. patent application Ser. No. 17/688,908, filed Mar. 8, 2022, which is a continuation of U.S. patent application Ser. No. 16/308,575, filed Dec. 10, 2018, which in turn is a 371 National Stage application of PCT Application No. PCT/US2017/036399, which was filed on Jun. 7, 2017, and claims priority to both U.S. Provisional Application No. 62/347,258, filed on Jun. 8, 2016, and U.S. Provisional Application No. 62/347,528, filed on Jun. 8, 2016, the entire contents of which are expressly incorporated herein by reference.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. Said XML copy, created on Sep. 3, 2024, is named A103017_1080US_C3_SL.xml and is 153,574 bytes in size.

BACKGROUND OF THE INVENTION

[0003] The human epidermal growth factor receptor (also known as HER-1 or Erb-B1, and referred to herein as “EGFR”) is a 170 kDa transmembrane receptor encoded by the c-erbB protooncogene, and exhibits intrinsic tyrosine kinase activity (Modjtahedi et al., Br. J. Cancer 73:228-235 (1996); Herbst and Shin, Cancer 94:1593-1611 (2002)). SwissProt database entry P00533 provides the sequence of human EGFR. EGFR regulates numerous cellular processes via tyrosine-kinase mediated signal transduction pathways, including, but not limited to, activation of signal transduction pathways that control cell proliferation, differentiation, cell survival, apoptosis, angiogenesis, mitogenesis, and metastasis (Atalay et al., Ann. Oncology 14:1346-1363 (2003); Tsao and Herbst, Signal 4:4-9 (2003); Herbst and Shin, Cancer 94:1593-1611 (2002); Modjtahedi et al., Br. J. Cancer 73:228-235 (1996)).

[0004] Known ligands of EGFR include EGF, TGFA/TGF-alpha, amphiregulin, epigen/EPGN, BTC/betacellulin, epiregulin/EREG and HBEGF/heparin-binding EGF. Ligand binding by EGFR triggers receptor homo- and/or heterodimerization and autophosphorylation of key cytoplasmic residues. The phosphorylated EGFR recruits adapter proteins like GRB2 which in turn activate complex downstream signaling cascades, including at least the following major downstream signaling cascades: the RAS-RAF-MEK-ERK, PI3 kinase-AKT, PLCgamma-PKC, and STATs modules. This autophosphorylation also elicits downstream activation and signaling by several other proteins that associate with the phosphorylated tyrosines through their own phosphotyrosine-binding SH2 domains.

[0005] These downstream signaling proteins initiate several signal transduction cascades, principally the MAPK, Akt and JNK pathways, leading to cell proliferation. Ligand binding by EGFR may also activate the NF-kappa-B signaling cascade. Ligand binding also directly phosphorylates other proteins like RGS16, activating its GTPase activity and potentially coupling the EGF receptor signaling to G protein-coupled receptor signaling. Ligand binding also phosphorylates MUC1 and increases its interaction with SRC and CTNNB1/beta-catenin.

[0006] Overexpression of EGFR has been reported in numerous human malignant conditions, including cancers of the bladder, brain, head and neck, pancreas, lung, breast, ovary, colon, prostate, and kidney. (Atalay et al., Ann. Oncology 14:1346-1363 (2003); Herbst and Shin, Cancer

94:1593-1611 (2002); and Modjtahedi et al., Br. J. Cancer 73:228-235 (1996)). In many of these conditions, the overexpression of EGFR correlates or is associated with poor prognosis of the patients. (Herbst and Shin, Cancer 94:1593-1611 (2002); and Modjtahedi et al., Br. J. Cancer 73:228-235 (1996)). EGFR is also expressed in the cells of normal tissues, particularly the epithelial tissues of the skin, liver, and gastrointestinal tract, although at generally lower levels than in malignant cells (Herbst and Shin, Cancer 94:1593-1611 (2002)).

[0007] A significant proportion of tumors containing amplifications of the EGFR gene also co-express a truncated version of the receptor (Wikstrand et al. (1998) J. Neurovirol. 4, 148-158) known as de2-7 EGFR, Δ EGFR, EGFRvIII, or Δ 2-7 (terms used interchangeably herein) (Olapade-Olaopa et al. (2000) Br. J. Cancer. 82, 186-94). The rearrangement seen in the de2-7 EGFR results in an in-frame mature mRNA lacking 801 nucleotides spanning exons 2-7 (Wong et al. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 2965-9; Yamazaki et al. (1990) Jpn. J. Cancer Res. 81, 773-9; Yamazaki et al. (1988) Mol. Cell. Biol. 8, 1816-20; and Sugawa et al. (1990) Proc. Natl. Acad. Sci. U.S.A. 87, 8602-6). The corresponding EGFR protein has a 267 amino acid deletion comprising residues 6-273 of the extracellular domain and a novel glycine residue at the fusion junction (Sugawa et al., 1990). This deletion, together with the insertion of a glycine residue, produces a unique junctional peptide at the deletion interface (Sugawa et al., 1990).

[0008] EGFRvIII has been reported in a number of tumor types including glioma, breast, lung, ovarian and prostate (Wikstrand et al. (1997) Cancer Res. 57, 4130-40; Olapade-Olaopa et al. (2000) Br. J. Cancer. 82, 186-94; Wikstrand, et al. (1995) Cancer Res. 55, 3140-8; Garcia de Palazzo et al. (1993) Cancer Res. 53, 3217-20). While this truncated receptor does not bind ligand, it possesses low constitutive activity and imparts a significant growth advantage to glioma cells grown as tumor xenografts in nude mice (Nishikawa et al. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 7727-31) and is able to transform NIH3T3 cells (Batra et al. (1995) Cell Growth Differ. 6, 1251-9) and MCF-7 cells. The cellular mechanisms utilized by the de2-7 EGFR in glioma cells are not fully defined but are reported to include a decrease in apoptosis (Nagane et al. (1996) Cancer Res. 56, 5079-86) and a small enhancement of proliferation (Nagane et al., 1996). As expression of this truncated receptor is restricted to tumor cells it represents a highly specific target for antibody therapy.

[0009] Antibody drug conjugates (ADC) represent a new class of therapeutics comprising an antibody conjugated to a cytotoxic drug via a chemical linker. The therapeutic concept of ADCs is to combine binding capabilities of an antibody with a drug, where the antibody is used to deliver the drug to a tumor cell by means of binding to a target surface antigen. Given the role of EGFR in cancer, there remains a need in the art for anti-EGFR ADCs that can be used for treatment of cancer.

SUMMARY OF THE INVENTION

[0010] It has been discovered that small molecule inhibitors of Bcl-xL are efficacious when administered in the form of antibody drug conjugates (ADCs) that bind to antigens expressed on the surface of cells, e.g. cells that express EGFR, where inhibition of Bcl-xL and consequent induction of apoptosis would be beneficial. This discovery provides the ability to target Bcl-xL inhibitory therapies to specific cells and/or tissues that express EGFR, such that the Bcl-xL inhibitor is delivered internally to a transformed cancer cell expressing EGFR. One advantage of the invention is the potential for lowering serum levels necessary to achieve desired therapeutic benefit and/or avoiding and/or ameliorating potential side effects associated with systemic administration of the small molecule Bcl-xL inhibitors per se.

[0011] ADCs may increase the therapeutic efficacy of antibodies in treating disease, e.g., cancer, due to the ability of the ADC to selectively deliver one or more drug moiety(s) to target tissues, such as a tumor-associated antigen, e.g., EGFR expressing tumors. Thus, in certain embodiments, the invention provides anti-EGFR ADCs for therapeutic use, e.g., treatment of cancer.

[0012] In one aspect, the invention features an anti-human Epidermal Growth Factor Receptor

(hEGFR) antibody drug conjugate (ADC) comprising an anti-hEGFR antibody, i.e., an antibody that specifically binds to human EGFR, linked to one or more Bcl-xL inhibitor(s).

[0013] In another aspect, the invention features an anti-human Epidermal Growth Factor Receptor (hEGFR) antibody drug conjugate (ADC) comprising a drug linked to an anti-human Epidermal Growth Factor (hEGFR) antibody by way of a linker, wherein the drug is a Bcl-xL inhibitor according to structural formula (IIa) or (IIb):

##STR00001##

wherein [0014] Ar.sup.1 is selected from

##STR00002##

and is optionally substituted with one or more substituents independently selected from halo, hydroxy, nitro, lower alkyl, lower heteroalkyl, C.sub.1-4alkoxy, amino, cyano and halomethyl;

[0015] Ar.sup.2 is selected from

##STR00003## ##STR00004##

and is optionally substituted with one or more substituents independently selected from halo, hydroxy, nitro, lower alkyl, lower heteroalkyl, C.sub.1-4alkoxy, amino, cyano and halomethyl, wherein the #—N(R.sup.4)—R.sup.13—Z.sup.2b— substituent of formula (IIb) is attached to Ar.sup.2 at any Ar.sup.2 atom capable of being substituted; [0016] Z.sup.1 is selected from N, CH, C-halo and C—CN; [0017] Z.sup.2a, Z.sup.2b, and Z.sup.2c are each, independent from one another, selected from a bond, NR.sup.6, CR.sup.6aR.sup.6b, O, S, S(O), SO.sub.2, NR.sup.6C(O), NR.sup.6aC(O)NR.sup.6b, and NR.sup.6C(O)O; [0018] R.sup.1 is selected from hydrogen, methyl, halo, halomethyl, ethyl and cyano; [0019] R.sup.2 is selected from hydrogen, methyl, halo, halomethyl and cyano; [0020] R.sup.3 is selected from hydrogen, lower alkyl and lower heteroalkyl; [0021] R.sup.4 is selected from hydrogen, lower alkyl, monocyclic cycloalkyl, monocyclic heterocyclyl, and lower heteroalkyl or is taken together with an atom of R.sup.13 to form a cycloalkyl or heterocyclyl ring having between 3 and 7 ring atoms, wherein the lower alkyl, monocyclic cycloalkyl, monocyclic heterocyclyl, and lower heteroalkyl are optionally substituted with one or more halo, cyano, hydroxy, C.sub.1-4alkoxy, monocyclic cycloalkyl, monocyclic heterocyclyl, C(O)NR.sup.6aR.sup.6b, S(O).sub.2NR.sup.6aR.sup.6b, NHC(O)CHR.sup.6aR.sup.6b, NHS(O)CHR.sup.6aR.sup.6b, NHS(O).sub.2CHR.sup.6aR.sup.6b, S(O).sub.2CHR.sup.6aR.sup.6b or S(O).sub.2NH.sub.2 groups; [0022] R.sup.6, R.sup.6a and R.sup.6b are each, independent from one another, selected from hydrogen, lower alkyl, lower heteroalkyl, optionally substituted monocyclic cycloalkyl and monocyclic heterocyclyl, or are taken together with an atom from R.sup.13 to form a cycloalkyl or heterocyclyl ring having between 3 and 7 ring atoms; [0023] R.sup.10 is selected from cyano, OR.sup.14, SR.sup.14, SOR.sup.14, SO.sub.2R.sup.14, SO.sub.2NR.sup.14aR.sup.14b, NR.sup.14aR.sup.14b, NHC(O)R.sup.14 and NHSO.sub.2R.sup.14; [0024] R.sup.1a and R.sup.1b are each, independently of one another, selected from hydrogen, halo, methyl, ethyl, halomethyl, hydroxyl, methoxy, CN, and SCH.sub.3; [0025] R.sup.12 is selected from hydrogen, halo, cyano, lower alkyl, lower heteroalkyl, cycloalkyl, and heterocyclyl, wherein the alkyl, heteroalkyl, cycloalkyl, and heterocyclyl are optionally substituted with one or more halo, cyano, C.sub.1-4alkoxy, monocyclic cycloalkyl, monocyclic heterocyclyl, NHC(O)CHR.sup.6aR.sup.6b, NHS(O)CHR.sup.6aR.sup.6b, NHS(O).sub.2CHR.sup.6aR.sup.6b or S(O).sub.2CHR.sup.6aR.sup.6b groups; [0026] R.sup.13 is selected from a bond, optionally substituted lower alkylene, optionally substituted lower heteroalkylene, optionally substituted cycloalkyl or optionally substituted heterocyclyl; [0027] R.sup.14 is selected from hydrogen, optionally substituted lower alkyl and optionally substituted lower heteroalkyl; [0028] R.sup.14a and R.sup.14b are each, independently of one another, selected from hydrogen, optionally substituted lower alkyl, and optionally substituted lower heteroalkyl, or are taken together with the nitrogen atom to which they are bonded to form an optionally substituted monocyclic cycloalkyl or monocyclic heterocyclyl ring; [0029] R.sup.15 is selected from hydrogen, halo, C.sub.1-6 alkanyl, C.sub.2-4 alkenyl, C.sub.2-4 alkynyl, and C.sub.1-4

haloalkyl and C.sub.1-4 hydroxyalkyl, with the proviso that when R.sup.15 is present, R.sup.4 is not C.sub.1-4 alkyl, C.sub.2-4 alkenyl, C.sub.2-4 alkynyl, C.sub.1-4 haloalkyl or C.sub.1-4 hydroxyalkyl, wherein the R.sup.4 C.sub.1-6 alkanyl, C.sub.2-4 alkenyl, C.sub.2-4 alkynyl, C.sub.1-4 haloalkyl and C.sub.1-4 hydroxyalkyl are optionally substituted with one or more substituents independently selected from OCH.sub.3, OCH.sub.2CH.sub.2OCH.sub.3, and OCH.sub.2CH.sub.2NHCH.sub.3; and [0030] #represents a point of attachment to a linker; and [0031] wherein the anti-hEGFR antibody has the following characteristics: [0032] binds to an epitope within the amino acid sequence CGADSYEMEEDGVRKC (SEQ ID NO: 45) or competes with a second anti-hEGFR antibody for binding to epidermal growth factor receptor variant III (EGFRvIII) (SEQ ID NO: 33) in a competitive binding assay, wherein the second anti-EGFR antibody comprises a heavy chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 1 and a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 5; and [0033] binds to EGFR(1-525) (SEQ ID NO: 47) with a dissociation constant (K.sub.d) of about 1×10^{-6} M or less, as determined by surface plasmon resonance. [0034] In one embodiment, the ADC is a compound according to structural formula (I):

##STR00005##

wherein: [0035] D is the Bcl-xL inhibitor drug of formula (IIa) or (IIb); [0036] L is the linker; [0037] Ab is the anti-hEGFR antibody; [0038] LK represents a covalent linkage linking the linker (L) to the anti-hEGFR antibody (Ab); and [0039] m is an integer ranging from 1 to 20.

[0040] In one embodiment, Ar.sup.1 is unsubstituted.

[0041] In a further embodiment, Ar.sup.1 is

##STR00006##

[0042] In one embodiment, Ar.sup.2 is unsubstituted.

[0043] In a further embodiment, Ar.sup.2 is

##STR00007##

which is optionally substituted at the 5-position with a group selected from hydroxyl, C.sub.1-4 alkoxy, and cyano; or [0044] Ar.sup.2 is

##STR00008##

or [0045] Ar.sup.2 is

or

##STR00009## [0046] Ar.sup.2 is

##STR00010##

[0047] In one embodiment of any one of the aspects and embodiments herein, Z.sup.1 is N.

[0048] In one embodiment of any one of the aspects and embodiments herein, Z.sup.2a is O.

[0049] In one embodiment of any one of the aspects and embodiments herein, R.sup.1 is methyl or chloro.

[0050] In one embodiment of any one of the aspects and embodiments herein, R.sup.2 is hydrogen or methyl.

[0051] In a further embodiment, R.sup.2 is hydrogen.

[0052] In one embodiment of any one of the aspects and embodiments herein, R.sup.4 is hydrogen or lower alkyl, wherein the lower alkyl is optionally substituted with C.sub.1-4 alkoxy or C(O)NR.sup.6aR.sup.6b.

[0053] In one embodiment, Z is N, Z.sup.2a is O, R.sup.1 is methyl or chloro, R.sup.2 is hydrogen, and Ar.sup.2 is

##STR00011##

wherein the

##STR00012##

is optionally substituted at the 5-position with a group selected from hydroxyl, C.sub.1-4 alkoxy, and cyano.

[0054] In a further embodiment, the drug is a Bcl-xL inhibitor according to structural formula (IIa).

[0055] In one embodiment of any one of the aspects and embodiments herein, the drug is a Bcl-xL inhibitor according to structural formula (IIa).

[0056] In a further embodiment, Z.sup.2a is CH.sub.2 or O.

[0057] In another further embodiment, R.sup.13 is selected from lower alkylene or lower heteroalkylene

##STR00013##

is

##STR00014##

[0058] In one embodiment, the group

##STR00015##

is

##STR00016##

[0059] In one embodiment, the group

##STR00017##

is selected from

##STR00018##

[0060] In one embodiment,

##STR00019##

is

##STR00020##

[0061] In one embodiment, Z.sup.2a is oxygen, R.sup.13 is CH.sub.2CH.sub.2, R.sup.4 is hydrogen or lower alkyl optionally substituted with C.sub.1-4 alkoxy or C(O)NR.sup.6aR.sup.6b.

[0062] In one embodiment of any one of the aspects and embodiments herein, the ADC is a compound according to structural formula (IIb).

[0063] In a further embodiment, Z.sup.2b is a bond, O, or NR.sup.6, or and R.sup.13 is ethylene or optionally substituted heterocyclyl.

[0064] In another further embodiment, Z.sup.2c is O and R.sup.12 is lower alkyl optionally substituted with one or more halo or C.sub.1-4alkoxy.

[0065] In one embodiment of the ADC of any one of the aspects and embodiments herein, the Bcl-xL inhibitor is selected from the group consisting of the following compounds modified in that the hydrogen corresponding to the #position of structural formula (IIa) or (IIb) is not present forming a monoradical: [0066] 6-[1-(1,3-benzothiazol-2-ylcarbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl]-3-[1-

({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid; [0067] 6-[4-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-

dihydro-2H-1,4-benzoxazin-6-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl)-5-methyl-1H-pyrazol-4-

yl]pyridine-2-carboxylic acid; [0068] 6-[4-(1,3-benzothiazol-2-ylcarbamoyl)-1-methyl-1,2,3,4-tetrahydroquinoxalin-6-yl]-3-[1-({3,5-dimethyl-7-[2-

(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid; [0069] 3-(1-{[3-(2-aminoethoxy)-5,7-

dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl}-5-methyl-1H-pyrazol-4-yl)-6-[1-(1,3-benzothiazol-2-ylcarbamoyl)-5,6-dihydroimidazo[1,5-a]pyrazin-7(8H)-yl]pyridine-2-carboxylic acid; [0070] 3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl}-5-

methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-hydroxy-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid; [0071] 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)naphthalen-2-yl]-3-[1-({3,5-dimethyl-7-[2-

(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid; [0072] 3-[1-({3,5-dimethyl-7-[2-

(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl]-6-[8-([1,3]thiazolo[5,4-b]pyridin-2-ylcarbamoyl)naphthalen-2-yl]pyridine-2-carboxylic acid; [0073] 3-

[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]-6-[8-([1,3]thiazolo[4,5-b]pyridin-2-ylcarbamoyl)naphthalen-2-yl]pyridine-2-carboxylic acid; [0074] 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.13]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid; [0075] 6-[5-(1,3-benzothiazol-2-ylcarbamoyl)quinolin-3-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid; [0076] 6-[4-(1,3-benzothiazol-2-ylcarbamoyl)quinolin-6-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid; [0077] 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{2-[(2-methoxyethyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid; [0078] 3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-cyano-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid; [0079] 6-[1-(1,3-benzothiazol-2-ylcarbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl]-3-{1-[(3-{2-[(2-methoxyethyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid; [0080] 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)naphthalen-2-yl]-3-{1-[(3-{2-[(2-methoxyethyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid; [0081] 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-[1-({3,5-dimethyl-7-[2-(oxetan-3-ylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid; [0082] 6-[6-(3-aminopyrrolidin-1-yl)-8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[3-(2-methoxyethoxy)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid; [0083] 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3,5-dimethyl-7-{2-[(2-sulfamoyl)ethyl]amino]ethoxy}tricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid; [0084] 3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1.sup.3']dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[3-(1,3-benzothiazol-2-ylcarbamoyl)-6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl]pyridine-2-carboxylic acid; [0085] 3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1.sup.3']dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[1-(1,3-benzothiazol-2-ylcarbamoyl)-3-(trifluoromethyl)-5,6-dihydroimidazo[1,5-a]pyrazin-7(8H)-yl]pyridine-2-carboxylic acid; [0086] 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-6-{methyl[2-(methylamino)ethyl]amino}-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[3-(2-methoxyethoxy)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid; [0087] 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-6-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid; [0088] 3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[4-(1,3-benzothiazol-2-ylcarbamoyl)quinolin-6-yl]pyridine-2-carboxylic acid; [0089] 6-[5-amino-8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid; [0090] 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-6-[3-(methylamino)prop-1-yn-1-yl]-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[3-(2-methoxyethoxy)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid; [0091] 6-[4-(1,3-benzothiazol-2-ylcarbamoyl)isoquinolin-6-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid; [0092] 6-[7-(1,3-benzothiazol-2-ylcarbamoyl)-1H-indol-2-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-

pyrazol-4-yl]pyridine-2-carboxylic acid; [0093] 3-(1-([3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl)-6-[7-(1,3-benzothiazol-2-ylcarbamoyl)-1H-indol-2-yl]pyridine-2-carboxylic acid; [0094] 6-[7-(1,3-benzothiazol-2-ylcarbamoyl)-3-methyl-1H-indol-2-yl]-3-[1-([3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid; [0095] 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-([3,5-dimethyl-7-(2-[1-(methylsulfonyl)piperidin-4-yl]amino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid; [0096] 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-([3,5-dimethyl-7-(2-[1-(methylsulfonyl)azetidin-3-yl]amino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid; [0097] 3-{1-[(3-{2-[(3-amino-3-oxopropyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid; [0098] 6-[3-(1,3-benzothiazol-2-ylcarbamoyl)-1H-indazol-5-yl]-3-[1-([3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid; [0099] 6-[3-(1,3-benzothiazol-2-ylcarbamoyl)-1H-indol-5-yl]-3-[1-([3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid; [0100] 6-[3-(1,3-benzothiazol-2-ylcarbamoyl)-1H-pyrrolo[2,3-b]pyridin-5-yl]-3-[1-([3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid; [0101] 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-((3-(2-((2-(N,N-dimethylsulfamoyl)ethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid; [0102] 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)naphthalen-2-yl]-3-{1-[(3-{2-[(3-hydroxypropyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid; [0103] 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-([3-(2-([3-(dimethylamino)-3-oxopropyl]amino)ethoxy)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid; [0104] 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-([3,5-dimethyl-7-(2-([3-(methylamino)-3-oxopropyl]amino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid; [0105] 3-(1-([3-(2-aminoacetamido)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]decan-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl)-6-{8-[(1,3-benzothiazol-2-yl)carbamoyl]-3,4-dihydroisoquinolin-2(1H)-yl}pyridine-2-carboxylic acid; [0106] 3-[1-([3-((2-aminoethyl)sulfanyl)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl]-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid; [0107] 3-(1-([3-(3-aminopropyl)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid; and [0108] 3-(1-([3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1.sup.3']decan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-{5-[(1,3-benzothiazol-2-yl)carbamoyl]quinolin-3-yl}pyridine-2-carboxylic acid.


[0109] In one embodiment of any one of the aspects and embodiments herein, the linker is cleavable by a lysosomal enzyme.

[0110] In a further embodiment, the lysosomal enzyme is Cathepsin B.

[0111] In one embodiment of any one of the aspects and embodiments herein, the linker comprises a segment according to structural formula (IVa), (IVb), (IVc), or (IVd):

##STR00021##

wherein: [0112] peptide represents a peptide (illustrated N—C, wherein peptide includes the amino and carboxy “termini”) a cleavable by a lysosomal enzyme; [0113] T represents a polymer


comprising one or more ethylene glycol units or an alkylene chain, or combinations thereof; [0114] R.sup.a is selected from hydrogen, C.sub.1-6alkyl, SO.sub.3H and CH.sub.2SO.sub.3H; [0115] R.sup.y is hydrogen or C.sub.1-4 alkyl-(O).sub.r—(C.sub.1-4 alkylene).sub.s-G.sup.1 or C.sub.1-4 alkyl-(N)—[(C.sub.1-4 alkylene)-G.sup.1].sub.2; [0116] R.sup.z is C.sub.1-4 alkyl-(O).sub.r—(C.sub.1-4 alkylene).sub.s-G.sup.2; [0117] G.sup.1 is SO.sub.3H, CO.sub.2H, PEG 4-32, or sugar moiety; [0118] G.sup.2 is SO.sub.3H, CO.sub.2H, or PEG 4-32 moiety; [0119] r is 0 or 1; [0120] s is 0 or 1; [0121] p is an integer ranging from 0 to 5; [0122] q is 0 or 1; [0123] x is 0 or 1; [0124] y is 0 or 1; [0125]  represents the point of attachment of the linker to the Bcl-xL inhibitor; and [0126] * represents the point of attachment to the remainder of the linker.

[0127] In a further embodiment, the peptide is selected from the group consisting of Val-Cit; Cit-Val; Ala-Ala; Ala-Cit; Cit-Ala; Asn-Cit; Cit-Asn; Cit-Cit; Val-Glu; Glu-Val; Ser-Cit; Cit-Ser; Lys-Cit; Cit-Lys; Asp-Cit; Cit-Asp; Ala-Val; Val-Ala; Phe-Lys; Lys-Phe; Val-Lys; Lys-Val; Ala-Lys; Lys-Ala; Phe-Cit; Cit-Phe; Leu-Cit; Cit-Leu; Ile-Cit; Cit-Ile; Phe-Arg; Arg-Phe; Cit-Trp; and Trp-Cit.

[0128] In another further embodiment, the lysosomal enzyme is β -glucuronidase or β -galactosidase.

[0129] In one embodiment of any one of the aspects and embodiments herein, the linker comprises a segment according to structural formula (Va), (Vb), (Vc), (Vd), or (Ve):

##STR00022##

wherein: [0130] q is 0 or 1; [0131] r is 0 or 1; [0132] X.sup.1 is CH.sub.2, O or NH; [0133]  represents the point of attachment of the linker to the drug; and [0134] * represents the point of attachment to the remainder of the linker.



[0135] In one embodiment of any one of the aspects and embodiments herein, the linker comprises a segment according to structural formula (VIIIa), (VIIIb), or (VIIIc):

##STR00023## ##STR00024##

or a hydrolyzed derivative thereof, wherein: [0136] R.sup.q is H or —O—

(CH.sub.2CH.sub.2O).sub.11—CH.sub.3; [0137] x is 0 or 1; [0138] y is 0 or 1; [0139] G.sup.3 is —CH.sub.2CH.sub.2CH.sub.2SO.sub.3H or —CH.sub.2CH.sub.2O—

(CH.sub.2CH.sub.2O).sub.11—CH.sub.3; [0140] R.sup.w is —O—CH.sub.2CH.sub.2SO.sub.3H or —NH(CO)—CH.sub.2CH.sub.2O—(CH.sub.2CH.sub.2O).sub.2—CH.sub.3; [0141] *

represents the point of attachment to the remainder of the linker; and [0142]  represents the point of attachment of the linker to the antibody, wherein when in the hydrolyzed form,  can be either at the α -position or β -position of the carboxylic acid next to it.

[0143] In one embodiment of any one of the aspects and embodiments herein, the linker comprises a polyethylene glycol segment having from 1 to 6 ethylene glycol units.

[0144] In one embodiment of any one of the aspects and embodiments herein, m is 2, 3 or 4.


[0145] In a further embodiment, the linker L comprises a segment according to structural formula (IVa) or (IVb).

[0146] In one embodiment of any one of the aspects and embodiments herein, the linker L is selected from the group consisting of IVa.1-IVa.8, IVb.1-IVb.19, IVc.1-IVc.7, IVd.1-IVd.4, Va.1-Va.12, Vb.1-Vb.10, Vc.1-Vc.11, Vd.1-Vd.6, Ve.1-Ve.2, VIa.1, VIc.1-VIc.2, VId.1-VId.4, VIIa.1-VIIa.4, VIIb.1-VIIb.8, VIIc.1-VIIc.6 in either the closed or open form.

[0147] In one embodiment of any one of the aspects and embodiments herein, the linker L is selected from the group consisting of IVb.2, IVc.5, IVc.6, IVc.7, IVd.4, Vb.9, Vc.11, VIIa.1, VIIa.3, VIIc.1, VIIc.4, and VIIc.5, wherein the maleimide of each linker has reacted with the antibody Ab, forming a covalent attachment as either a succinimide (closed form) or succinamide (open form).

[0148] In one embodiment of any one of the aspects and embodiments herein, the linker L is selected from the group consisting of IVb.2, IVc.5, IVc.6, IVd.4, Vc.11, VIIa.1, VIIa.3, VIIc.1,

VIIc.4, VIIc.5, wherein the maleimide of each linker has reacted with the antibody Ab, forming a covalent attachment as either a succinimide (closed form) or succinamide (open form).

[0149] In one embodiment of any one of the aspects and embodiments herein, the linker L is selected from the group consisting of IVb.2, Vc.11, VIIa.3, IVc.6, and VIIc.1, wherein  custom-character is the attachment point to drug D and @ is the attachment point to the LK, wherein when the linker is in the open form as shown below, @ can be either at the α -position or β -position of the carboxylic acid next to it:

##STR00025## ##STR00026## ##STR00027##

[0150] In one embodiment of any one of the aspects and embodiments herein, LK is a linkage formed with an amino group on the anti-hEGFR antibody Ab.

[0151] In a further embodiment, LK is an amide or a thiourea.

[0152] In one embodiment of any one of the aspects and embodiments herein, LK is a linkage formed with a sulfhydryl group on the anti-hEGFR antibody Ab.

[0153] In a further embodiment, LK is a thioether.

[0154] In one embodiment of any one of the aspects and embodiments herein, LK is selected from the group consisting of amide, thiourea and thioether; and m is an integer ranging from 1 to 8.

[0155] In one embodiment of any one of the aspects and embodiments herein, D is the Bcl-xL inhibitor as defined in the aspects and embodiments herein; L is selected from the group consisting of linkers IVa.1-IVa.8, IVb.1-IVb.19, IVc.1-IVc.7, IVd.1-IVd.4, Va.1-Va.12, Vb.1-Vb.10, Vc.1-Vc.11, Vd.1-Vd.6, Ve.1-Ve.2, VIa.1, VIc.1-VIc.2, VID.1-VID.4, VIIa.1-VIIa.4, VIIb.1-VIIb.8, and VIIc.1-VIIc.6, wherein each linker has reacted with the antibody, Ab, forming a covalent attachment; LK is thioether; and m is an integer ranging from 1 to 8.

[0156] In one embodiment of any one of the aspects and embodiments herein, D is the Bcl-xL inhibitor selected from the group consisting of the following compounds modified in that the hydrogen corresponding to the #position of structural formula (IIa) or (IIb) is not present, forming a monoradical:

[0157] 3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[1-(1,3-benzothiazol-2-ylcarbamoyl)-5,6-dihydroimidazo[1,5-a]pyrazin-7(8H)-yl]pyridine-2-carboxylic acid; [0158] 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)naphthalen-2-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid; [0159] 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{2-[(2-methoxyethyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.13]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid; [0160] 3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-cyano-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid; [0161] 6-[4-(1,3-benzothiazol-2-ylcarbamoyl)isoquinolin-6-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid; and [0162] 3-{1-[(3-{2-[(3-amino-3-oxopropyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1.sup.3°]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid; [0163] L is selected from the group consisting of linkers IVb.2, IVc.5, IVc.6, IVc.7, IVd.4, Vb.9, Vc.11, VIIa.1, VIIa.3, VIIc.1, VIIc.4, and VIIc.5 in either closed or open forms; [0164] LK is thioether; and [0165] m is an integer ranging from 2 to 4.

[0166] In one embodiment, the ADC of any one of the aspects and embodiments herein is selected from the group consisting of AbA-ZT, AbA-ZZ, AbA-XW, AbA-SE, AbA-SR, AbA-YG, AbA-KZ, AbB-ZT, AbB-ZZ, AbB-XW, AbB-SE, AbB-SR, AbB-YG, AbB-KZ, AbG-ZT, AbG-ZZ, AbG-XW, AbG-SE, AbG-SR, AbG-YG, AbG-KZ, AbK-ZT, AbK-ZZ, AbK-XW, AbK-SE, AbK-SR, AbK-YG, and AbK-KZ, wherein KZ, SR, SE, XW, YG, ZT and ZZ are synthons disclosed in Table 5, and wherein the synthons are either in open or closed form.

[0167] In one embodiment, the ADC of any one of the aspects and embodiments herein is selected

from the group consisting of AbA-ZT, AbA-ZZ, AbA-SE, AbA-SR, AbB-ZT, AbB-ZZ, AbB-SE, AbB-SR, AbG-ZT, AbG-ZZ, AbG-SE, AbG-SR, AbK-ZT, AbK-ZZ, AbK-SE, AbK-SR, wherein AbA, AbB, AbG, and AbK are the anti-hEGFR antibodies and KZ, SR, SE, XW, YG, ZT and ZZ are synthons disclosed in Table 5, and wherein the synthons are either in open or closed form. [0168] In one embodiment, the ADC of any one of the aspects and embodiments herein is selected from the group consisting of formulae i-xiv:

##STR00028## ##STR00029## ##STR00030## ##STR00031## ##STR00032## ##STR00033## ##STR00034##

wherein m is an integer from 1 to 6. In a specific embodiment, m is 2. In a specific embodiment, Ab is the hEGFR antibody, wherein the hEGFR antibody comprises the heavy and light chain CDRs of AbA. In other embodiments, the hEGFR ADC comprises an antibody comprising a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 12, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 11, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 10; and a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 8, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 7, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 6. In yet another embodiment, the hEGFR ADC comprises an antibody comprising a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 9, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 5. In other embodiments, the hEGFR ADC comprises an antibody comprising a heavy chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 41 and/or a light chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 43. In a further embodiment, the hEGFR ADC comprises an antibody comprising a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 15, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 13. In a further embodiment, the hEGFR ADC comprises an antibody comprising a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 102, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 13. In another specific embodiment, Ab is the hEGFR antibody, wherein the hEGFR antibody comprises the heavy and light chain CDRs of AbG. In other embodiments, the hEGFR ADC comprises an antibody comprising a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 18, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 17, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 16; and a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 25, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 24, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 23. In yet another embodiment, the hEGFR ADC comprises an antibody comprising a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 72, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 73. In other embodiments, the hEGFR ADC comprises an antibody comprising a heavy chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 41 and/or a light chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 43. In a further embodiment, the hEGFR ADC comprises an antibody comprising a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 93, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 95. In a further embodiment, the hEGFR ADC comprises an antibody comprising a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 94, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 95.

[0169] In a further embodiment, m is an integer from 2 to 6.

[0170] In one embodiment of any one of the aspects and embodiments herein, the antibody binds to EGFR (1-525) (SEQ ID NO: 47) with a $K_{\text{sub.d}}$ of between about 1×10^{-6} M and about

1×10^{sup.}-10 M, as determined by surface plasmon resonance.

[0171] In one embodiment of any one of the aspects and embodiments herein, the antibody binds to EGFR (1-525) (SEQ ID NO: 47) with a K_{sub.d} of between about 1×10^{sup.}-6 M and about 1×10^{sup.}-7 M, as determined by surface plasmon resonance.

[0172] In one embodiment of any one of the aspects and embodiments herein, the antibody binds to EGFRvIII (SEQ ID NO: 33) with a K_{sub.d} of about 8.2×10^{sup.}-9 M or less, as determined by surface plasmon resonance.

[0173] In one embodiment of any one of the aspects and embodiments herein, the antibody binds to EGFRvIII (SEQ ID NO: 33) with a K_{sub.d} of between about 8.2×10^{sup.}-9 M and about 6.3×10^{sup.}-10 M, as determined by surface plasmon resonance.

[0174] In one embodiment of any one of the aspects and embodiments herein, the antibody binds to EGFRvIII (SEQ ID NO: 33) with a K_{sub.d} of between about 8.2×10^{sup.}-9 M and about 2.0×10^{sup.}-9 M, as determined by surface plasmon resonance.

[0175] In one embodiment of any one of the aspects and embodiments herein, the anti-hEGFR antibody comprises a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 12, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 11, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 10; a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 8, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 7, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 6;

[0176] In one embodiment of any one of the aspects and embodiments herein, the antibody comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 9, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 5.

[0177] In one embodiment of any one of the aspects and embodiments herein, the antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 15, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 13.

[0178] In one embodiment of any one of the aspects and embodiments herein, the antibody comprises a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 40, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 39, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 38; and a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 37, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 36, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 35.

[0179] In one embodiment of any one of the aspects and embodiments herein, the antibody comprises a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID Nos: 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, and 78; and a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID Nos: 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, and 79.

[0180] In one embodiment of any one of the aspects and embodiments herein, the antibody comprises a heavy chain CDR set (CDR1, CDR2, and CDR3) selected from the group consisting of SEQ ID NOs: 10, 11, and 12; SEQ ID NOs: 16, 17, and 18; SEQ ID NOs: 10, 11, and 19; SEQ ID NOs: 20, 11, and 12; SEQ ID NOs: 21, 3, and 22; SEQ ID NOs: 16, 17, and 19; SEQ ID NOs: 2, 3, and 4; SEQ ID NOs: 10, 3, and 12; SEQ ID NOs: 80, 11, and 18; SEQ ID NOs: 80, 3, and 18; SEQ ID NOs: 20, 3, and 12; SEQ ID NOs: 80, 11, and 12; and SEQ ID NOs: 81, 11, and 22; and a light chain CDR set (CDR1, CDR2, and CDR3) selected from the group consisting of SEQ ID NOs: 6, 7, and 8; SEQ ID NOs: 23, 24, and 25; SEQ ID NOs: 26, 27, and 28; SEQ ID NOs: 29, 30, and 31; SEQ ID NOs: 6, 7, and 84; SEQ ID NOs: 82, 83, and 31; and SEQ ID NOs: 82, 27, and 85, wherein the antibody does not comprise both the heavy chain CDR set of SEQ ID NOs: 2, 3, and 4,

and the light chain CDR set of SEQ ID NOs: 6, 7, and 8.

[0181] In one embodiment of any one of the aspects and embodiments herein, the antibody comprises a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 8, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 7, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 6; and a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 19, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 17, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 16.

[0182] In one embodiment of any one of the aspects and embodiments herein, the antibody comprises a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 25, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 24, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 23; and a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 18, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 17, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 16.

[0183] In one embodiment of any one of the aspects and embodiments herein, the antibody comprises a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 28, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 27, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 26; and a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 19, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 11, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 10.

[0184] In one embodiment of any one of the aspects and embodiments herein, the antibody comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 64, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 65.

[0185] In one embodiment of any one of the aspects and embodiments herein, the antibody comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 72, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 73.

[0186] In one embodiment of any one of the aspects and embodiments herein, the antibody comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 74, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 75.

[0187] In one embodiment of any one of the aspects and embodiments herein, the antibody is an monoclonal IgG antibody.

[0188] In a further embodiment, the antibody is an IgG1 antibody.

[0189] In another further embodiment, the light chain is a lambda light chain or a kappa light chain.

[0190] In another aspect, the invention features a pharmaceutical composition comprising an effective amount of an ADC according to any one of the aspects and embodiments herein, and a pharmaceutically acceptable carrier.

[0191] In another aspect, the invention features a pharmaceutical composition comprising an ADC mixture comprising a plurality of the ADC of any one of the aspects and embodiments herein, and a pharmaceutically acceptable carrier.

[0192] In one embodiment, the ADC mixture has an average drug to antibody ratio (DAR) of 2 to 4.

[0193] In another embodiment, the ADC mixture comprises ADCs each having a DAR of 2 to 8.

[0194] In another aspect, the invention features a method for treating cancer, comprising administering a therapeutically effective amount of the ADC of any one of the aspects and embodiments herein to a subject in need thereof.

[0195] In one embodiment, the cancer is selected from the group consisting of non small cell lung cancer, breast cancer, ovarian cancer, a glioblastoma, prostate cancer, pancreatic cancer, colon cancer, head and neck cancer, and kidney cancer.

[0196] In another embodiment, the cancer is a squamous cell carcinoma. In a further embodiment, the squamous cell carcinoma is squamous lung cancer or squamous head and neck cancer.

[0197] In another embodiment, the cancer is triple negative breast cancer.

[0198] In another embodiment, the cancer is non-small cell lung cancer. In a further embodiment, the ADC is administered with taxane.

[0199] In one embodiment of any one of the aspects and embodiments herein, the cancer is characterized as having EGFR expression, or as being EGFRvIII positive.

[0200] In one embodiment of any one of the aspects and embodiments herein, the cancer is characterized as having EGFR overexpression or EGFR amplification.

[0201] In another aspect, the invention features a method for inhibiting or decreasing solid tumor growth in a subject having a solid tumor, said method comprising administering an effective amount of the ADC of any one of the aspects and embodiments herein to the subject having the solid tumor, such that the solid tumor growth is inhibited or decreased.

[0202] In one embodiment, the solid tumor is selected from the group consisting of non-small cell lung carcinoma, breast cancer, ovarian cancer, and glioblastoma.

[0203] In another embodiment the solid tumor is a squamous cell carcinoma.

[0204] In one embodiment of any one of the aspects and embodiments herein, the solid tumor is an EGFRvIII positive solid tumor, is a solid tumor characterized as having EGFR amplification, or is a solid tumor characterized as having EGFR overexpression.

[0205] In one embodiment, the cancer is characterized as having an activating EGFR mutation.

[0206] In a further embodiment, the EGFR mutation is selected from the group consisting of an exon 19 deletion mutation, a single-point substitution mutation L858R in exon 21, a T790M point mutation, and combinations thereof.

[0207] In one embodiment of any one of the aspects and embodiments herein, the ADC is administered in combination with an additional agent or an additional therapy.

[0208] In a further embodiment, the additional agent is selected from the group consisting of an anti-PD1 antibody (e.g. pembrolizumab), an anti-PD-L1 antibody (e.g. atezolizunab), an anti-CTLA-4 antibody (e.g. ipilimumab), a MEK inhibitor (e.g. trametinib), an ERK inhibitor, a BRAF inhibitor (e.g. dabrafenib), osimertinib, erlotinib, gefitinib, sorafenib, a CDK9 inhibitor (e.g. dinaciclib), a MCL-1 inhibitor, temozolomide, a Bcl-xL inhibitor, a Bel-2 inhibitor (e.g. venetoclax), ibrutinib, a mTOR inhibitor (e.g. everolimus), a PI3K inhibitor (e.g. buparlisib), duvelisib, idelalisib, an AKT inhibitor, a HER2 inhibitor (e.g. lapatinib), a taxane (e.g. docetaxel, paclitaxel, nab-paclitaxel), an ADC comprising an auristatin, an ADC comprising a PBD (e.g. rovalpituzumab tesirine), an ADC comprising a maytansinoid (e.g. TDM1), a TRAIL agonist, a proteasome inhibitor (e.g. bortezomib), and a nicotinamide phosphoribosyltransferase (NAMPT) inhibitor.

[0209] In another further embodiment, the additional therapy is radiation.

[0210] In another further embodiment, the additional agent is a chemotherapeutic agent.

[0211] In another aspect, the invention features a process for the preparation of an ADC according to structural formula (I):

##STR00035##

wherein: [0212] D is the Bcl-xL inhibitor drug of formula (IIa) or (IIb) as disclosed herein; [0213] L is the linker as disclosed herein; [0214] Ab is an hEGFR antibody, wherein the hEGFR antibody comprises the heavy and light chain CDRs of AbA; AbB; AbG; or AbK; [0215] LK represents a covalent linkage linking linker L to antibody Ab; and [0216] m is an integer ranging from 1 to 20; [0217] the process comprising: [0218] treating an antibody in an aqueous solution with an effective amount of a disulfide reducing agent at 30-40° C. for at least 15 minutes, and then cooling the

antibody solution to 20-27° C.; [0219] adding to the reduced antibody solution a solution of water/dimethyl sulfoxide comprising a synthon selected from the group of 2.1 to 2.31 and 2.34 to 2.72 (Table 5); [0220] adjusting the pH of the solution to a pH of 7.5 to 8.5; [0221] allowing the reaction to run for 48 to 80 hours to form the ADC; [0222] wherein the mass is shifted by 18±2 amu for each hydrolysis of a succinimide to a succinamide as measured by electron spray mass spectrometry; and [0223] wherein the ADC is optionally purified by hydrophobic interaction chromatography.

[0224] In one embodiment, m is 2.

[0225] In another aspect, the invention features an ADC prepared by the process as described above.

Description

BRIEF DESCRIPTION OF THE DRAWINGS

[0226] FIG. 1 shows a schematic of EGFR and the regions bound by Ab1 and Ab2.

[0227] FIG. 2 provides the variable heavy (VH) and variable light (VL) chain region amino acid sequences of Ab1 (SEQ ID NOs: 1 and 5) and AbA (SEQ ID NOs: 9 and 5). CDR sequences within the VH and VL regions are boxed, and differences between the Ab1 VH sequence and the AbA VH sequence are shaded.

[0228] FIG. 3 describes the full length light and heavy chains for Ab1 (SEQ ID NOs: 13 and 14) and AbA (SEQ ID NOs: 13 and 15). Differences between the Ab1 sequence and the AbA sequence in the heavy chain are highlighted.

[0229] FIG. 4 shows a representation of antibody reduction, modification with a maleimide derivative to give a thiosuccinimide intermediate, and subsequent hydrolysis of thiosuccinimide moiety.

[0230] FIG. 5 shows mass spectrometry (MS) characterization of light chain and heavy chain of an exemplary antibody 1) prior to conjugation, 2) after conjugation to a maleimide derivative to give a thiosuccinimide intermediate and 3) post pH8-mediated hydrolysis of the thiosuccinimide ring.

DETAILED DESCRIPTION OF THE INVENTION

[0231] Numerous Bcl-xL inhibitors have been developed for treatment of diseases (e.g., cancer) that involve dysregulated apoptotic pathways. However, Bcl-xL inhibitors can act on cells other than the target cells (e.g., cancer cells). For instance, pre-clinical studies have shown that pharmacological inactivation of Bcl-xL reduces platelet half-life and causes thrombocytopenia (see Mason et al., 2007, Cell 128:1173-1186).

[0232] Given the importance of Bcl-xL in regulating apoptosis, there remains a need in the art for agents that inhibit Bcl-xL activity, either selectively or non-selectively, as an approach towards the treatment of diseases in which apoptosis is dysregulated via expression or over-expression of anti-apoptotic Bel-2 family proteins, such as Bcl-xL. Accordingly, new Bcl-xL inhibitors with reduced dose-limiting toxicity are needed.

[0233] One potential means of delivering a drug to a cell which has not been explored for Bcl-xL inhibitors is delivery through the use of antibody drug conjugates (ADCs). Antibody drug conjugates (ADC) represent a new class of therapeutics comprising an antibody conjugated to a cytotoxic drug via a chemical linker. The therapeutic concept of ADCs is to combine binding capabilities of an antibody with a drug, where the antibody is used to deliver the drug to a tumor cell by means of binding to a target surface antigen.

[0234] Accordingly, the development of new ADCs that can selectively deliver Bcl-xL to target cancer cells, e.g., EGFRvIII expressing cells, would be a significant discovery.

[0235] Various aspects of the invention relate to new anti-EGFR antibody drug conjugates (ADCs; also called immunoconjugates), and pharmaceutical compositions thereof. In particular, the present

disclosure concerns new anti-EGFR ADCs comprising Bcl-xL inhibitors, synthons useful for synthesizing the ADCs, compositions comprising the ADCs, methods of making the ADCs, and various methods of using the ADCs.

[0236] As will be appreciated by skilled artisans, the ADCs disclosed herein are “modular” in nature. Throughout the instant disclosure, various specific embodiments of the various “modules” comprising the ADCs, as well as the synthons useful for synthesizing the ADCs, are described. As specific non-limiting examples, specific embodiments of antibodies, linkers, and Bcl-xL inhibitors that may comprise the ADCs and synthons are described. It is intended that all of the specific embodiments described may be combined with each other as though each specific combination were explicitly described individually.

[0237] It will also be appreciated by skilled artisans that the various Bcl-xL inhibitors, ADCs and/or ADC synthons described herein may be in the form of salts, and in certain embodiments, particularly pharmaceutically acceptable salts. The compounds of the present disclosure that possess a sufficiently acidic, a sufficiently basic, or both functional groups, can react with any of a number of inorganic bases, and inorganic and organic acids, to form a salt. Alternatively, compounds that are inherently charged, such as those with a quaternary nitrogen, can form a salt with an appropriate counterion, e.g., a halide such as a bromide, chloride, or fluoride.

[0238] Acids commonly employed to form acid addition salts are inorganic acids such as hydrochloric acid, hydrobromic acid, hydroiodic acid, sulfuric acid, phosphoric acid, and the like, and organic acids such as p-toluenesulfonic acid, methanesulfonic acid, oxalic acid, p-bromophenyl-sulfonic acid, carbonic acid, succinic acid, citric acid, etc. Base addition salts include those derived from inorganic bases, such as ammonium and alkali or alkaline earth metal hydroxides, carbonates, bicarbonates, and the like.

[0239] In the disclosure below, if both structural diagrams and nomenclature are included and if the nomenclature conflicts with the structural diagram, the structural diagram controls.

1. Definitions

[0240] In order that the invention may be more readily understood, certain terms are first defined. In addition, it should be noted that whenever a value or range of values of a parameter are recited, it is intended that values and ranges intermediate to the recited values are also intended to be part of this invention. Further, unless otherwise defined herein, scientific and technical terms used in connection with the present disclosure shall have the meanings that are commonly understood by those of ordinary skill in the art.

[0241] Various chemical substituents are defined below. In some instances, the number of carbon atoms in a substituent (e.g., alkyl, alkanyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, heteroaryl, and aryl) is indicated by the prefix “C.sub.x-C.sub.y” or “C.sub.x-y” wherein x is the minimum and y is the maximum number of carbon atoms. Thus, for example, “C.sub.1-C.sub.6 alkyl” refers to an alkyl containing from 1 to 6 carbon atoms. Illustrating further, “C.sub.3-C.sub.8 cycloalkyl” means a saturated hydrocarbon ring containing from 3 to 8 carbon ring atoms. If a substituent is described as being “substituted,” a hydrogen atom on a carbon or nitrogen is replaced with a non-hydrogen group. For example, a substituted alkyl substituent is an alkyl substituent in which at least one hydrogen atom on the alkyl is replaced with a non-hydrogen group. To illustrate, monofluoroalkyl is alkyl substituted with a fluoro radical, and difluoroalkyl is alkyl substituted with two fluoro radicals. It should be recognized that if there is more than one substitution on a substituent, each substitution may be identical or different (unless otherwise stated). If a substituent is described as being “optionally substituted”, the substituent may be either (1) not substituted or (2) substituted. Possible substituents include, but are not limited to, C.sub.1-C.sub.6 alkyl, C.sub.2-C.sub.6 alkenyl, C.sub.2-C.sub.6 alkynyl, aryl, cycloalkyl, heterocyclyl, heteroaryl, halogen, C.sub.1-C.sub.6 haloalkyl, oxo, —CN, NO.sub.2, —OR.sup.xa, —OC(O)R.sup.xz, —OC(O)N(R.sup.xa).sub.2, —SR.sup.xa, —S(O).sub.2R.sup.xa, —S(O).sub.2N(R.sup.xa).sub.2, —C(O)R.sup.xa, —C(O)OR.sup.xa, —C(O)N(R.sup.xa).sub.2, —

$\text{C(O)N(R.sup.xa)S(O).sub.2R.sup.xz}$, $\text{—N(R.sup.xa).sub.2}$, $\text{—N(R.sup.xa)C(O)R.sup.xz}$, $\text{—N(R.sup.xa)S(O).sub.2R.sup.x}$, $\text{—N(R.sup.xa)C(O)O(R.sup.x)}$, $\text{—N(R.sup.xa)C(O)N(R.sup.xa).sub.2}$, $\text{—N(R.sup.xa)S(O).sub.2N(R.sup.xa).sub.2}$, $\text{—(C.sub.1-C.sub.6 alkylenyl)-CN}$, $\text{—(C.sub.1-C.sub.6 alkylenyl)-OR.sup.xa}$, $\text{—(C.sub.1-C.sub.6 alkylenyl)-OC(O)R.sup.xz}$, $\text{—(C.sub.1-C.sub.6 alkylenyl)-OC(O)N(R.sup.xa).sub.2}$, $\text{—(C.sub.1-C.sub.6 alkylenyl)-SR.sup.xa}$, $\text{—(C.sub.1-C.sub.6 alkylenyl)-S(O).sub.2R.sup.xa}$, $\text{—(C.sub.1-C.sub.6 alkylenyl)-S(O).sub.2N(R.sup.xa).sub.2}$, $\text{—(C.sub.1-C.sub.6 alkylenyl)-C(O)R.sup.xa}$, $\text{—(C.sub.1-C.sub.6 alkylenyl)-C(O)OR.sup.xa}$, $\text{—(C.sub.1-C.sub.6 alkylenyl)-C(O)N(R.sup.xa).sub.2}$, $\text{—(C.sub.1-C.sub.6 alkylenyl)-C(O)N(R.sup.xa)S(O).sub.2R.sup.x}$, $\text{—(C.sub.1-C.sub.6 alkylenyl)-N(R.sup.xa).sub.2}$, $\text{—(C.sub.1-C.sub.6 alkylenyl)-N(R.sup.xa)C(O)R.sup.xz}$, $\text{—(C.sub.1-C.sub.6 alkylenyl)-N(R.sup.xa)S(O).sub.2R.sup.x}$, $\text{—(C.sub.1-C.sub.6 alkylenyl)-N(R.sup.xa)C(O)O(R.sup.x)}$ — $\text{(C.sub.1-C.sub.6 alkylenyl)-N(R.sup.xa)C(O)N(R.sup.xa).sub.2}$, or $\text{—(C.sub.1-C.sub.6 alkylenyl)-N(R.sup.xa)S(O).sub.2N(R.sup.xa).sub.2}$; wherein R.sup.xa, at each occurrence, is independently hydrogen, aryl, cycloalkyl, heterocyclyl, heteroaryl, C.sub.1-C.sub.6 alkyl, or C.sub.1-C.sub.6 haloalkyl; and R.sup.xz, at each occurrence, is independently aryl, cycloalkyl, heterocyclyl, heteroaryl, C.sub.1-C.sub.6 alkyl or C1-C.sub.6 haloalkyl.

[0242] Various ADCs, synthons and Bcl-xL inhibitors comprising the ADCs and/or synthons are described in some embodiments herein by reference to structural formulae including substituents. It is to be understood that the various groups comprising substituents may be combined as valence and stability permit. Combinations of substituents and variables envisioned by this disclosure are only those that result in the formation of stable compounds. As used herein, the term “stable” refers to compounds that possess stability sufficient to allow manufacture and that maintain the integrity of the compound for a sufficient period of time to be useful for the purpose detailed herein.

[0243] As used herein, the following terms are intended to have the following meanings: The term “alkoxy” refers to a group of the formula —OR.sup.xa , where R.sup.xa is an alkyl group.

[0244] Representative alkoxy groups include methoxy, ethoxy, propoxy, tert-butoxy and the like.

[0245] The term “alkoxyalkyl” refers to an alkyl group substituted with an alkoxy group and may be represented by the general formula —R.sup.bOR.sup.xa where R.sup.b is an alkylene group and R.sup.xa is an alkyl group.

[0246] The term “alkyl” by itself or as part of another substituent refers to a saturated or unsaturated branched, straight-chain or cyclic monovalent hydrocarbon radical that is derived by the removal of one hydrogen atom from a single carbon atom of a parent alkane, alkene or alkyne. Typical alkyl groups include, but are not limited to, methyl; ethyls such as ethanyl, ethenyl, ethynyl; propyls such as propan-1-yl, propan-2-yl, cyclopropan-1-yl, prop-1-en-1-yl, prop-1-en-2-yl, prop-2-en-1-yl, cycloprop-1-en-1-yl; cycloprop-2-en-1-yl, prop-1-yn-1-yl, prop-2-yn-1-yl, etc.; butyls such as butan-1-yl, butan-2-yl, 2-methyl-propan-1-yl, 2-methyl-propan-2-yl, cyclobutan-1-yl, but-1-en-1-yl, but-1-en-2-yl, 2-methyl-prop-1-en-1-yl, but-2-en-1-yl, but-2-en-2-yl, buta-1,3-dien-1-yl, buta-1,3-dien-2-yl, cyclobut-1-en-1-yl, cyclobut-1-en-3-yl, cyclobuta-1,3-dien-1-yl, but-1-yn-1-yl, but-1-yn-3-yl, but-3-yn-1-yl, etc.; and the like. Where specific levels of saturation are intended, the nomenclature “alkanyl,” “alkenyl” and/or “alkynyl” are used, as defined below. The term “lower alkyl” refers to alkyl groups with 1 to 6 carbons.

[0247] The term “alkanyl” by itself or as part of another substituent refers to a saturated branched, straight-chain or cyclic alkyl derived by the removal of one hydrogen atom from a single carbon atom of a parent alkane. Typical alkanyl groups include, but are not limited to, methyl; ethanyl; propanyls such as propan-1-yl, propan-2-yl (isopropyl), cyclopropan-1-yl, etc.; butanyls such as butan-1-yl, butan-2-yl (sec-butyl), 2-methyl-propan-1-yl (isobutyl), 2-methyl-propan-2-yl (t-butyl), cyclobutan-1-yl, etc.; and the like.

[0248] The term “alkenyl” by itself or as part of another substituent refers to an unsaturated branched, straight-chain or cyclic alkyl having at least one carbon-carbon double bond derived by the removal of one hydrogen atom from a single carbon atom of a parent alkene. Typical alkenyl

groups include, but are not limited to, ethenyl; propenyls such as prop-1-en-1-yl, prop-1-en-2-yl, prop-2-en-1-yl, prop-2-en-2-yl, cycloprop-1-en-1-yl; cycloprop-2-en-1-yl; butenyls such as but-1-en-1-yl, but-1-en-2-yl, 2-methyl-prop-1-en-1-yl, but-2-en-1-yl, but-2-en-2-yl, buta-1,3-dien-1-yl, buta-1,3-dien-2-yl, cyclobut-1-en-1-yl, cyclobut-1-en-3-yl, cyclobuta-1,3-dien-1-yl, etc.; and the like.

[0249] The term “alkynyl” by itself or as part of another substituent refers to an unsaturated branched, straight-chain or cyclic alkyl having at least one carbon-carbon triple bond derived by the removal of one hydrogen atom from a single carbon atom of a parent alkyne. Typical alkynyl groups include, but are not limited to, ethynyl; propynyls such as prop-1-yn-1-yl, prop-2-yn-1-yl, etc.; butynyls such as but-1-yn-1-yl, but-1-yn-3-yl, but-3-yn-1-yl, etc.; and the like.

[0250] The term “alkylamine” refers to a group of the formula $\text{—NR}^{\text{sup.xa}}$ and “dialkylamine” refers to a group of the formula $\text{—NR}^{\text{sup.xa}}\text{R}^{\text{sup.xa}}$, where each $\text{R}^{\text{sup.xa}}$ is, independently of the others, an alkyl group.

[0251] The term “alkylene” refers to an alkane, alkene or alkyne group having two terminal monovalent radical centers derived by the removal of one hydrogen atom from each of the two terminal carbon atoms. Typical alkylene groups include, but are not limited to, methylene; and saturated or unsaturated ethylene; propylene; butylene; and the like. The term “lower alkylene” refers to alkylene groups with 1 to 6 carbons.

[0252] The term “heteroalkylene” refers to a divalent alkylene having one or more $\text{—CH}^{\text{sub.2}}$ groups replaced with a thio, oxy, or $\text{—NR}^{\text{sup.x3}}$ — where $\text{R}^{\text{sup.x3}}$ is selected from hydrogen, lower alkyl and lower heteroalkyl. The heteroalkylene can be linear, branched, cyclic, bicyclic, or a combination thereof and can include up to 10 carbon atoms and up to 4 heteroatoms. The term “lower heteroalkylene” refers to alkylene groups with 1 to 4 carbon atoms and 1 to 3 heteroatoms.

[0253] The term “aryl” means an aromatic carbocyclyl containing from 6 to 14 carbon ring atoms. An aryl may be monocyclic or polycyclic (i.e., may contain more than one ring). In the case of polycyclic aromatic rings, only one ring the polycyclic system is required to be aromatic while the remaining ring(s) may be saturated, partially saturated or unsaturated. Examples of aryls include phenyl, naphthalenyl, indenyl, indanyl, and tetrahydronaphthyl.

[0254] The term “arylene” refers to an aryl group having two monovalent radical centers derived by the removal of one hydrogen atom from each of the two ring carbons. An exemplary arylene group is a phenylene.

[0255] An alkyl group may be substituted by a “carbonyl” which means that two hydrogen atoms from a single alkanylene carbon atom are removed and replaced with a double bond to an oxygen atom.

[0256] The prefix “halo” indicates that the substituent which includes the prefix is substituted with one or more independently selected halogen radicals. For example, haloalkyl means an alkyl substituent in which at least one hydrogen radical is replaced with a halogen radical. Typical halogen radicals include chloro, fluoro, bromo and iodo. Examples of haloalkyls include chloromethyl, 1-bromoethyl, fluoromethyl, difluoromethyl, trifluoromethyl, and 1,1,1-trifluoroethyl. It should be recognized that if a substituent is substituted by more than one halogen radical, those halogen radicals may be identical or different (unless otherwise stated).

[0257] The term “haloalkoxy” refers to a group of the formula $\text{—OR}^{\text{sup.c}}$, where $\text{R}^{\text{sup.c}}$ is a haloalkyl.

[0258] The terms “heteroalkyl,” “heteroalkanyl,” “heteroalkenyl,” “heteroalkynyl,” and “heteroalkylene” refer to alkyl, alkanyl, alkenyl, alkynyl, and alkylene groups, respectively, in which one or more of the carbon atoms, e.g., 1, 2 or 3 carbon atoms, are each independently replaced with the same or different heteroatoms or heteroatomic groups. Typical heteroatoms and/or heteroatomic groups which can replace the carbon atoms include, but are not limited to, —O— , —S— , —S—O— , $\text{—NR}^{\text{sup.c}}$ —, —PH— , —S(O)— , $\text{—S(O)}^{\text{sub.2}}$ —, $\text{—S(O)NR}^{\text{sup.c}}$ —, $\text{—S(O)}^{\text{sub.2}}\text{NR}^{\text{sup.c}}$ —, and the like, including combinations thereof, where each $\text{R}^{\text{sup.c}}$ is

independently hydrogen or C.sub.1-C.sub.6 alkyl. The term “lower heteroalkyl” refers to between 1 and 4 carbon atoms and between 1 and 3 heteroatoms.

[0259] The terms “cycloalkyl” and “heterocyclyl” refer to cyclic versions of “alkyl” and “heteroalkyl” groups, respectively. For heterocyclyl groups, a heteroatom can occupy the position that is attached to the remainder of the molecule. A cycloalkyl or heterocyclyl ring may be a single-ring (monocyclic) or have two or more rings (bicyclic or polycyclic).

[0260] Monocyclic cycloalkyl and heterocyclyl groups will typically contains from 3 to 7 ring atoms, more typically from 3 to 6 ring atoms, and even more typically 5 to 6 ring atoms. Examples of cycloalkyl groups include, but are not limited to, cyclopropyl; cyclobutyls such as cyclobutanyl and cyclobutenyl; cyclopentyls such as cyclopentanyl and cyclopentenyl; cyclohexyls such as cyclohexanyl and cyclohexenyl; and the like. Examples of monocyclic heterocyclyls include, but are not limited to, oxetane, furanyl, dihydrofuranyl, tetrahydrofuranyl, tetrahydropyranyl, thiophenyl (thiofuranyl), dihydrothiophenyl, tetrahydrothiophenyl, pyrrolyl, pyrrolinyl, pyrrolidinyl, imidazolyl, imidazolinyl, imidazolidinyl, pyrazolyl, pyrazolinyl, pyrazolidinyl, triazolyl, tetrazolyl, oxazolyl, oxazolidinyl, isoxazolidinyl, isoxazolyl, thiazolyl, isothiazolyl, thiazolinyl, isothiazolinyl, thiazolidinyl, isothiazolidinyl, thiodiazolyl, oxadiazolyl (including 1,2,3-oxadiazolyl, 1,2,4-oxadiazolyl, 1,2,5-oxadiazolyl (furazanyl), or 1,3,4-oxadiazolyl), oxatriazolyl (including 1,2,3,4-oxatriazolyl or 1,2,3,5-oxatriazolyl), dioxazolyl (including 1,2,3-dioxazolyl, 1,2,4-dioxazolyl, 1,3,2-dioxazolyl, or 1,3,4-dioxazolyl), 1,4-dioxanyl, dioxothiomorpholinyl, oxathiazolyl, oxathieryl, oxathiolanyl, pyranyl, dihydropyranyl, thiopyranyl, tetrahydrothiopyranyl, pyridinyl (azinyl), piperidinyl, diazinyl (including pyridazinyl (1,2-diazinyl), pyrimidinyl (1,3-diazinyl), or pyrazinyl (1,4-diazinyl)), piperazinyl, triazinyl (including 1,3,5-triazinyl, 1,2,4-triazinyl, and 1,2,3-triazinyl)), oxazinyl (including 1,2-oxazinyl, 1,3-oxazinyl, or 1,4-oxazinyl)), oxathiazinyl (including 1,2,3-oxathiazinyl, 1,2,4-oxathiazinyl, 1,2,5-oxathiazinyl, or 1,2,6-oxathiazinyl)), oxadiazinyl (including 1,2,3-oxadiazinyl, 1,2,4-oxadiazinyl, 1,4,2-oxadiazinyl, or 1,3,5-oxadiazinyl)), morpholinyl, azepinyl, oxepinyl, thiopinyl, diazepinyl, pyridonyl (including pyrid-2(1H)-onyl and pyrid-4(1H)-onyl), furan-2(5H)-onyl, pyrimidonyl (including pyrimid-2(1H)-onyl and pyrimid-4(3H)-onyl), oxazol-2(3H)-onyl, 1H-imidazol-2(3H)-onyl, pyridazin-3(2H)-onyl, and pyrazin-2(1H)-onyl.

[0261] Polycyclic cycloalkyl and heterocyclyl groups contain more than one ring, and bicyclic cycloalkyl and heterocyclyl groups contain two rings. The rings may be in a bridged, fused or spiro orientation. Polycyclic cycloalkyl and heterocyclyl groups may include combinations of bridged, fused and/or spiro rings. In a spirocyclic cycloalkyl or heterocyclyl, one atom is common to two different rings. An example of a spirocycloalkyl is spiro[4.5]decane and an example of a spiroheterocyclyls is a spiropyrazoline.

[0262] In a bridged cycloalkyl or heterocyclyl, the rings share at least two common non-adjacent atoms. Examples of bridged cycloalkyls include, but are not limited to, adamantyl and norbornanyl rings. Examples of bridged heterocyclyls include, but are not limited to, 2-oxatricyclo[3.3.1.1.sup.3,7]decanyl.

[0263] In a fused-ring cycloalkyl or heterocyclyl, two or more rings are fused together, such that two rings share one common bond. Examples of fused-ring cycloalkyls include decalin, naphthylene, tetralin, and anthracene. Examples of fused-ring heterocyclyls containing two or three rings include imidazopyrazinyl (including imidazo[1,2-a]pyrazinyl), imidazopyridinyl (including imidazo[1,2-a]pyridinyl), imidazopyridazinyl (including imidazo[1,2-b]pyridazinyl), thiazolopyridinyl (including thiazolo[5,4-c]pyridinyl, thiazolo[5,4-b]pyridinyl, thiazolo[4,5-b]pyridinyl, and thiazolo[4,5-c]pyridinyl), indolizinyl, pyranopyrrolyl, 4H-quinolizinyl, purinyl, naphthyridinyl, pyridopyridinyl (including pyrido[3,4-b]-pyridinyl, pyrido[3,2-b]-pyridinyl, or pyrido[4,3-b]-pyridinyl), and pteridinyl. Other examples of fused-ring heterocyclyls include benzo-fused heterocyclyls, such as dihydrochromenyl, tetrahydroisoquinolinyl, indolyl, isoindolyl (isobenzazolyl, pseudoisoindolyl), indoleninyl (pseudoindolyl), isoindazolyl (benzpyrazolyl),

benzazinyl (including quinolinyl (1-benzazinyl) or isoquinolinyl (2-benzazinyl)), phthalazinyl, quinoxaliny, quinazolinyl, benzodiaziny (including cinnolinyl (1,2-benzodiaziny) or quinazolinyl (1,3-benzodiaziny)), benzopyranyl (including chromanyl or isochromanyl), benzoxaziny (including 1,3,2-benzoxaziny, 1,4,2-benzoxaziny, 2,3,1-benzoxaziny, or 3,1,4-benzoxaziny), benzo[d]thiazolyl, and benzisoxaziny (including 1,2-benzisoxaziny or 1,4-benzisoxaziny).

[0264] The term “heteroaryl” refers to an aromatic heterocyclyl containing from 5 to 14 ring atoms. A heteroaryl may be a single ring or 2 or 3 fused rings. Examples of heteroaryls include 6-membered rings such as pyridyl, pyrazyl, pyrimidinyl, pyridaziny, and 1,3,5-, 1,2,4- or 1,2,3-triaziny; 5-membered ring substituents such as triazolyl, pyrrolyl, imidazyl, furanyl, thiophenyl, pyrazolyl, oxazolyl, isoxazolyl, thiazolyl, 1,2,3-, 1,2,4-, 1,2,5-, or 1,3,4-oxadiazolyl and isothiazolyl; 6/5-membered fused ring substituents such as imidazopyraziny (including imidazo[1,2-a]pyraziny)imidazopyridiny (including imidazo[1,2-a]pyridiny), imidazopyridaziny (including imidazo[1,2-b]pyridaziny), thiazolopyridiny (including thiazolo[5,4-c]pyridiny, thiazolo[5,4-b]pyridiny, thiazolo[4,5-b]pyridiny, and thiazolo[4,5-c]pyridiny), benzo[d]thiazolyl, benzothiofuranyl, benzisoxazolyl, benzoxazolyl, puriny, and anthranily; and 6/6-membered fused rings such as benzopyranyl, quinolinyl, isoquinolinyl, cinnolinyl, quinazolinyl, and benzoxaziny.

[0265] Heteroaryls may also be heterocycles having aromatic (4N+2 pi electron) resonance contributors such as pyridonyl (including pyrid-2(1H)-onyl and pyrid-4(1H)-onyl), pyrimidonyl (including pyramid-2(1H)-onyl and pyramid-4(3H)-onyl), pyridazin-3(2H)-onyl and pyrazin-2(1H)-onyl.

[0266] The term “sulfonate” as used herein means a salt or ester of a sulfonic acid.

[0267] The term “methyl sulfonate” as used herein means a methyl ester of a sulfonic acid group.

[0268] The term “carboxylate” as used herein means a salt or ester of a carboxylic acid.

[0269] The term “polyol”, as used herein, means a group containing more than two hydroxyl groups independently or as a portion of a monomer unit. Polyols include, but are not limited to, reduced C.sub.2-C.sub.6 carbohydrates, ethylene glycol, and glycerin.

[0270] The term “sugar” when used in context of “G” includes O-glycoside, N-glycoside, S-glycoside and C-glycoside (C-glycosyl) carbohydrate derivatives of the monosaccharide and disaccharide classes and may originate from naturally-occurring sources or may be synthetic in origin.

[0271] For example “sugar” when used in context of “G.sup.1” includes derivatives such as but not limited to those derived from glucuronic acid, galacturonic acid, galactose, and glucose among others. Suitable sugar substitutions include but are not limited to hydroxyl, amine, carboxylic acid, sulfonic acid, phosphonic acid, esters, and ethers.

[0272] The term “NHS ester” means the N-hydroxysuccinimide ester derivative of a carboxylic acid.

[0273] The term “amine” includes primary, secondary and tertiary aliphatic amines, including cyclic versions.

[0274] The term salt when used in context of “or salt thereof” include salts commonly used to form alkali metal salts and to form addition salts of free acids or free bases. In general, these salts typically may be prepared by conventional means by reacting, for example, the appropriate acid or base with a compound of the invention Where a salt is intended to be administered to a patient (as opposed to, for example, being in use in an in vitro context), the salt preferably is pharmaceutically acceptable and/or physiologically compatible. The term “pharmaceutically acceptable” is used adjectivally in this patent application to mean that the modified noun is appropriate for use as a pharmaceutical product or as a part of a pharmaceutical product. The term “pharmaceutically acceptable salt” includes salts commonly used to form alkali metal salts and to form addition salts of free acids or free bases. In general, these salts typically may be prepared by conventional means by reacting, for example, the appropriate acid or base with a compound of the invention.

[0275] The term “anti-Epidermal Growth Factor Receptor (EGFR) antibody” as used herein, refers

to an antibody that specifically binds to EGFR. An antibody “which binds” an antigen of interest, i.e., EGFR, is one capable of binding that antigen with sufficient affinity such that the antibody is useful in targeting a cell expressing the antigen. In a preferred embodiment, the antibody specifically binds to human EGFR (hEGFR). Examples of anti-EGFR antibodies are disclosed below.

[0276] Unless otherwise indicated, the term “anti-EGFR antibody” is meant to refer to an antibody which binds to wild type EGFR or any variant of EGFR, such as EGFRvIII.

[0277] The amino acid sequence of wild type human EGFR is provided below as SEQ ID NO: 32, where the signal peptide (amino acid residues 1-24) is underlined, and the amino acid residues of the extracellular domain (ECD, amino acid residues 25-645) are highlighted in bold. A truncated wild type ECD of the EGFR (also referred to herein as EGFR(1-525)) corresponds to SEQ ID NO: 47 and is equivalent to amino acids 1-525 of SEQ ID NO: 32. The mature form of wild type EGFR corresponds to the protein without the signal peptide, i.e., amino acid residues 25 to 1210 of SEQ ID NO: 32.

TABLE-US-00001 (SEQ ID NO: 32) 1 mrpsgtagaa llallaalcp asraleekkv **cqgtsnkl****tg**
lgtfedhfls **lqrmfnncev** 61 **vlgnleityv** **qrnydlsf1k** **tiqevagyvl** **ialntverip** **lenlqiirgn**
myyensyala 121 **vlсныdankt** **glkelpmrnl** **qeilhгavrf** **snnpalcnve** **siqwrdivss**
dfslnmsmdf 181 **qnhlгscqkc** **dpсpngscw** **gаgeencqkl** **tkiicaqqcs** **grcrgkspsd**
cchnqcaagc 241 **tgpresdclv** **crkfrdeatc** **kdtcpplmly** **npttyqmdvn** **pegkysfgat**
cvkkcprnyv 301 **vtdhgscvra** **cgadsyemee** **dgvrkckkce** **gperkvengi** **gigefkdsls**
inatnikhfk 361 **nctsisgdlh** **ilpvafrgds** **fthtppldpq** **eldilktvke** **itgflliqaw** **penrtdlhaf** 421
enleiirgt **kqhгqfslav** **vslnitslgl** **rslkeisdgd** **viisgnknlc** **yantinwkk1** 481 **fgtsggqtki**
isnrgensck **atgqvchalc** **speгewgpep** **rdcvscrnvs** **rgrecvdkcn** 541 **llegeprefv**
enseciqchp **eclpqamnit** **ctgrgpdnci** **qcahyidgph** **cvktcpagvm** 601 **genntlvwky**
adaghvchlc **hpnctygctg** **pglegcptng** **pkipsiatgm** **vgalllllvv** 661 **alig1fmr** **r**
rhivrkrtr
rlqerelve **pltpsgeapn** **qallrilket** **efkkikvlgs** 721 **gafgtvykgl** **wipegekvki** **pvaikelrea**
tspkankeil **deayvmasvd** **nphvcrlgi** 781 **cltstvqlit** **qlmpfgclld** **yvrehkdng** **sqyllnwcvq**
iakgmnyled **rrlvhrdlaa** 841 **rnvlvktpqh** **vkitdfglak** **lgaekeyh** **aeggvkpiw** **malesilhri**
ythqsdvwsy 901 **gvtvwelmtf** **gskpydgipa** **seissilekg** **erlpqpict** **idvymimvkc** **wmidadsrpk**
961 **freliefsk** **mardpqrylv** **iqgdermhlп** **sptdsnfyra** **lmdeedmddv** **vdadeylipq** 1021
qgffsspsts **rtpllsslsa** **tsnnstvaci** **drnglqscpi** **kedsflqrys** **sdptgalted** 1081 **siddtflpvp**
eyinqsvpkr **pagsvqnpvy** **hnqplnpaps** **rdphyqdphs** **tavgnpeyln** 1141 **tvqptcvnst**
fdspahwaqk **gshqisldnp** **dyqqdffpke** **akpngifkgs** **taenaeylr** 1201 **apqssefiga**

[0278] The amino acid sequence of the ECD of human EGFR is provided below as SEQ ID NO: 34, and includes the signal sequence (underlined).

TABLE-US-00002 (SEQ ID NO: 34) 1 mrpsgtagaa llallaalcp asraleekkv
cqgtsnkl**tg** **lgtfedhfls** **lqrmfnncev** 61 **vlgnleityv** **qrnydlsflk** **tiqevagyvl** **ialntverip**
lenlqiirgn **myyensyala** 121 **vlсныdankt** **glkelpmrnl** **qeilhгavrf** **snnpalcnve** **siqwrdivss**
dfslnmsmdf 181 **qnhlгscqkc** **dpсpngscw** **gаgeencqkl** **tkiicaqqcs** **grcrgkspsd** **cchnqcaagc**
241 **tgpresdclv** **crkfrdeatc** **kdtcpplmly** **npttyqmdvn** **pegkysfgat** **cvkkcprnyv** 301
vtdhgscvra **cgadsyemee** **dgvrkckkce** **gpcrkvcngi** **gigefkdsls** **inatnikhfk** 361 **nctsisgdlh**
ilpvafrgds **fthtppldpq** **eldilktvke** **itgflliqaw** **penrtdlhaf** 421 **enleiirgt** **kqhгqfslav**
vslnitslgl **rslkeisdgd** **viisgnknlc** **yantinwkk1** 481 **fgtsggqtki** **isnrgensck** **atgqvchalc**
speгcwgpep **rdcvscrnvs** **rgrecvdkcn** 541 **llegeprefv** **enseciqchp** **eclpqamnit** **ctgrgpdnci**
qcahyidgph **cvktcpagvm** 601 **genntlvwky** **adaghvchlc** **hpnctygctg** **pglegcptng** **pkips**

[0279] The overall structure of EGFR is described in FIG. 1. The ECD of EGFR has four domains (Cochran et al. (2004) J. Immunol. Methods, 287, 147-158). Domains I and III have been suggested to contribute to the formation of high affinity binding sites for ligands. Domains II and IV are cysteine rich, laminin-like regions that stabilize protein folding and contain a possible EGFR dimerization interface.

[0280] EGFR variants may result from gene rearrangement accompanied by EGFR gene amplification.

[0281] EGFRvIII is the most commonly occurring variant of the EGFR in human cancers (Kuan et al. Endocr Relat Cancer. 8(2):83-96 (2001)). During the process of gene amplification, a 267 amino acid deletion occurs in the extracellular domain of EGFR with a glycine residue inserted at the fusion junction. Thus, EGFRvIII lacks amino acids 6-273 of the extracellular domain of wild type EGFR and includes a glycine residue insertion at the junction. The EGFRvIII variant of EGFR contains a deletion of 267 amino acid residues in the extracellular domain where a glycine is inserted at the deletion junction. The EGFRvIII amino acid sequence is shown below as SEQ ID NO: 33 (the ECD is highlighted in bold and corresponds to SEQ ID NO: 46 the signal sequence is underlined).

TABLE-US-00003 (SEQ ID NO: 33) mrpsgtagaallallaalcpasra**leekkgnyvvdthgscvracgadsye**
meedgvrkckkcegpckvcngigigefkdslsinatnikhfkncsisg
dlhilpvafrgdsfthtppldpqeldilktvkeitgflliqawpenrtdl
hafenleirgrtkqhggqfslavvslnitslglrsllkeisdgdviisgnk
nlcyantinwkkllfgtsgqtkiisnrgensckatgqychalcspegcwg
peprdcvscrnvsgrecvdkcnllegeprefvenseciqchpeclpqam
nitctgrgpdnciqcahyidgphcvktcpagvmgenntlvwkyadaghvc
hlchpnctygtgpglegcptngpkipsiatgmvgaalllllvalgiglf
mrrrhivrkrtrllrllqerelveltpsggeapnqallrilketefkkikv
lgsgafgtvykglwipegekvpkipvaikelreatspkankeildeayvma
svdnphvcrlgicltstvlitqlmpfgclldyvrehkdnigsqyllnw
cvqiakgmnyledrrlvhrdlaarnvlvktpqhvkitdfglakllgaeek
eyhaeggkvpikwmalesilhriythqsdvwsygvvtvwelmtfgskpydg
ipaseissilekgerlpqppictidvymimvkcwmidadsrpkfrelie
fskmardpqrylviqgdermhlpstpsnfyralmdeedmddvvdadeyl
ipqqgffsspstsrtpllsslsatsnnstvacidrnglqscpikedsflq
ryssdptgaltedsiddtflvppeyinqsvpkpagsvqnpvyhnpqplnp
apsrdphyqdphstavgnppeylntvqptcvnstfdspahwaqkgshqisl
dnpdyqqdffpkeakpngifkgstaenaeylr vapqssefiga

[0282] EGFRvIII contributes to tumor progression through constitutive signaling in a ligand independent manner. EGFRvIII is not known to be expressed in normal tissues (Wikstrand et al. Cancer Research 55(14): 3140-3148 (1995); Olapade-Olaopa et al. Br J Cancer. 82(1):186-94 (2000)), but shows significant expression in tumor cells, including breast cancers, gliomas, NSCL cancers, ovarian cancers, and prostate cancers (Wikstrand et al. Cancer Research 55(14): 3140-3148 (1995); Ge et al. Int J Cancer. 98(3):357-61 (2002); Wikstrand et al. Cancer Research 55(14): 3140-3148 (1995); Moscatello et al. Cancer Res. 55(23):5536-9 (1995); Garcia de Palazzo et al. Cancer Res. 53(14):3217-20 (1993); Moscatello et al. Cancer Res. 55(23):5536-9 (1995); and Olapade-Olaopa et al. 2(1):186-94 (2000)).

[0283] “Biological activity of EGFR” as used herein, refers to all inherent biological properties of the EGFR, including, but not limited to, binding to epidermal growth factor (EGF), binding to tumor growth factor α (TGF α), homodimerization, activation of JAK2 kinase activity, activation of MAPK kinase activity, and activation of transmembrane receptor protein tyrosine kinase activity.

[0284] The term “gene amplification”, as used herein, refers to a cellular process characterized by the production of multiple copies of any particular piece of DNA. For example, a tumor cell may amplify, or copy, chromosomal segments as a result of cell signals and sometimes environmental events. The process of gene amplification leads to the production of additional copies of the gene. In one embodiment, the gene is EGFR, i.e., “EGFR amplification.” In one embodiment, the compositions and methods disclosed herein are used to treat a subject having EGFR amplified cancer.

[0285] The terms “specific binding” or “specifically binding”, as used herein, in reference to the interaction of an antibody or an ADC with a second chemical species, mean that the interaction is dependent upon the presence of a particular structure (e.g., an antigenic determinant or epitope) on the chemical species; for example, an antibody recognizes and binds to a specific protein structure rather than to proteins generally. If an antibody or ADC is specific for epitope “A”, the presence of a molecule containing epitope A (or free, unlabeled A), in a reaction containing labeled “A” and the antibody, will reduce the amount of labeled A bound to the antibody or ADC.

[0286] The phrase “specifically binds to hEGFR” or “specific binding to hEGFR”, as used herein, refers to the ability of an anti-EGFR antibody or ADC to bind to hEGFR with a K_{d} of at least about 1×10^{-6} M, 1×10^{-7} M, 1×10^{-8} M, 1×10^{-9} M, 1×10^{-10} M, 1×10^{-11} M, 1×10^{-12} M, or more, and/or bind to an antigen with an affinity that is at least two-fold greater than its affinity for a nonspecific antigen. It shall be understood, however, that the antibody or ADC may be capable of specifically binding to two or more antigens which are related in sequence. For example, in one embodiment, an antibody can specifically bind to both human and a non-human (e.g., mouse or non-human primate) orthologs of EGFR. In one embodiment, the antigen is EGFR(1-525).

[0287] The term “antibody” refers to an immunoglobulin molecule that specifically binds to an antigen and comprises a heavy (H) chain(s) and a light (L chain(s). Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR or VL) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. An antibody can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY) and class (e.g., IgG1, IgG2, IgG 3, IgG4, IgA1 and IgA2) or subclass.

[0288] While the term “antibody” is not intended to include antigen binding portions of an antibody (defined below), it is intended, in certain embodiments, to include a small number of amino acid deletions from the carboxy end of the heavy chain(s). In one embodiment, an antibody comprises a heavy chain having 1-5 amino acid deletions the carboxy end of the heavy chain. In a one embodiment, an antibody is a monoclonal antibody which is an IgG, having four polypeptide chains, two heavy (H) chains, and two light (L chains) that can bind to hEGFR. In one embodiment, an antibody is a monoclonal IgG antibody comprising a lambda or a kappa light chain.

[0289] An IgG is a class of antibody comprising two heavy chains and two light chains arranged in a Y-shape. An IgG constant domain refers to a heavy or light chain constant domain. Exemplary human IgG heavy chain and light chain constant domain amino acid sequences are known in the art and represented below in Table 1.

TABLE-US-00004 TABLE 1 Sequence of human IgG heavy chain constant domain and light chain constant domain Sequence Identifier Sequence Ig SEQ ID
ASTKGPSVFPLAPSSKSTSGGTAALGCLV gamma-1 NO: 41
KDYFPEPVTWSNSGALTSGVHTFPAVLQ constant
SSGLYSLSVVTVPSSSLGTQTYICNVNH region
KPSNTKVDKKVEPKSCDKTHTCPPCPAPE LLGGPSVFLFPPKPKDTLMISRTPEVTCV
VVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYK
CKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSREEMTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTTPVLDSDGSFFLY SKLTVDKSRWQQGNVVFSCSVMHEALHNHY
TQKSLSLSPGK Ig SEQ ID ASTKGPSVFPLAPSSKSTSGGTAALGCLV gamma-1 NO: 42

KDYFPTVTSVNGSALTSGVHTFPAVLQ constant
SSGLYSLSSVVTVPSSSLGTQTYICNVNH region
KPSNTKVDKKVEPKSCDKTHTCPPCPAPE mutant
AAGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKALPAPIEKTISKAKGQPREPQVY
TLPPSREEMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTPPVLDSDGSFFLY
SKLTVDKSRWQQGNVFCSSVMHEALHNHY TQKSLSLSPGK Ig Kappa SEQ ID
RTVAAPSVFIFPPSDEQLKSGTASVVCLL constant NO: 43
NNFYPREAKVQWKVDNALQSGNSQESVTE region
QDSKDSTYLSSTLTLSKADYEKHKVYAC EVTHQGLSSPVTKSFNRGEC Ig Lambda
SEQ ID QPKAAPSVTLFPPSSEELQANKATLVCLI constant NO: 44
SDFYPGAFTVAWKADSSPVKAGVETTTPS region
KQSNNKYAASSYLSLTPEQWKSHRSYSCQ VTHEGSTVEKTVAPTECS

[0290] An “isolated antibody”, as used herein, is intended to refer to an antibody that is substantially free of other antibodies having different antigenic specificities (e.g., an isolated antibody that specifically binds EGFR is substantially free of antibodies that specifically bind antigens other than EGFR). An isolated antibody that specifically binds EGFR may, however, have cross-reactivity to other antigens, such as EGFR molecules from other species. Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals.

[0291] The term “humanized antibody” refers to an antibody which comprises heavy and light chain variable region sequences from a nonhuman species (e.g., a mouse) but in which at least a portion of the VH and/or VL sequence has been altered to be more “human-like”, i.e., more similar to human germline variable sequences. In particular, the term “humanized antibody” is an antibody or a variant, derivative, analog or fragment thereof which immunospecifically binds to an antigen of interest and which comprises a framework (FR) region having substantially the amino acid sequence of a human antibody and a complementary determining region (CDR) having substantially the amino acid sequence of a non-human antibody. As used herein, the term “substantially” in the context of a CDR refers to a CDR having an amino acid sequence at least 80%, preferably at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the amino acid sequence of a non-human antibody CDR. A humanized antibody comprises substantially all of at least one, and typically two, variable domains (Fab, Fab', F(ab').sub.2, FabC, Fv) in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin (i.e., donor antibody) and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. Preferably, a humanized antibody also comprises at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. In some embodiments, a humanized antibody contains both the light chain as well as at least the variable domain of a heavy chain. The antibody also may include the CH1, hinge, CH2, CH3, and CH4 regions of the heavy chain. In some embodiments, a humanized antibody only contains a humanized light chain. In other embodiments, a humanized antibody only contains a humanized heavy chain. In specific embodiments, a humanized antibody only contains a humanized variable domain of a light chain and/or humanized heavy chain.

[0292] The humanized antibody can be selected from any class of immunoglobulins, including IgM, IgG, IgD, IgA and IgE, and any isotype, including without limitation IgG1, IgG2, IgG3 and IgG4. The humanized antibody may comprise sequences from more than one class or isotype, and particular constant domains may be selected to optimize desired effector functions using techniques well-known in the art.

[0293] The terms “Kabat numbering,” “Kabat definitions,” and “Kabat labeling” are used interchangeably herein. These terms, which are recognized in the art, refer to a system of numbering amino acid residues which are more variable (i.e., hypervariable) than other amino acid residues in the heavy and light chain variable regions of an antibody, or an antigen binding portion

thereof (Kabat et al. (1971) *Ann. NY Acad. Sci.* 190:382-391 and, Kabat, E. A., et al. (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). For the heavy chain variable region, the hypervariable region ranges from amino acid positions 31 to 35 for CDR1, amino acid positions 50 to 65 for CDR2, and amino acid positions 95 to 102 for CDR3. For the light chain variable region, the hypervariable region ranges from amino acid positions 24 to 34 for CDR1, amino acid positions 50 to 56 for CDR2, and amino acid positions 89 to 97 for CDR3.

[0294] As used herein, the term “CDR” refers to the complementarity determining region within antibody variable sequences. There are three CDRs in each of the variable regions of the heavy chain (HC) and the light chain (LC), which are designated CDR1, CDR2 and CDR3 (or specifically HC CDR1, HC CDR2, HC CDR3, LC CDR1, LC CDR2, and LC CDR3), for each of the variable regions. The term “CDR set” as used herein refers to a group of three CDRs that occur in a single variable region capable of binding the antigen. The exact boundaries of these CDRs have been defined differently according to different systems. The system described by Kabat (Kabat et al., *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md. (1987) and (1991)) not only provides an unambiguous residue numbering system applicable to any variable region of an antibody, but also provides precise residue boundaries defining the three CDRs. These CDRs may be referred to as Kabat CDRs. Chothia and coworkers (Chothia & Lesk, *J. Mol. Biol.* 196:901-917 (1987) and Chothia et al., *Nature* 342:877-883 (1989)) found that certain sub-portions within Kabat CDRs adopt nearly identical peptide backbone conformations, despite having great diversity at the level of amino acid sequence. These sub-portions were designated as L1, L2 and L3 or H1, H2 and H3 where the “L” and the “H” designates the light chain and the heavy chains regions, respectively. These regions may be referred to as Chothia CDRs, which have boundaries that overlap with Kabat CDRs. Other boundaries defining CDRs overlapping with the Kabat CDRs have been described by Padlan (*FASEB J.* 9:133-139 (1995)) and MacCallum (*J Mol Biol* 262(5):732-45 (1996)). Still other CDR boundary definitions may not strictly follow one of the above systems, but will nonetheless overlap with the Kabat CDRs, although they may be shortened or lengthened in light of prediction or experimental findings that particular residues or groups of residues or even entire CDRs do not significantly impact antigen binding. The methods used herein may utilize CDRs defined according to any of these systems, although preferred embodiments use Kabat or Chothia defined CDRs.

[0295] As used herein, the term “framework” or “framework sequence” refers to the remaining sequences of a variable region minus the CDRs. Because the exact definition of a CDR sequence can be determined by different systems, the meaning of a framework sequence is subject to correspondingly different interpretations. The six CDRs (CDR-L1, CDR-L2, and CDR-L3 of light chain and CDR-H1, CDR-H2, and CDR-H3 of heavy chain) also divide the framework regions on the light chain and the heavy chain into four sub-regions (FR1, FR2, FR3 and FR4) on each chain, in which CDR1 is positioned between FR1 and FR2, CDR2 between FR2 and FR3, and CDR3 between FR3 and FR4. Without specifying the particular sub-regions as FR1, FR2, FR3 or FR4, a framework region, as referred by others, represents the combined FR's within the variable region of a single, naturally occurring immunoglobulin chain. As used herein, a FR represents one of the four sub-regions, and FRs represents two or more of the four sub- regions constituting a framework region.

[0296] The framework and CDR regions of a humanized antibody need not correspond precisely to the parental sequences, e.g., the donor antibody CDR or the consensus framework may be mutagenized by substitution, insertion and/or deletion of at least one amino acid residue so that the CDR or framework residue at that site does not correspond to either the donor antibody or the consensus framework. In a preferred embodiment, such mutations, however, will not be extensive.

[0297] Usually, at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% of the humanized antibody residues will correspond to those of the parental

FR and CDR sequences. As used herein, the term “consensus framework” refers to the framework region in the consensus immunoglobulin sequence. As used herein, the term “consensus immunoglobulin sequence” refers to the sequence formed from the most frequently occurring amino acids (or nucleotides) in a family of related immunoglobulin sequences (See e.g., Winnaker, *From Genes to Clones* (Verlagsgesellschaft, Weinheim, Germany 1987). In a family of immunoglobulins, each position in the consensus sequence is occupied by the amino acid occurring most frequently at that position in the family. If two amino acids occur equally frequently, either can be included in the consensus sequence.

[0298] The term “antigen binding portion” or “antigen binding fragment” of an antibody (or simply “antibody portion” or “antigen fragment”), as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen (e.g., hEGFR). It has been shown that the antigen binding function of an antibody can be performed by fragments of a full-length antibody. Such antibody embodiments may also be bispecific, dual specific, or multi-specific formats; specifically binding to two or more different antigens. Examples of binding fragments encompassed within the term “antigen binding portion” of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a F(ab').sub.2 fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward et al., (1989) *Nature* 341:544-546, Winter et al., PCT publication WO 90/05144 A1 herein incorporated by reference), which comprises a single variable domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see e.g., Bird et al. (1988) *Science* 242:423-426; and Huston et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883). Such single chain antibodies are also intended to be encompassed within the term “antigen binding portion” of an antibody. In certain embodiments of the invention, scFv molecules may be incorporated into a fusion protein. Other forms of single chain antibodies, such as diabodies are also encompassed. Diabodies are bivalent, bispecific antibodies in which VH and VL domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (see e.g., Holliger, P., et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:6444-6448; Poljak, R. J., et al. (1994) *Structure* 2:1121-1123). Such antibody binding portions are known in the art (Kontermann and Dubel eds., *Antibody Engineering* (2001) Springer-Verlag. New York. 790 pp. (ISBN 3-540-41354-5).

[0299] “Percent (%) amino acid sequence identity” with respect to a peptide or polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific peptide or polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. In one embodiment, the invention includes an amino acid sequence having at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identity to an amino acid sequence set forth in any one of SEQ ID NOs: 1 to 31, 35-40, or 50 to 85.

[0300] The term “multivalent antibody” is used herein to denote an antibody comprising two or

more antigen binding sites. In certain embodiments, the multivalent antibody may be engineered to have the three or more antigen binding sites, and is generally not a naturally occurring antibody. [0301] The term “multispecific antibody” refers to an antibody capable of binding two or more unrelated antigens. In one embodiment, the multispecific antibody is a bispecific antibody that is capable of binding to two unrelated antigens, e.g., a bispecific antibody, or antigen-binding portion thereof, that binds EGFR (e.g., EGFRvIII) and CD3.

[0302] The term “activity” includes activities such as the binding specificity/affinity of an antibody or ADC for an antigen, for example, an anti-hEGFR antibody that binds to an hEGFR antigen and/or the neutralizing potency of an antibody, for example, an anti-hEGFR antibody whose binding to hEGFR inhibits the biological activity of hEGFR, e.g., inhibition of phosphorylation of EGFR in an EGFR expressing cell line, e.g., the human lung carcinoma cell line H292, or inhibition of proliferation of EGFR expressing cell lines, e.g., human H292 lung carcinoma cells, human H1703 lung carcinoma cells, or human EBC1 lung carcinoma cells.

[0303] The term “non small-cell lung carcinoma (NSCLC) xenograft assay,” as used herein, refers to an in vivo assay used to determine whether an anti-EGFR antibody or ADC, can inhibit tumor growth (e.g., further growth) and/or decrease tumor growth resulting from the transplantation of NSCLC cells into an immunodeficient mouse. An NSCLC xenograft assay includes transplantation of NSCLC cells into an immunodeficient mouse such that a tumor grows to a desired size, e.g., 200-250 mm^{sup.3}, whereupon the antibody or ADC is administered to the mouse to determine whether the antibody or ADC can inhibit and/or decrease tumor growth. In certain embodiments, the activity of the antibody or ADC is determined according to the percent tumor growth inhibition (% TGI) relative to a control antibody, e.g., a human IgG antibody (or collection thereof) which does not specifically bind tumor cells, e.g., is directed to an antigen not associated with cancer or is obtained from a source which is noncancerous (e.g., normal human serum). In such embodiments, the antibody (or ADC) and the control antibody are administered to the mouse at the same dose, with the same frequency, and via the same route. In one embodiment, the mouse used in the NSCLC xenograft assay is a severe combined immunodeficiency (SCID) mouse and/or an athymic CD-1 nude mouse. Examples of NSCLC cells that may be used in the NSCLC xenograft assay include, but are not limited to, H292 cells (e.g., NCIH292 [H292](ATCC CRL1848).

[0304] The term “epitope” refers to a region of an antigen that is bound by an antibody or ADC. In certain embodiments, epitope determinants include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl, or sulfonyl, and, in certain embodiments, may have specific three dimensional structural characteristics, and/or specific charge characteristics. In certain embodiments, an antibody is said to specifically bind an antigen when it preferentially recognizes its target antigen in a complex mixture of proteins and/or macromolecules. In one embodiment, the antibodies of the invention bind to an epitope defined by the amino acid sequence CGADSYEMEEDGVRKC (SEQ ID NO: 45) (which corresponds to amino acid residues 287-302 of the mature form of hEGFR).

[0305] The term “surface plasmon resonance”, as used herein, refers to an optical phenomenon that allows for the analysis of real-time biospecific interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIAcore system (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, NJ). For further descriptions, see Jönsson, U., et al. (1993) *Ann. Biol. Clin.* 51:19-26; Jönsson, U., et al. (1991) *Biotechniques* 11:620-627; Johnsson, B., et al. (1995) *J. Mol. Recognit.* 8:125-131; and Johnsson, B., et al. (1991) *Anal. Biochem.* 198:268-277. In one embodiment, surface plasmon resonance is determined according to the methods described in Example 2

[0306] The term “k_{sub.on}” or “k_{sub.a}”, as used herein, is intended to refer to the on rate constant for association of an antibody to the antigen to form the antibody/antigen complex.

[0307] The term “k_{sub.off}” or “k_{sub.d}”, as used herein, is intended to refer to the off rate constant for dissociation of an antibody from the antibody/antigen complex.

[0308] The term “K.sub.D”, as used herein, is intended to refer to the equilibrium dissociation constant of a particular antibody-antigen interaction (e.g., AbA antibody and EGFR). K.sub.D is calculated by $k_{\text{sub.a}}/k_{\text{sub.d}}$.

[0309] The term “competitive binding”, as used herein, refers to a situation in which a first antibody competes with a second antibody, for a binding site on a third molecule, e.g., an antigen. In one embodiment, competitive binding between two antibodies is determined using FACS analysis.

[0310] The term “competitive binding assay” is an assay used to determine whether two or more antibodies bind to the same epitope. In one embodiment, a competitive binding assay is a competition fluorescent activated cell sorting (FACS) assay which is used to determine whether two or more antibodies bind to the same epitope by determining whether the fluorescent signal of a labeled antibody is reduced due to the introduction of a non-labeled antibody, where competition for the same epitope will lower the level of fluorescence.

[0311] The term “antibody-drug-conjugate” or “ADC” refers to a binding protein, such as an antibody or antigen binding fragment thereof, chemically linked to one or more chemical drug(s) (also referred to herein as agent(s), warhead(s), or payload(s)) that may optionally be therapeutic or cytotoxic agents. In a preferred embodiment, an ADC includes an antibody, a cytotoxic or therapeutic drug, and a linker that enables attachment or conjugation of the drug to the antibody. An ADC typically has anywhere from 1 to 8 drugs conjugated to the antibody, including drug loaded species of 2, 4, 6, or 8. In a preferred embodiment, the ADC of the invention comprises an anti-EGFR antibody conjugated via a linker to a Bcl-xL inhibitor. In a preferred embodiment, the ADC of the invention comprises an anti-EGFR monoclonal IgG antibody conjugated via a linker to a Bcl-xL inhibitor.

[0312] The terms “anti-Epidermal Growth Factor antibody drug conjugate,” “anti-EGFR antibody drug conjugate,” or “anti-EGFR ADC”, used interchangeably herein, refer to an ADC comprising an antibody that specifically binds to EGFR, whereby the antibody is conjugated to one or more chemical agent(s). In one embodiment, an anti-EGFR ADC comprises antibody AbA conjugated to a Bcl-xL inhibitor. In one embodiment, an anti-EGFR ADC comprises antibody AbB conjugated to a Bcl-xL inhibitor. In one embodiment, an anti-EGFR ADC comprises antibody AbK conjugated to a Bcl-xL inhibitor. In one embodiment, an anti-EGFR ADC comprises antibody AbG conjugated to a Bcl-xL inhibitor.

[0313] The term “drug-to-antibody ratio” or “DAR” refers to the number of drugs, e.g., a Bcl-xL inhibitor, attached to the antibody of the ADC. The DAR of an ADC can range from 1 to 8, although higher loads, e.g., 20, are also possible depending on the number of linkage site on an antibody. The term DAR may be used in reference to the number of drugs loaded onto an individual antibody, or, alternatively, may be used in reference to the average or mean DAR of a group of ADCs.

[0314] The term “undesired ADC species”, as used herein, refers to any drug loaded species which is to be separated from an ADC species having a different drug load. In one embodiment, the term undesired ADC species may refer to drug loaded species of 6 or more, i.e., ADCs with a DAR of 6 or more, including DAR6, DAR7, DAR8, and DAR greater than 8 (i.e., drug loaded species of 6, 7, 8, or greater than 8). In a separate embodiment, the term undesired ADC species may refer to drug loaded species of 8 or more, i.e., ADCs with a DAR of 8 or more, including DAR8, and DAR greater than 8 (i.e., drug loaded species of 8, or greater than 8).

[0315] The term “ADC mixture”, as used herein, refers to a composition containing a heterogeneous DAR distribution of ADCs. In one embodiment, an ADC mixture contains ADCs having a distribution of DARs of 1 to 8, e.g., 2, 4, 6, and 8 (i.e., drug loaded species of 2, 4, 6, and 8). Notably, degradation products may result such that DARs of 1, 3, 5, and 7 may also be included in the mixture. Further, ADCs within the mixture may also have DARs greater than 8. The ADC mixture results from interchain disulfide reduction followed by conjugation. In one embodiment,

the ADC mixture comprises both ADCs with a DAR of 4 or less (i.e., a drug loaded species of 4 or less) and ADCs with a DAR of 6 or more (i.e., a drug loaded species of 6 or more).

[0316] The term “cancer” is meant to refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. Examples of cancer include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include small cell lung cancer, glioblastoma, non-small cell lung cancer, lung cancer, colon cancer, colorectal cancer, head and neck cancer, breast cancer (e.g., triple negative breast cancer), pancreatic cancer, squamous cell tumors, squamous cell carcinoma (e.g., squamous cell lung cancer or squamous cell head and neck cancer), anal cancer, skin cancer, and vulvar cancer. In one embodiment, the ADCs of the invention are administered to a patient having a tumor(s) containing amplifications of the EGFR gene, whereby the tumor expresses the truncated version of the EGFR, EGFRvIII. In one embodiment, the ADCs of the invention are administered to a patient having a solid tumor which is likely to overexpress EGFR. In one embodiment, the ADCs of the invention are administered to a patient having squamous cell Non-Small Cell Lung Cancer (NSCLC). In one embodiment, the ADCs of the invention are administered to a patient having solid tumors, including advanced solid tumors.

[0317] The term “EGFR expressing tumor,” as used herein, refers to a tumor which expresses EGFR protein. In one embodiment, EGFR expression in a tumor is determined using immunohistochemical staining of tumor cell membranes, where any immunohistochemical staining above background level in a tumor sample indicates that the tumor is an EGFR expressing tumor. Methods for detecting expression of EGFR in a tumor are known in the art, e.g., the EGFR pharmDx™ Kit (Dako). In contrast, an “EGFR negative tumor” is defined as a tumor having an absence of EGFR membrane staining above background in a tumor sample as determined by immunohistochemical techniques.

[0318] The term “EGFRvIII positive tumor,” as used herein, refers to a tumor which expresses EGFRvIII protein. In one embodiment, EGFRvIII expression in a tumor is determined using immunohistochemical staining of tumor cell membranes, where any immunohistochemical staining above background level in a tumor sample indicates that the tumor is an EGFRvIII expressing tumor.

[0319] Methods for detecting expression of EGFR in a tumor are known in the art, and include immunohistochemical assays. In contrast, an “EGFRvIII negative tumor” is defined as a tumor having an absence of EGFRvIII membrane staining above background in a tumor sample as determined by immunohistochemical techniques.

[0320] The terms “overexpress,” “overexpression,” or “overexpressed” interchangeably refer to a gene that is transcribed or translated at a detectably greater level, usually in a cancer cell, in comparison to a normal cell. Overexpression therefore refers to both overexpression of protein and RNA (due to increased transcription, post transcriptional processing, translation, post translational processing, altered stability, and altered protein degradation), as well as local overexpression due to altered protein traffic patterns (increased nuclear localization), and augmented functional activity, e.g., as in an increased enzyme hydrolysis of substrate. Thus, overexpression refers to either protein or RNA levels. Overexpression can also be by 50%, 60%, 70%, 80%, 90% or more in comparison to a normal cell or comparison cell. In certain embodiments, the anti-EGFR ADCs of the invention are used to treat solid tumors likely to overexpress EGFR.

[0321] The term “administering” as used herein is meant to refer to the delivery of a substance (e.g., an anti-EGFR ADC) to achieve a therapeutic objective (e.g., the treatment of an EGFR-associated disorder). Modes of administration may be parenteral, enteral and topical. Parenteral administration is usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion.

[0322] The term “combination therapy”, as used herein, refers to the administration of two or more therapeutic substances, e.g., an anti-EGFR ADC and an additional therapeutic agent. The additional therapeutic agent may be administered concomitant with, prior to, or following the administration of the anti-EGFR ADC.

[0323] As used herein, the term “effective amount” or “therapeutically effective amount” refers to the amount of a drug, e.g., an antibody or ADC, which is sufficient to reduce or ameliorate the severity and/or duration of a disorder, e.g., cancer, or one or more symptoms thereof, prevent the advancement of a disorder, cause regression of a disorder, prevent the recurrence, development, onset or progression of one or more symptoms associated with a disorder, detect a disorder, or enhance or improve the prophylactic or therapeutic effect(s) of another therapy (e.g., prophylactic or therapeutic agent). The effective amount of an antibody or ADC may, for example, inhibit tumor growth (e.g., inhibit an increase in tumor volume), decrease tumor growth (e.g., decrease tumor volume), reduce the number of cancer cells, and/or relieve to some extent one or more of the symptoms associated with the cancer. The effective amount may, for example, improve disease free survival (DFS), improve overall survival (OS), or decrease likelihood of recurrence.

[0324] Various aspects of the invention are described in further detail in the following subsections.

2. Anti-EGFR Antibody Drug Conjugates (ADCs): Anti-EGFR Antibodies

[0325] One aspect of the invention features an anti-human Epidermal Growth Factor Receptor (anti-hEGFR) Antibody Drug Conjugate (ADC) comprising an anti-hEGFR antibody conjugated to a drug via a linker, wherein the drug is a Bcl-xL inhibitor. Exemplary anti-EGFR antibodies (and sequences thereof) that can be used in the ADCs set forth herein are described below, as well as in US 2015-0337042, incorporated by reference in its entirety herein.

[0326] The anti-EGFR antibodies described herein provide the ADCs of the invention with the ability to bind to EGFR such that the cytotoxic Bcl-xL drug attached to the antibody may be delivered to the EGFR-expressing cell.

[0327] While the term “antibody” is used throughout, it should be noted that antibody fragments (i.e., antigen-binding portions of an anti-EGFR antibody) may also be conjugated to the Bcl-xL inhibitors described herein. Thus, it is within the scope of the invention that in certain embodiments, antibody fragments of the anti-EGFR antibodies described herein are conjugated to Bcl-xL inhibitors (including those described below in Section 3) via linkers (including those described below in Section 4). In certain embodiments, the anti-EGFR antibody binding portion is a Fab, a Fab', a F(ab')₂, a Fv, a disulfide linked Fv, an scFv, a single domain antibody, or a diabody.

[0328] Anti-EGFR antibodies that may be used in the ADCs of the invention have characteristics making them advantageous for use in an ADC. In one embodiment, an anti-EGFR antibody has characteristics including, but not limited to, binding to tumor cells expressing EGFRvIII, binding to wild type EGFR on tumor cells expressing EGFR, recognizing the epitope

CGADSYEMEEDGVRKC (SEQ ID NO: 45) on EGFR, binding to EGFR on normal human epithelial keratinocytes, and decreasing or inhibiting xenograft tumor growth in a mouse model. In one embodiment, an anti-EGFR antibody which may be used in the ADC of the invention is capable of binding an epitope of human EGFR defined by SEQ ID NO: 45 and/or is able to compete with any antibody disclosed herein (e.g., Ab1, AbA, AbB, AbC, AbD, AbE, AbF, AbG, AbH, AbJ, AbK) for binding to human EGFR. Binding of the antibody to EGFR may be assessed according to, e.g. competition assay analysis, as described in US 2015-0337042 A1, incorporated by reference in its entirety herein. In one embodiment of the invention, an anti-EGFR antibody that may be used in an ADC of the invention has a dissociation constant ($K_{sub.d}$) of between about 1×10^{-6} M and about 1×10^{-10} M, as determined by surface plasmon resonance, to 1-525 of EGFR (SEQ ID NO: 47). In other embodiments of the foregoing aspects, the ADC of the invention comprises an anti-EGFR antibody that binds EGFRvIII, binds EGFR on cells overexpressing EGFR, and recognizes the epitope CGADSYEMEEDGVRKC (SEQ ID NO: 45) on EGFR. In a further embodiment, the anti-EGFR antibody binds EGFRvIII at an epitope which is

distinct from the EGFRvIII junctional peptide. In additional embodiments of the foregoing aspects, the anti-EGFR antibody used in an ADC of the invention, does not compete with cetuximab for binding to human EGFR.

[0329] In one embodiment, an ADC of the invention comprises an anti-EGFR antibody that binds to EGFR(1-525) (SEQ ID NO: 47) with a dissociation constant ($K_{sub.d}$) of about $1 \times 10^{sup.-6}$ M or less, as determined by surface plasmon resonance. Alternatively, an anti-EGFR antibody may bind to EGFR (1-525) (SEQ ID NO: 47) with a $K_{sub.d}$ of between about $1 \times 10^{sup.-6}$ M and about $1 \times 10^{sup.-6}$ M, as determined by surface plasmon resonance. In a further alternative, an anti-EGFR antibody binds to EGFR (1-525) (SEQ ID NO: 47) with a $K_{sub.d}$ of between about $1 \times 10^{sup.-6}$ M and about $1 \times 10^{sup.-7}$ M, as determined by surface plasmon resonance. Alternatively, antibodies used in the invention may bind to EGFR (1-525) (SEQ ID NO: 47) with a $K_{sub.d}$ of between about $1 \times 10^{sup.-6}$ M and about $5 \times 10^{sup.-10}$ M; a $K_{sub.d}$ of between about $1 \times 10^{sup.-6}$ M and about $1 \times 10^{sup.-9}$ M; a $K_{sub.d}$ of between about $1 \times 10^{sup.-6}$ M and about $5 \times 10^{sup.-9}$ M; a $K_{sub.d}$ of between about $1 \times 10^{sup.-6}$ M and about $1 \times 10^{sup.-8}$ M; a $K_{sub.d}$ of between about $1 \times 10^{sup.-6}$ M and about $5 \times 10^{sup.-8}$ M; a $K_{sub.d}$ of between about $5.9 \times 10^{sup.-7}$ M and about $1.7 \times 10^{sup.-9}$ M; a $K_{sub.d}$ of between about $5.9 \times 10^{sup.-7}$ M and about $2.2 \times 10^{sup.-7}$ M, as determined by surface plasmon resonance. In certain embodiments, the dissociation constant ($K_{sub.d}$) of the anti-hEGFR antibody used in the ADC of the invention is lower than the dissociation constant for Ab1 but higher than the dissociation constant of anti-EGFR antibody cetuximab (i.e., the antibody binds to EGFR more tightly than Ab1 but not as tightly as cetuximab).

[0330] One advantage of the anti-EGFR antibodies described herein, is that the antibodies are capable of binding to tumor cells expressing EGFRvIII, thus making the ADCs of the invention specific for malignant cells. While EGFRvIII is associated with certain types of cancer, many anti-EGFR antibodies known in the art, e.g., cetuximab, are not effective at inhibiting or decreasing tumor growth in EGFRvIII expressing tumors. Thus, in one embodiment, an antibody used in an ADC of the invention binds to EGFRvIII (SEQ ID NO: 33) with a $K_{sub.d}$ of about $8.2 \times 10^{sup.-9}$ M or less, as determined by surface plasmon resonance. Alternatively, an antibody used in an ADC of the invention binds to EGFRvIII (SEQ ID NO: 33) with a $K_{sub.d}$ of between about $8.2 \times 10^{sup.-9}$ M and about $6.3 \times 10^{sup.-10}$ M; a $K_{sub.d}$ of between about $8.2 \times 10^{sup.-9}$ M and about $2.0 \times 10^{sup.-9}$ M; a $K_{sub.d}$ of between about $2.3 \times 10^{sup.-9}$ M and about $1.5 \times 10^{sup.-10}$ M, as determined by surface plasmon resonance.

[0331] An anti-EGFR antibody used in an ADC of the invention is able, in one embodiment, to inhibit or decrease tumor growth in an in vivo xenograft mouse model. For example, in certain embodiments, an anti-EGFR antibody is able to inhibit tumor growth by at least about 50% in an in vivo human non-small-cell lung carcinoma (NSCLC) xenograft assay relative to a human IgG antibody which is not specific for EGFR. In certain embodiments, an anti-EGFR antibody is able to inhibit or decrease tumor growth in an in vivo human non-small-cell lung carcinoma (NSCLC) xenograft assay relative to a human IgG antibody which is not specific for EGFR by at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, or at least about 80%, when administered at the same dose and dosing periodicity.

[0332] The term a “xenograft assay”, as used herein, refers to a human tumor xenograft assay, wherein human tumor cells are transplanted, either under the skin or into the organ type in which the tumor originated, into immunocompromised mice that do not reject human cells.

[0333] It should be noted that anti-EGFR antibodies having combinations of the aforementioned characteristics are also considered to be embodiments of the invention. For example, an anti-EGFR antibody may bind to EGFR(1-525) (SEQ ID NO: 47) with a dissociation constant ($K_{sub.d}$) of about $1 \times 10^{sup.-6}$ M or less, as determined by surface plasmon resonance, and bind to an epitope within the amino acid sequence CGADSYEMEEDGVRKC (SEQ ID NO: 45) and compete with Ab1 (or an anti-EGFR antibody comprising a heavy chain variable domain comprising the amino

acid sequence set forth in SEQ ID NO: 1 and a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 5) for binding to EGFRvIII (SEQ ID NO: 33) in a competitive binding assay. In certain embodiments, an anti-EGFR ADC of the invention comprises an anti-EGFR antibody that binds to an epitope within the amino acid sequence CGADSYEMEEDGVRKC (SEQ ID NO: 45) and competes with Ab1 (or an anti-EGFR antibody comprises a heavy chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 1 and a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 5) for binding to EGFRvIII (SEQ ID NO: 33) in a competitive binding assay; and bind to EGFRvIII (SEQ ID NO: 33) with a $K_{sub.D}$ of about 8.2×10^{-9} M or less, as determined by surface plasmon resonance.

[0334] In one embodiment, anti-EGFR antibodies used in an ADC of the invention exhibits a high capacity to reduce or to neutralize EGFR activity, e.g., as assessed by any one of several in vitro and in vivo assays known in the art. For example, inhibition of phosphorylation of EGFR in an EGFR expressing cell line, e.g., the h292 cell line, can be measured. In certain embodiments, an anti-EGFR antibody binds human EGFR, wherein the antibody dissociates from human EGFR (EGFR 1-525) with a $K_{sub.D}$ rate constant of about 5.9×10^{-7} M or less, as determined by surface plasmon resonance. In a further embodiment, the antibody may dissociate from human EGFR (1-525) with a $K_{sub.D}$ rate constant of about 4.2×10^{-7} M, as determined by surface plasmon resonance. Alternatively, the antibody may dissociate from human EGFR (1-525) with a $k_{sub.off}$ rate constant of about 2.5×10^{-7} M, as determined by surface plasmon resonance. In certain embodiments, the anti-EGFR antibodies of the invention have a $K_{sub.D}$ rate constant of between 5.9×10^{-7} M and 5×10^{-9} M. Alternatively, the antibody may dissociate from human EGFRvIII with a $K_{sub.D}$ rate constant of about 6.1×10^{-9} M or less, as determined by surface plasmon resonance. Alternatively, the antibody may dissociate from human EGFRvIII with a $K_{sub.D}$ rate constant of about 3.9×10^{-9} M or less, as determined by surface plasmon resonance. Alternatively, the antibody may dissociate from human EGFRvIII with a $K_{sub.D}$ rate constant of about 2.3×10^{-9} M or less, as determined by surface plasmon resonance.

[0335] Exemplary anti-EGFR antibodies that may be used in the ADCs described herein include, but are not limited to, Antibody 1 (Ab1), Antibody A (AbA), Antibody B (AbB), Antibody C (AbC), Antibody D (AbD), Antibody E (AbE), Antibody F (AbF), Antibody G (AbG), Antibody H (AbH), Antibody J (AbJ), Antibody K (AbK), Antibody L (AbL), Antibody M (AbM), Antibody N (AbN), Antibody O (AbO), Antibody P (AbP), and Antibody Q (AbQ).

[0336] In one embodiment, the invention features an anti-EGFR ADC comprising Ab1 conjugated via a linker to a Bcl-xL inhibitor. Ab1 is a humanized anti-EGFR antibody. The light and heavy chain sequences of Ab1 are described in SEQ ID NO: 13 and SEQ ID NO: 14, respectively (see also US Patent Application Publication No. 20120183471, incorporated by reference herein). The light chain variable region of Ab1 is described in SEQ ID NO: 5, and comprises a CDR1 amino acid sequence set forth in SEQ ID NO: 6, a CDR2 amino acid sequence set forth in SEQ ID NO: 7, and a CDR3 amino acid sequence set forth in SEQ ID NO: 8. The heavy chain variable region of Ab1 is described in SEQ ID NO: 1, and comprises a CDR1 amino acid sequence set forth in SEQ ID NO: 2, a CDR2 amino acid sequence set forth in SEQ ID NO: 3, and a CDR3 amino acid sequence set forth in SEQ ID NO: 4. In one embodiment, an ADC of the invention comprises an anti-EGFR antibody that binds to an epitope within the amino acid sequence set forth in SEQ ID NO: 45 and competes with an anti-EGFR antibody comprising a heavy chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 1 and a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 5 for binding to EGFRvIII in a competitive binding assay.

[0337] In one embodiment, the invention features an anti-hEGFR ADC comprising an anti-hEGFR antibody which is antibody AbA conjugated via a linker to a Bcl-xL inhibitor. The term "AbA" is

meant to include an IgG antibody having at least the six CDRs of AbA. The AbA antibody has the same light chain as that of Ab1, but has a heavy chain containing six amino acid sequence changes relative to parental antibody Ab1 (four amino acid changes in the variable region and two changes in the constant region of the heavy chain). The AbA antibody comprises a heavy chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 12, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 11, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 10, and a light chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 8, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 7, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 6. The heavy chain variable region of AbA is defined by the amino acid sequence set forth in SEQ ID NO: 9, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 5. The full length heavy chain of antibody AbA is set forth in the amino acid sequence described in SEQ ID NO: 15, while the full length light chain of antibody AbA is set forth in the amino acid sequence described in SEQ ID NO: 13 (see FIG. 3). The nucleic acid sequence of the heavy chain of AbA is provided below:

TABLE-US-00005 (SEQ ID NO: 86) gaggtgcaactccaagagagcgggccccggcctcgtgaagccctctcagac
tctgtccctgacttgcaactgtgagcgggtattccatcagcagagacttcg
catggaactggatccgccagcctcccggtaagggactggagtggatgggg
tacatcagctacaacggtaatacacgctatcagccctccctgaagtctcg
cattaccattagtcgcatcctccaagaaccagttctttctgaaactca
acagcgtgacagccgctgacaccgccactactgctgaccgccagc
aggggggttcccttactggggccagggcactctgggtcacggtttcttctgc
gtcgaccaagggcccatcggtcttccccctggcacccctcctccaagagca
cctctgggggacagcggccctgggctgcctgggtcaaggactacttccc
gaaccggtgacgggtgctggtgaactcaggcgccctgaccagcggcgtgca
caccttccgggtgctctacagtcctcaggactctactccctcagcagcg
tggtgaccgtgccctccagcagcttgggcacccagacctacatctgcaac
gtgaatcacaagcccagcaacaccaaggtggacaagaaagttgagcccaa
atcttgtagaaaaactcacacatgccaccgtgccagcacctgaactcc
tggggggaccgtcagttcttcttccccccaaaacccaaggacaccctc
atgatctccggaccctgaggtcacatgctggtgggtggacgtgagcca
cgaagacctgaggtcaagttcaactggtacgtggacggcgtggaggtgc
ataatgccaagacaaagccgcgggaggagcagtacaacagcacgtaccgt
gtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaagga
gtacaagtgaaggttccaacaaagccctccagcccccatcgagaaaa
ccatctccaaagccaaagggcagccccgagaaccacaggtgtacaccctg
cccccatcccgcgaggagatgaccaagaaccaggtcagcctgacctgcct
ggtaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatg
ggcagccggagaacaactacaagaccacgcctcccgctgctggactccgac
ggctccttcttctctacagcaagctcaccgtggacaagagcaggtggca
gcaggggaacgttcttctcatgctccgtgatgcatgaggctctgcacaacc actacacgcagaagagcctctccctgtctccgggtaaa

The nucleic acid sequence of the light chain of AbA is provided below:

TABLE-US-00006 (SEQ ID NO: 87) Gacatccagatgaccagtccccctccagtatgtctgtgtctgtgggcga
ccgtgtgaccattacctgccactcctcccaggacatcaatagcaatatcg
gttggttgcaacagaagccaggcaagtccttcaaagggctgatttaccat
ggtaccaacctggacgacgggggttctagtcgtttcagcggctccgggtc
cggaaccgattacactctgaccatcagcagtttgagcctgaggactttg
ctacctattattgtgtgagctacgctcagttcccatggacttccggcggg
ggcaccaaaactggagatcaaactgacgggtggctgcaccatctgtcttcat
cttcccgccatctgatgagcagttgaaatctggaactgcctctgtgtgt

gcctgctgaataacttctatcccagagagggccaaagtacagtgggaaggtg
gataacgccctccaatcgggtaactcccaggagagtgtcacagagcagga
cagcaaggacagcacctacagcctcagcagcaccctgacgctgagcaaag
cagactacgagaaacacaaagtctacgcctgcgaagtcacccatcagggc
ctgagctcgcccgtcacaaagagcttcaacaggggagagtgt

The amino acid sequence of the heavy chain of AbA is provided below:

TABLE-US-00007 (SEQ ID NO: 15)

EVQLQESGPGLVKPSQTLSTCTVSGYSISRDFAWNWIRQPPGKGLEWMG
YISYNGNTRYQPSLKSRITISRDTSKNQFFLKLNSVTAADTATYYCVTAS
RGFPYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFP
EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN
VNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL
MISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL
PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSD
GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG

In another embodiment, the amino acid sequence of the heavy chain of AbA is provided below:

TABLE-US-00008 (SEQ ID NO: 102)

EVQLQESGPGLVKPSQTLSTCTVSGYSISRDFAWNWIRQPPGKGLEWMG
YISYNGNTRYQPSLKSRITISRDTSKNQFFLKLNSVTAADTATYYCVTAS
RGFPYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFP
EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN
VNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL
MISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL
PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSD
GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

The amino acid sequence of the light chain of AbA is provided below:

TABLE-US-00009 (SEQ ID NO: 13)

DIQMTQSPSSMSVSVGDRVTITCHSSQDINSNIGWLQQKPGKSFKGLIYH
GTNLDGVPSPRFSGSGSGTDYTLTISSLQPEDFATYYCVQYAQFPWTFGG
GTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV
DNALQSGNSQESVTEQDSKSTYSLSTLTLSKADYEKHKVYACEVTHQG
LSSPVTKSFNRGEC

[0338] FIGS. 2 and 3 provide an alignment of the amino acid sequences of the VH and VL regions (FIG. 2) and the complete heavy and light chains (FIG. 3) of Ab1 and AbA. The light chain amino acid sequences of Ab1 and AbA are the same (SEQ ID NO: 13). The heavy chain amino acid sequences of Ab1 and AbA, however, have six amino acid differences between the two sequences, three of which are in the CDRs. Differences between the Ab1 VH amino acid sequence and the AbA VH amino acid sequence are shaded in FIG. 2 and are found in each of the VH CDRs. The CDR1 domain of the variable heavy chain of AbA included an amino acid change from a serine (Ab1) to an arginine. The CDR2 domain of the variable heavy chain included an amino acid change from a serine in Ab1 to an asparagine in AbA. Finally, the CDR3 domain of the variable heavy chain included an amino acid change from a glycine in Ab1 to a serine in AbA. Two of the amino acid changes within AbA are in the constant region of the heavy chain (D354E and L356M). The Fc region amino acid mutations in AbA represent human IgG allotype changes from a z, a allotype to a z, non-a allotype.

[0339] In addition to the other changes, the first amino acid was changed from a glutamine (Q) to a glutamic acid (E), as described, for example, in FIG. 3.

[0340] Thus, in one embodiment, the invention features an ADC comprising an anti-hEGFR

antibody conjugated via a linker to a Bcl-xL inhibitor wherein the antibody comprises a heavy chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 12, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 11, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 10, and a light chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 8, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 7, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 6. In one embodiment, the invention features an ADC comprising an anti-hEGFR antibody conjugated via a linker to a Bcl-xL inhibitor, wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 9, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 5.

[0341] In one embodiment, the invention features an anti-EGFR ADC comprising antibody AbB conjugated via a linker to a Bcl-xL inhibitor. The AbB antibody comprises a heavy chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 19, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 17, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 16, and a light chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 8, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 7, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 6. In further embodiments, the invention provides an antibody having a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 64 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 65. Thus, in one embodiment, the ADC of the invention comprises an anti-hEGFR antibody having the CDR amino acid sequences of AbB. In a separate embodiment, the ADC of the invention comprises an anti-hEGFR antibody having heavy and light chain variable regions comprising the amino acid sequences of AbB.

[0342] In one embodiment, the invention features an anti-EGFR ADC comprising antibody AbC conjugated via a linker to a Bcl-xL inhibitor. The AbC antibody comprises a heavy chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 4, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 3, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 2, and a light chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 84, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 7, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 6. In further embodiments, the invention provides an antibody having a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 66 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 67. Thus, in one embodiment, the ADC of the invention comprises an anti-hEGFR antibody having the CDR amino acid sequences of AbC. In a separate embodiment, the ADC of the invention comprises an anti-hEGFR antibody having heavy and light chain variable regions comprising the amino acid sequences of AbC.

[0343] In one embodiment, the invention features an anti-EGFR ADC comprising antibody AbD conjugated via a linker to a Bcl-xL inhibitor. The AbD antibody comprises a heavy chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 4, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 3, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 2, and a light chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 31, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 83, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 82. In further embodiments, the invention provides an antibody having a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 68 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 69. Thus, in one embodiment, the ADC of the invention comprises an anti-hEGFR antibody having the CDR amino acid

sequences of AbD. In a separate embodiment, the ADC of the invention comprises an anti-hEGFR antibody having heavy and light chain variable regions comprising the amino acid sequences of AbD.

[0344] In one embodiment, the invention features an anti-EGFR ADC comprising antibody AbE conjugated via a linker to a Bcl-xL inhibitor. The AbE antibody comprises a heavy chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 4, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 3, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 2, and a light chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 85, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 27, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 82. In further embodiments, the invention provides an antibody having a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 50 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 51. Thus, in one embodiment, the ADC of the invention comprises an anti-hEGFR antibody having the CDR amino acid sequences of AbE. In a separate embodiment, the ADC of the invention comprises an anti-hEGFR antibody having heavy and light chain variable regions comprising the amino acid sequences of AbE.

[0345] In one embodiment, the invention features an anti-EGFR ADC comprising antibody AbF conjugated via a linker to a Bcl-xL inhibitor. The AbF antibody comprises a heavy chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 12, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 3, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 10, and a light chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 8, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 7, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 6. In further embodiments, the invention provides an antibody having a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 52 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 53. Thus, in one embodiment, the ADC of the invention comprises an anti-hEGFR antibody having the CDR amino acid sequences of AbF. In a separate embodiment, the ADC of the invention comprises an anti-hEGFR antibody having heavy and light chain variable regions comprising the amino acid sequences of AbF.

[0346] In one embodiment, the invention features an anti-EGFR ADC comprising antibody AbG conjugated via a linker to a Bcl-xL inhibitor. The AbG antibody comprises a heavy chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 18, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 17, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 16, and a light chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 25, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 24, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 23. In further embodiments, the invention provides an antibody having a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 72 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 73. Thus, in one embodiment, the ADC of the invention comprises an anti-hEGFR antibody having the CDR amino acid sequences of AbG. In a separate embodiment, the ADC of the invention comprises an anti-hEGFR antibody having heavy and light chain variable regions comprising the amino acid sequences of AbG.

[0347] In one embodiment, the invention features an anti-EGFR ADC comprising antibody AbH conjugated via a linker to a Bcl-xL inhibitor. The AbH antibody comprises a heavy chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 18, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 11, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 80, and a light chain variable region

comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 25, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 24, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 23. In further embodiments, the invention provides an antibody having a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 54 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 55. Thus, in one embodiment, the ADC of the invention comprises an anti-hEGFR antibody having the CDR amino acid sequences of AbH. In a separate embodiment, the ADC of the invention comprises an anti-hEGFR antibody having heavy and light chain variable regions comprising the amino acid sequences of AbH.

[0348] In one embodiment, the invention features an anti-EGFR ADC comprising antibody AbJ conjugated via a linker to a Bcl-xL inhibitor. The AbJ antibody comprises a heavy chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 18, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 3, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 80, and a light chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 25, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 24, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 23. In further embodiments, the invention provides an antibody having a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 56 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 57. Thus, in one embodiment, the ADC of the invention comprises an anti-hEGFR antibody having the CDR amino acid sequences of AbJ. In a separate embodiment, the ADC of the invention comprises an anti-hEGFR antibody having heavy and light chain variable regions comprising the amino acid sequences of AbJ.

[0349] In one embodiment, the invention features an anti-EGFR ADC comprising antibody AbK conjugated via a linker to a Bcl-xL inhibitor. The AbK antibody comprises a heavy chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 19, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 11, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 10, and a light chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 28, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 27, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 26. In further embodiments, the invention provides an antibody having a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 74 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 75. Thus, in one embodiment, the ADC of the invention comprises an anti-hEGFR antibody having the CDR amino acid sequences of AbK. In a separate embodiment, the ADC of the invention comprises an anti-hEGFR antibody having heavy and light chain variable regions comprising the amino acid sequences of AbK.

[0350] In one embodiment, the invention features an anti-EGFR ADC comprising antibody AbL conjugated via a linker to a Bcl-xL inhibitor. The AbL antibody comprises a heavy chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 18, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 11, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 80, and a light chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 28, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 27, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 26. In further embodiments, the invention provides an antibody having a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 58 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 59. Thus, in one embodiment, the ADC of the invention comprises an anti-hEGFR antibody having the CDR amino acid sequences of AbL. In a separate embodiment, the ADC of the invention comprises an anti-hEGFR antibody having heavy and light chain variable regions comprising the amino acid

sequences of AbL.

[0351] In one embodiment, the invention features an anti-EGFR ADC comprising antibody AbM conjugated via a linker to a Bcl-xL inhibitor. The AbM antibody comprises a heavy chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 12, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 11, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 20, and a light chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 28, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 27, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 26. In further embodiments, the invention provides an antibody having a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 76 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 77. Thus, in one embodiment, the ADC of the invention comprises an anti-hEGFR antibody having the CDR amino acid sequences of AbM. In a separate embodiment, the ADC of the invention comprises an anti-hEGFR antibody having heavy and light chain variable regions comprising the amino acid sequences of AbM.

[0352] In one embodiment, the invention features an anti-EGFR ADC comprising antibody AbN conjugated via a linker to a Bcl-xL inhibitor. The AbN antibody comprises a heavy chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 12, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 3, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 20, and a light chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 28, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 27, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 26. In further embodiments, the invention provides an antibody having a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 60 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 61. Thus, in one embodiment, the ADC of the invention comprises an anti-hEGFR antibody having the CDR amino acid sequences of AbN. In a separate embodiment, the ADC of the invention comprises an anti-hEGFR antibody having heavy and light chain variable regions comprising the amino acid sequences of AbN.

[0353] In one embodiment, the invention features an anti-EGFR ADC comprising antibody AbO conjugated via a linker to a Bcl-xL inhibitor. The AbO antibody comprises a heavy chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 12, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 11, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 80, and a light chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 28, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 27, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 26. In further embodiments, the invention provides an antibody having a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 62 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 63. Thus, in one embodiment, the ADC of the invention comprises an anti-hEGFR antibody having the CDR amino acid sequences of AbO. In a separate embodiment, the ADC of the invention comprises an anti-hEGFR antibody having heavy and light chain variable regions comprising the amino acid sequences of AbO.

[0354] In one embodiment, the invention features an anti-EGFR ADC comprising antibody AbP conjugated via a linker to a Bcl-xL inhibitor. The AbP antibody comprises a heavy chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 22, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 3, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 21, and a light chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 31, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 30, and a CDR1 domain comprising

the amino acid sequence of SEQ ID NO: 29. In further embodiments, the invention provides an antibody having a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 78 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 79. Thus, in one embodiment, the ADC of the invention comprises an anti-hEGFR antibody having the CDR amino acid sequences of AbP. In a separate embodiment, the ADC of the invention comprises an anti-hEGFR antibody having heavy and light chain variable regions comprising the amino acid sequences of AbP.

[0355] In one embodiment, the invention features an anti-EGFR ADC comprising antibody AbQ conjugated via a linker to a Bcl-xL inhibitor. The AbQ antibody comprises a heavy chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 22, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 11, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 81, and a light chain variable region comprising a CDR3 domain comprising the amino acid sequence of SEQ ID NO: 31, a CDR2 domain comprising the amino acid sequence of SEQ ID NO: 30, and a CDR1 domain comprising the amino acid sequence of SEQ ID NO: 29. In further embodiments, the invention provides an antibody having a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 70 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 71. Thus, in one embodiment, the ADC of the invention comprises an anti-hEGFR antibody having the CDR amino acid sequences of AbQ. In a separate embodiment, the ADC of the invention comprises an anti-hEGFR antibody having heavy and light chain variable regions comprising the amino acid sequences of AbQ.

[0356] As described in Table 2, shown below, the antibody sequences disclosed herein provide amino acid consensus sequences that represent CDR domains resulting in improved binding to the Ab1 EGFR epitope. Thus, in one embodiment, the invention features an anti-EGFR antibody comprising a light chain variable region comprising a CDR3 domain comprising the amino acid sequence set forth as SEQ ID NO: 40, a CDR2 domain comprising the amino acid sequence set forth as SEQ ID NO: 39, and a CDR1 domain comprising the amino acid sequence set forth as SEQ ID NO: 38; and a heavy chain variable region comprising a CDR3 domain comprising the amino acid sequence set forth as SEQ ID NO: 37, a CDR2 domain comprising the amino acid sequence set forth as SEQ ID NO: 36, and a CDR1 domain comprising the amino acid sequence set forth as SEQ ID NO: 35. In a further embodiment, the anti-EGFR antibody of the invention comprises a heavy chain variable region comprising a CDR3 domain comprising an amino acid sequence as set forth in SEQ ID NO: 12, 18, 19, and 22; a CDR2 domain comprising an amino acid sequence as set forth in SEQ ID NO: 11 or 17; and a CDR1 domain comprising an amino acid sequence as set forth in SEQ ID NO: 10, 16, 20, and 21; and a light chain variable region comprising a CDR3 domain comprising an amino acid sequence as set forth in SEQ ID NO: 8, 25, 28, and 31; a CDR2 domain comprising an amino acid sequence as set forth in SEQ ID NO: 7, 24, 27, and 30; and a CDR1 domain comprising an amino acid sequence as set forth in SEQ ID NO: 6, 23, 26, and 29.

TABLE-US-00010 TABLE 2 Heavy and Light Chain CDR Sequence Comparison of Ab1 vs. AbA, AbG, AbK, AbM, and AbP Variants

HEAVY CHAIN CDRS	SEQ	SEQ	SEQ	Variable Heavy Chain ID	ID	ID	(VH)	CDR1 NO:	VH	CDR2 NO:	VH	CDR3 NO:
Ab1	G	Y	S	I	S	S	D	F	A	W	N	2
AbA	R	10	N	11	S	12	AbG	N	16	K	17	S
AbK	R	10	N	11	S	W	19	AbM	G	R	20	N
AbP	H	21	3	S	W	L	W	22	LIGHT CHAIN CDRS	SEQ	Variable	Light Chain ID
(VL)	CDR1 NO:	VL	CDR2	SEQ	ID	NO:	VL	CDR3	SEQ	ID	NO:	Ab1
S	N	I	G	6	H	G	T	N	L	D	D	7
Y	F	P	W	T	8	AbA	6	7	8	AbG	T	Y
23	A	24	E	25	Ab	T	Y	V	26	S	H	27
28	AbM	T	Y	V	26	S	H	27	28	AbP	M	V
29	A	I	30	E	31							

[0357] In one embodiment, the ADC of the invention includes an anti-hEGFR antibody comprises a heavy chain variable region comprising an amino acid sequence selected from the group

consisting of 50, 52, 53, 56, 58, 60, 62, 64, 66, and 68; and a light chain variable region comprising an amino acid sequence selected from the group consisting of 51, 53, 55, 57, 59, 61, 63, 65, 67, and 69.

[0358] The foregoing anti-EGFR antibody CDR sequences establish a novel family of EGFR binding proteins, isolated in accordance with this invention, and comprising polypeptides that include the CDR sequences listed in Tables 2-4.

[0359] Table 2, above, provides an alignment of the amino acid sequences of the heavy and light chain CDRs for Ab1 variant antibodies AbA, AbG, AbK, AbM, and AbP in comparison to Ab1.

[0360] As described in Table 3, below, the Ab1 variant antibodies AbA, AbG, AbK, AbM, AbP each has a serine residue in the variable heavy chain of CDR3 in place of a glycine (shown in bold/underlined in Table 3).

TABLE-US-00011 TABLE 3 CDR Consensus Sequences for Ab1 Variants from Table 2

CDR	CDR Consensus	region SEQ ID NO:	Sequences for Ab1 Variants												
VH CDR1	SEQ ID NO: 35	W N	VH CDR2	SEQ ID NO: 36	P S L K S	VH CDR3	SEQ ID NO: 37	VL CDR1	SEQ ID NO: 38	(I/V)G	VL CDR2	SEQ ID NO: 39	V Q Y(A/D)(Q/E/D)	F P W T	NO: 40

[0361] A comparison of the VH and VL CDR sequences of Ab1 versus antibodies AbB, AbC, AbD, AbE, AbF, AbH, AbJ, AbL, AbN, AbO, and AbQ is described in Table 4. In addition to the CDR changes described in Table 4, below, AbG has an amino acid residue change within the framework 2 regions of the VH.

[0362] In one embodiment, the invention includes an anti-hEGFR antibody comprising a heavy chain variable region comprising an amino acid sequence selected from the group consisting of 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, and 78; and a light chain variable region comprising an amino acid sequence selected from the group consisting of 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, and 79.

[0363] In one embodiment, the invention includes an anti-hEGFR antibody comprising an HC CDR set (CDR1, CDR2, and CDR3) selected from the group consisting of SEQ ID NOs: 10, 11, and 12; SEQ ID NOs: 16, 17, and 18; SEQ ID NOs: 10, 11, and 19; SEQ ID NOs: 20, 11, and 12; SEQ ID NOs: 21, 3, and 22; SEQ ID NOs: 16, 17, and 19; SEQ ID NOs: 2, 3, and 4; SEQ ID NOs: 10, 3, and 12; SEQ ID NOs: 80, 11, and 18; SEQ ID NOs: 80, 3, and 18; SEQ ID NOs: 20, 3, and 12; SEQ ID NOs: 80, 11, and 12; and SEQ ID NOs: 81, 11, and 22; and an LC light chain CDR set (CDR1, CDR2, and CDR3) selected from the group consisting of SEQ ID NOs: 6, 7, and 8; SEQ ID NOs: 23, 24, and 25; SEQ ID NOs: 26, 27, and 28; SEQ ID NOs: 29, 30, and 31; SEQ ID NOs: 6, 7, and 84; SEQ ID NOs: 82, 83, and 31; and SEQ ID NOs: 82, 27, and 85, wherein the antibody, or antigen binding portion thereof, does not comprise both the HC CDR set of SEQ ID NOs: 2, 3, and 4, and the LC CDR set of SEQ ID NOs: 6, 7, and 8. In one embodiment, the invention includes an anti-hEGFR antibody comprising an LC CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 40, an LC CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 39, and an LC CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 38; and an HC CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 37, an HC CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 36, and an HC CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 35.

TABLE-US-00012 TABLE 4 Heavy and Light Chain CDR Sequence Comparison of Ab1 vs. Certain Ab1 Variants

HEAVY CHAIN CDRS	SEQ ID NO:	HEAVY CHAIN CDRS	SEQ ID NO:	HEAVY CHAIN CDRS	SEQ ID NO:	HEAVY CHAIN CDRS	SEQ ID NO:
Variable Heavy Chain	ID NO: 10	ID NO: 11	ID NO: 12	ID NO: 16	ID NO: 17	ID NO: 18	ID NO: 19
Y S I S S D F A W N	2	Y I S	Y S G N T R Y	Q P S L K S	3	A G R G F P Y	4
AbB	N 16	K 17	S W 19	AbC	2	3	4
AbD	2	3	4	AbE	2	3	4
AbF	R 10	3	S 12	AbH	G K	80	N 11
AbJ	G K 80	3	S L 18	AbL	G K 80	N 11	S L 18
AbN	G R 20	3	S 12	AbO	G K	80	N 11

80 N 11 S 12 AbQ H 81 N 11 S W L W 22 LIGHT CHAIN CDRS SEQ Variable Light
Chain ID (VL) CDR1 NO: VL CDR2 SEQ ID NO: VL CDR3 SEQ ID NO: Ab1 H S
S Q D I N S N I G 6 H G T N L D D 7 V Q Y A Q F P W T 8 AbB 6 7 8 AbC
6 7 E 84 AbD L 82 A H 83 E 31 AbE L 82 S H 27 D 85 AbF 6 7 8 AbH T Y 23 A 24 D
E 25 AbJ T Y 23 A 24 D E 25 AbL T Y V 26 S H 27 D D 28 AbN T Y V 26 S H 27 D D 28 AbO T
Y V 26 S H 27 D D 28 AbQ M V 29 A I 30 E 31

[0364] The full length heavy and light chain sequences of AbB are provided below:

TABLE-US-00013 AbB Heavy chain (SEQ ID NO: 90)

EVQLQESGPGGLVKPSQTLSTCTVSGYSISNDFAWNWIQQPGKGLEWMG
YISYKGNTRYQPSLKSRITISRDTSKNQFFLKLNSVTAADTATYYCVTAS
RGFPWWGQGTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFP
EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN
VNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL
MISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
VVSVELTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL
PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD
GSFFLYSKLTVDKSRWQQGNVVFSCSVMHEALHNHYTQKSLSLSPGK

[0365] In one embodiment, the above AbB heavy chain sequence contains two alanine substitutions at the positions marked with two bold leucines (see also SEQ ID NO: 91).

TABLE-US-00014 AbB Light chain (SEQ ID NO: 92)

DIQMTQSPSSMSVSVGDRVITITCHSSQDINSNIGWLQKPGKSFKGLIYH
GTNLDDGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCVQYAQFPWTFGG
GTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV
DNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQG
LSSPVTKSFNRGEC

[0366] In one embodiment, the ADC comprises an anti-EGFR antibody comprising a heavy chain comprising SEQ ID NO: 90 or 91 and a light chain comprising SEQ ID NO: 92.

[0367] The full length heavy and light chain sequences of AbG are provided below:

TABLE-US-00015 AbG Heavy chain (SEQ ID NO: 93)

EVQLQESGPGGLVKPSQTLSTCTVSGYSISNDFAWNWIQLPGKGLEWMG
YISYKGNTRYQPSLKSRITISRDTSKNQFFLKLNSVTAADTATYYCVTAS
RGLPYWGQGTLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFP
EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN
VNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL
MISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
VVSVELTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL
PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD
GSFFLYSKLTVDKSRWQQGNVVFSCSVMHEALHNHYTQKSLSLSPGK

[0368] In one embodiment, the above AbG heavy chain sequence contains two alanine substitutions at the positions marked with two bold leucines (see also SEQ ID NO: 94).

TABLE-US-00016 AbG Light chain (SEQ ID NO: 95)

DIQMTQSPSSMSVSVGDRVITITCHSSQDITYNIGWLQKPGKSFKGLIYH
GANLDDGVPSRFSGSGSGTDYTLTISSLQPEDFATYYCVQYDEFPWTFGG
GTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV
DNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVTHQG
LSSPVTKSFNRGEC

[0369] In one embodiment, the ADC comprises an anti-EGFR antibody comprising a heavy chain comprising SEQ ID NO: 93 or 94 and a light chain comprising SEQ ID NO: 95.

[0370] The full length heavy and light chain sequences of AbK are provided below:

TABLE-US-00017 AbK Heavy chain (SEQ ID NO: 96)

EVQLQESGPGQLVKPSQTLTLTCTVSGISRDFAWNWIRQPPGKGLEWMG
YISYNGNTRYQPSLKSRTISRDTSKNQFFLKLNSVTAADTATYYCVTAS
RGFPWWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFP
EPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN
VNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL
MISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYR
VVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL
PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD
GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[0371] In one embodiment, the above AbK heavy chain sequence contains two alanine substitutions at the positions marked with two bold leucines (see also SEQ ID NO: 97).

TABLE-US-00018 AbK Light chain (SEQ ID NO: 98)

DIQMTQSPSSMSVSVGDRVITITCHSSQDITYNVGWLQQKPGKSKGLIYH
GSNLDHGVPSRFSGSGSGTDYTLTISSLPEDFATYYCVQYDDFPWTFGG
GTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKV
DNALQSGNSQESVTEQDSKSTYSLSSTLTLSKADYEKHKVYACEVTHQG
LSSPVTKSFNRGEC

[0372] In one embodiment, the ADC comprises an anti-EGFR antibody comprising a heavy chain comprising SEQ ID NO: 96 or 97 and a light chain comprising SEQ ID NO: 98.

[0373] To generate and to select CDRs having preferred EGFR binding and/or neutralizing activity with respect to hEGFR, standard methods known in the art for generating antibodies, or antigen binding portions thereof, and assessing the EGFR binding and/or neutralizing characteristics of those antibodies, or antigen binding portions thereof, may be used, including but not limited to those specifically described herein.

[0374] In certain embodiments, the antibody comprises a heavy chain constant region, such as an IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM, or IgD constant region. In certain embodiments, the anti-EGFR antibody comprises a heavy chain immunoglobulin constant domain selected from the group consisting of a human IgG constant domain, a human IgM constant domain, a human IgE constant domain, and a human IgA constant domain. In further embodiments, the antibody, or antigen binding portion thereof, has an IgG1 heavy chain constant region, an IgG2 heavy chain constant region, an IgG3 constant region, or an IgG4 heavy chain constant region. Preferably, the heavy chain constant region is an IgG1 heavy chain constant region or an IgG4 heavy chain constant region. Furthermore, the antibody can comprise a light chain constant region, either a kappa light chain constant region or a lambda light chain constant region. In one embodiment, the antibody comprises a kappa light chain constant region.

[0375] In certain embodiments, the anti-EGFR antibody is a multispecific antibody, e.g. a bispecific antibody.

[0376] In certain embodiments, the anti-EGFR antibody comprises a heavy chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 41 and/or a light chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 43.

[0377] Replacements of amino acid residues in the Fc portion to alter antibody effector function have been described (Winter, et al. U.S. Pat. Nos. 5,648,260 and 5,624,821, incorporated by reference herein). The Fc portion of an antibody mediates several important effector functions e.g. cytokine induction, ADCC, phagocytosis, complement dependent cytotoxicity (CDC) and half-life/clearance rate of antibody and antigen-antibody complexes. In some cases these effector functions are desirable for therapeutic antibody but in other cases might be unnecessary or even deleterious, depending on the therapeutic objectives. Certain human IgG isotypes, particularly IgG1 and IgG3, mediate ADCC and CDC via binding to FcγRs and complement C1q, respectively. Neonatal Fc receptors (FcRn) are the critical components determining the circulating half-life of antibodies. In still another embodiment at least one amino acid residue is replaced in the constant

region of the antibody, for example the Fc region of the antibody, such that effector functions of the antibody are altered.

[0378] One embodiment of the invention includes a labeled anti-EGFR antibody where the antibody is derivatized or linked to one or more functional molecule(s) (e.g., another peptide or protein) in addition to the Bcl-xL inhibitors described below. For example, a labeled antibody can be derived by functionally linking an anti-EGFR antibody (by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody (e.g., a bispecific antibody or a diabody), a detectable agent, a pharmaceutical agent, a protein or peptide that can mediate the association of the antibody or antibody portion with another molecule (such as a streptavidin core region or a polyhistidine tag), and/or a cytotoxic or therapeutic agent selected from the group consisting of a mitotic inhibitor, an antitumor antibiotic, an immunomodulating agent, a vector for gene therapy, an alkylating agent, an antiangiogenic agent, an antimetabolite, a boron-containing agent, a chemoprotective agent, a hormone, an antihormone agent, a corticosteroid, a photoactive therapeutic agent, an oligonucleotide, a radionuclide agent, a topoisomerase inhibitor, a kinase inhibitor, a radiosensitizer, and a combination thereof.

[0379] Useful detectable agents with which an antibody or ADC may be derivatized include fluorescent compounds. Exemplary fluorescent detectable agents include fluorescein, fluorescein isothiocyanate, rhodamine, 5-dimethylamine-1-naphthalenesulfonyl chloride, phycoerythrin and the like. An antibody may also be derivatized with detectable enzymes, such as alkaline phosphatase, horseradish peroxidase, glucose oxidase and the like. When an antibody is derivatized with a detectable enzyme, it is detected by adding additional reagents that the enzyme uses to produce a detectable reaction product. For example, when the detectable agent horseradish peroxidase is present the addition of hydrogen peroxide and diaminobenzidine leads to a colored reaction product, which is detectable. An antibody or ADC may also be derivatized with biotin, and detected through indirect measurement of avidin or streptavidin binding.

[0380] In one embodiment, the antibody or ADC is conjugated to an imaging agent. Examples of imaging agents that may be used in the compositions and methods described herein include, but are not limited to, a radiolabel (e.g., indium), an enzyme, a fluorescent label, a luminescent label, a bioluminescent label, a magnetic label, and biotin.

[0381] In one embodiment, the antibodies or ADCs are linked to a radiolabel, such as, but not limited to, indium (¹¹¹In). ¹¹¹Indium may be used to label the antibodies and ADCs described herein for use in identifying EGFR positive tumors. In a certain embodiment, anti-EGFR antibodies (or ADCs) described herein are labeled with ¹¹¹I via a bifunctional chelator which is a bifunctional cyclohexyl diethylenetriaminepentaacetic acid (DTPA) chelate (see U.S. Pat. Nos. 5,124,471; 5,434,287; and 5,286,850, each of which is incorporated herein by reference).

[0382] Another embodiment of the invention provides a glycosylated binding protein wherein the anti-EGFR antibody comprises one or more carbohydrate residues. Nascent in vivo protein production may undergo further processing, known as post-translational modification. In particular, sugar (glycosyl) residues may be added enzymatically, a process known as glycosylation. The resulting proteins bearing covalently linked oligosaccharide side chains are known as glycosylated proteins or glycoproteins. Antibodies are glycoproteins with one or more carbohydrate residues in the Fc domain, as well as the variable domain. Carbohydrate residues in the Fc domain have important effect on the effector function of the Fc domain, with minimal effect on antigen binding or half-life of the antibody (R. Jefferis, *Biotechnol. Prog.* 21 (2005), pp. 11-16). In contrast, glycosylation of the variable domain may have an effect on the antigen binding activity of the antibody. Glycosylation in the variable domain may have a negative effect on antibody binding affinity, likely due to steric hindrance (Co, M. S., et al., *Mol. Immunol.* (1993) 30:1361-1367), or result in increased affinity for the antigen (Wallick, S. C., et al., *Exp. Med.* (1988) 168:1099-1109; Wright, A., et al., *EMBO J.* (1991) 10:2717-2723).

[0383] One aspect of the invention is directed to generating glycosylation site mutants in which the O- or N-linked glycosylation site of the binding protein has been mutated. One skilled in the art can generate such mutants using standard well-known technologies. Glycosylation site mutants that retain the biological activity, but have increased or decreased binding activity, are another object of the invention.

[0384] In still another embodiment, the glycosylation of the anti-EGFR antibody is modified. For example, an aglycosylated antibody can be made (i.e., the antibody lacks glycosylation).

Glycosylation can be altered to, for example, increase the affinity of the antibody for antigen. Such carbohydrate modifications can be accomplished by, for example, altering one or more sites of glycosylation within the antibody sequence. For example, one or more amino acid substitutions can be made that result in elimination of one or more variable region glycosylation sites to thereby eliminate glycosylation at that site. Such aglycosylation may increase the affinity of the antibody for antigen. Such an approach is described in further detail in PCT Publication WO2003016466A2, and U.S. Pat. Nos. 5,714,350 and 6,350,861, each of which is incorporated herein by reference in its entirety.

[0385] Additionally or alternatively, a modified anti-EGFR antibody can be made that has an altered type of glycosylation, such as a hypofucosylated antibody having reduced amounts of fucosyl residues or an antibody having increased bisecting GlcNAc structures. Such altered glycosylation patterns have been demonstrated to increase the ADCC ability of antibodies. Such carbohydrate modifications can be accomplished by, for example, expressing the antibody in a host cell with altered glycosylation machinery. Cells with altered glycosylation machinery have been described in the art and can be used as host cells in which to express recombinant antibodies of the invention to thereby produce an antibody with altered glycosylation. See, for example, Shields, R. L. et al. (2002) *J. Biol. Chem.* 277:26733-26740; Umana et al. (1999) *Nat. Biotech.* 17:176-1, as well as, European Patent No: EP 1,176,195; PCT Publications WO 03/035835; WO 99/54342 80, each of which is incorporated herein by reference in its entirety.

[0386] Protein glycosylation depends on the amino acid sequence of the protein of interest, as well as the host cell in which the protein is expressed. Different organisms may produce different glycosylation enzymes (e.g., glycosyltransferases and glycosidases), and have different substrates (nucleotide sugars) available. Due to such factors, protein glycosylation pattern, and composition of glycosyl residues, may differ depending on the host system in which the particular protein is expressed. Glycosyl residues useful in the invention may include, but are not limited to, glucose, galactose, mannose, fucose, n-acetylglucosamine and sialic acid. Preferably the glycosylated binding protein comprises glycosyl residues such that the glycosylation pattern is human.

[0387] Differing protein glycosylation may result in differing protein characteristics. For instance, the efficacy of a therapeutic protein produced in a microorganism host, such as yeast, and glycosylated utilizing the yeast endogenous pathway may be reduced compared to that of the same protein expressed in a mammalian cell, such as a CHO cell line. Such glycoproteins may also be immunogenic in humans and show reduced half-life in vivo after administration. Specific receptors in humans and other animals may recognize specific glycosyl residues and promote the rapid clearance of the protein from the bloodstream. Other adverse effects may include changes in protein folding, solubility, susceptibility to proteases, trafficking, transport, compartmentalization, secretion, recognition by other proteins or factors, antigenicity, or allergenicity. Accordingly, a practitioner may prefer a therapeutic protein with a specific composition and pattern of glycosylation, for example glycosylation composition and pattern identical, or at least similar, to that produced in human cells or in the species-specific cells of the intended subject animal.

[0388] Expressing glycosylated proteins different from that of a host cell may be achieved by genetically modifying the host cell to express heterologous glycosylation enzymes. Using recombinant techniques, a practitioner may generate antibodies or antigen binding portions thereof exhibiting human protein glycosylation. For example, yeast strains have been genetically modified

to express non-naturally occurring glycosylation enzymes such that glycosylated proteins (glycoproteins) produced in these yeast strains exhibit protein glycosylation identical to that of animal cells, especially human cells (U.S. patent Publication Nos. 20040018590 and 20020137134 and PCT publication WO2005100584 A2).

[0389] Antibodies may be produced by any of a number of techniques. For example, expression from host cells, wherein expression vector(s) encoding the heavy and light chains is (are) transfected into a host cell by standard techniques. The various forms of the term “transfection” are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, e.g., electroporation, calcium-phosphate precipitation, DEAE-dextran transfection and the like. Although it is possible to express antibodies in either prokaryotic or eukaryotic host cells, expression of antibodies in eukaryotic cells is preferable, and most preferable in mammalian host cells, because such eukaryotic cells (and in particular mammalian cells) are more likely than prokaryotic cells to assemble and secrete a properly folded and immunologically active antibody.

[0390] Preferred mammalian host cells for expressing the recombinant antibodies of the invention include Chinese Hamster Ovary (CHO cells) (including dhfr-CHO cells, described in Urlaub and Chasm, (1980) *Proc. Natl. Acad. Sci. USA* 77:4216-4220, used with a DHFR selectable marker, e.g., as described in R. J. Kaufman and P. A. Sharp (1982) *Mol. Biol.* 159:601-621), NS0 myeloma cells, COS cells and SP2 cells. When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more preferably, secretion of the antibody into the culture medium in which the host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods.

[0391] Host cells can also be used to produce functional antibody fragments, such as Fab fragments or scFv molecules. It will be understood that variations on the above procedure are within the scope of the invention. For example, it may be desirable to transfect a host cell with DNA encoding functional fragments of either the light chain and/or the heavy chain of an antibody of this invention.

[0392] Recombinant DNA technology may also be used to remove some, or all, of the DNA encoding either or both of the light and heavy chains that is not necessary for binding to the antigens of interest. The molecules expressed from such truncated DNA molecules are also encompassed by the antibodies of the invention. In addition, bifunctional antibodies may be produced in which one heavy and one light chain are an antibody of the invention and the other heavy and light chain are specific for an antigen other than the antigens of interest by crosslinking an antibody of the invention to a second antibody by standard chemical crosslinking methods.

[0393] In a preferred system for recombinant expression of an antibody a recombinant expression vector encoding both the antibody heavy chain and the antibody light chain is introduced into dhfr-CHO cells by calcium phosphate-mediated transfection. Within the recombinant expression vector, the antibody heavy and light chain genes are each operatively linked to CMV enhancer/AdMLP promoter regulatory elements to drive high levels of transcription of the genes. The recombinant expression vector also carries a DHFR gene, which allows for selection of CHO cells that have been transfected with the vector using methotrexate selection/amplification. The selected transformant host cells are cultured to allow for expression of the antibody heavy and light chains and intact antibody is recovered from the culture medium. Standard molecular biology techniques are used to prepare the recombinant expression vector, transfect the host cells, select for transformants, culture the host cells and recover the antibody from the culture medium. Still further the invention provides a method of synthesizing a recombinant antibody of the invention by culturing a host cell in a suitable culture medium until a recombinant antibody is synthesized. Recombinant antibodies of the invention may be produced using nucleic acid molecules corresponding to the amino acid sequences disclosed herein. In one embodiment, the nucleic acid

molecules set forth in SEQ ID NOs: 86 and/or 87 are used in the production of a recombinant antibody. The method can further comprise isolating the recombinant antibody from the culture medium.

[0394] The antibodies and the sequences of the antibodies recited herein are also described in U.S. Pat. No. 9,493,568 (AbbVie Inc.), which is incorporated by reference herein.

3. Anti-EGFR Antibody Drug Conjugates (ADCs): Bcl-xL Inhibitors and Linkers

[0395] Dysregulated apoptotic pathways have also been implicated in the pathology of cancer. The implication that down-regulated apoptosis (and more particularly the Bel-2 family of proteins) is involved in the onset of cancerous malignancy has revealed a novel way of targeting this still elusive disease. Research has shown, for example, the anti-apoptotic proteins, Bel 2 and Bcl-xL, are over-expressed in many cancer cell types. See, Zhang, 2002, *Nature Reviews/Drug Discovery* 1:101; Kirkin et al., 2004, *Biochimica Biophysica Acta* 1644:229-249; and Amundson et al., 2000, *Cancer Research* 60:6101-6110. The effect of this deregulation is the survival of altered cells which would otherwise have undergone apoptosis in normal conditions. The repetition of these defects associated with unregulated proliferation is thought to be the starting point of cancerous evolution.

[0396] Aspects of the disclosure concern anti-hEGFR ADCs comprising an anti-hEGFR antibody conjugated to a drug via a linker, wherein the drug is a Bcl-xL inhibitor. In specific embodiments, the ADCs are compounds according to structural formula (I) below, or a pharmaceutically acceptable salt thereof, wherein Ab represents the anti-hEGFR antibody, D represents a Bcl-xL inhibitor drug (i.e., a compound of formula IIa or IIb as shown below), L represents a linker, LK represents a covalent linkage linking the linker (L) to the anti-hEGFR antibody (Ab) and m represents the number of D-L-LK units linked to the antibody, which is an integer ranging from 1 to 20. In certain embodiments, m is 2, 3 or 4. In some embodiments, m ranges from 1 to 8, 1 to 7, 1 to 6, 2 to 6, 1 to 5, 1 to 4, or 2 to 4.

[0397] In some embodiments, the ADC has the following formula (formula I):

##STR00036##

wherein Ab is the antibody, e.g., anti-EGFR antibody AbA, AbB, AbG, or AbK, and (D-L-LK) is a Drug-Linker-Covalent Linkage. The Drug-Linker moiety is made of L- which is a Linker, and -D, which is a drug moiety having, for example, cytostatic, cytotoxic, or otherwise therapeutic activity against a target cell, e.g., a cell expressing EGFR; and m is an integer from 1 to 20. In some embodiments, m ranges from 1 to 8, 1 to 7, 1 to 6, 2 to 6, 1 to 5, 1 to 4, 1 to 3, 1 to 2, 1.5 to 8, 1.5 to 7, 1.5 to 6, 1.5 to 5, 1.5 to 4, 2 to 6, 1 to 5, 1 to 4, 1 to 3, 1 to 2, or 2 to 4. The DAR of an ADC is equivalent to the “m” referred to in Formula I. In one embodiment, the ADC has a formula of Ab-(LK-L-D).sub.m, wherein Ab is an anti-EGFR antibody, e.g. AbA, AbB, AbG, or AbK, L is a linker, D is a drug, e.g., a Bcl-xL inhibitor, LK is a covalent linker, e.g. —S—, and m is 1 to 8 (or a DAR of 2-4).

[0398] Additional details regarding drugs (D of Formula I) and linkers (L of Formula I) that may be used in the ADCs of the invention, as well as alternative ADC structures, are described below.

[0399] Specific embodiments of various Bcl-xL inhibitors per se, and various Bcl-xL inhibitors (D), linkers (L) and anti-EGFR antibodies (Ab) that can comprise the ADCs described herein, as well as the number of Bcl-xL inhibitors linked to the ADCs, are described in more detail below.

[0400] Examples of Bcl-xL inhibitors that may be used in the anti-EGFR ADC of the invention are provided below, as are linkers that may be used to conjugate the antibody and the one or more Bcl-xL inhibitor(s). The terms “linked” and “conjugated” are also used interchangeably herein and indicate that the antibody and moiety are covalently linked.

[0401] Bcl-xL inhibitors and linkers that may be used in the ADCs described herein and methods of making the same, are described in US 2016-0158377 (AbbVie Inc.), which is incorporated by reference herein.

3.1. Bcl-xL Inhibitors

[0402] The Bcl-xL inhibitors may be used as compounds or salts per se in the various methods

described herein, or may be included as a component part of an ADC, e.g., as the drug (D) in formula (I).

[0403] Specific embodiments of Bcl-xL inhibitors that may be used in unconjugated form, or that may be included as part of an ADC include compounds according to structural formula (IIa) or (IIb). In the present invention, when the Bcl-xL inhibitors are included as part of an ADC, #shown in formula (IIa) or (IIb) below represents a point of attachment to a linker, which indicates that they are represented in a monoradical form.

##STR00037##

or salts thereof, wherein: [0404] Ar.sup.1 is selected from

##STR00038##

and is optionally substituted with one or more substituents independently selected from halo, hydroxy, nitro, lower alkyl, lower heteroalkyl, C.sub.1-4alkoxy, amino, cyano and halomethyl;

[0405] Ar.sup.2 is selected from

##STR00039##

and is optionally substituted with one or more substituents independently selected from halo, hydroxy, nitro, lower alkyl, lower heteroalkyl, C.sub.1-4alkoxy, amino, cyano and halomethyl, wherein the #—N(R.sup.4)—R.sup.13—Z.sup.2b— substituent of formula (IIb) is attached to Ar.sup.2 at any Ar.sup.2 atom capable of being substituted; [0406] Z.sup.1 is selected from N, CH, C-halo and C—CN; [0407] Z.sup.2a, Z.sup.2, and Z.sup.2c are each, independent from one another, selected from a bond, NR.sup.6, CR.sup.6aR.sup.6b, O, S, S(O), SO.sub.2, NR.sup.6C(O), NR.sup.6aC(O)NR.sup.6b, and NR.sup.6C(O)O; [0408] R.sup.1 is selected from hydrogen, methyl, halo, halomethyl, ethyl and cyano; [0409] R.sup.2 is selected from hydrogen, methyl, halo, halomethyl and cyano; [0410] R.sup.3 is selected from hydrogen, lower alkyl and lower heteroalkyl; [0411] R.sup.4 is selected from hydrogen, lower alkyl, monocyclic cycloalkyl, monocyclic heterocyclyl, lower heteroalkyl or is taken together with an atom of R.sup.13 to form a cycloalkyl or heterocyclyl ring having between 3 and 7 ring atoms, wherein the lower alkyl, monocyclic cycloalkyl, monocyclic heterocyclyl, lower heteroalkyl are optionally substituted with one or more halo, cyano, C.sub.1-4alkoxy, monocyclic cycloalkyl, monocyclic heterocyclyl, NHC(O)CR.sup.6aR.sup.6b, NHS(O)CR.sup.6aR.sup.6b, NHS(O).sub.2CR.sup.6aR.sup.6b, S(O).sub.2CR.sup.6aR.sup.6b or S(O).sub.2NH.sub.2 groups; [0412] R.sup.6, R.sup.6a and R.sup.6b are each, independent from one another, selected from hydrogen, lower alkyl, lower heteroalkyl, optionally substituted monocyclic cycloalkyl and monocyclic heterocyclyl, or are taken together with an atom from R.sup.13 to form a cycloalkyl or heterocyclyl ring having between 3 and 7 ring atoms; [0413] R.sup.10 is selected from cyano, OR.sup.14, SR.sup.14, SOR.sup.14, SO.sub.2R.sup.14, SO.sub.2NR.sup.14aR.sup.14b, NR.sup.14aR.sup.14b, NHC(O)R.sup.14 and NHSO.sub.2R.sup.14; [0414] R.sup.11a and R.sup.11b are each, independently of one another, selected from hydrogen, halo, methyl, ethyl, halomethyl, hydroxyl, methoxy, CN, and SCH.sub.3; [0415] R.sup.12 is selected from hydrogen, halo, cyano, lower alkyl, lower heteroalkyl, cycloalkyl, or heterocyclyl, wherein the alkyl, heteroalkyl, cycloalkyl, or heterocyclyl are optionally substituted with one or more halo, cyano, C.sub.1-4alkoxy, monocyclic cycloalkyl, monocyclic heterocyclyl, NHC(O)CR.sup.6aR.sup.6b, NHS(O)CR.sup.6aR.sup.6b, NHS(O).sub.2CR.sup.6aR.sup.6b or S(O).sub.2CR.sup.6aR.sup.6b groups; [0416] R.sup.13 is selected from a bond, optionally substituted lower alkylene, optionally substituted lower heteroalkylene, optionally substituted cycloalkyl or optionally substituted heterocyclyl; [0417] R.sup.14 is selected from hydrogen, optionally substituted lower alkyl and optionally substituted lower heteroalkyl; [0418] R.sup.14a and R.sup.14b are each, independently of one another, selected from hydrogen, optionally substituted lower alkyl, optionally substituted lower heteroalkyl, or are taken together with the nitrogen atom to which they are bonded to form a monocyclic cycloalkyl or monocyclic heterocyclyl ring; [0419] R.sup.15 is selected from hydrogen, halo, C.sub.1-6 alkanyl, C.sub.2-4 alkenyl, C.sub.2-4 alkynyl, and C.sub.1-4 haloalkyl and C.sub.1-4 hydroxyalkyl, with

the proviso that when R¹⁵ is present, R⁴ is not C₁₋₄ alkyl, C₂₋₄ alkenyl, C₂₋₄ alkynyl, C₁₋₄ haloalkyl or C₁₋₄ hydroxyalkyl, wherein the R⁴ C₁₋₆ alkanyl, C₂₋₄ alkenyl, C₂₋₄ alkynyl, C₁₋₄ haloalkyl and C₁₋₄ hydroxyalkyl are optionally substituted with one or more substituents independently selected from OCH₃, OCH₂CH₂OCH₃, and OCH₂CH₂NHCH₃; and [0420] # represents a point of attachment to a linker or a hydrogen atom.

[0421] Specific embodiments of Bcl-xL inhibitors that may be used in unconjugated form, or that may be included as part of an ADC include compounds according to structural formula (IIa) or (IIb):

##STR00040##

or salts thereof, wherein: [0422] Ar¹ is selected from,

##STR00041##

and is optionally substituted with one or more substituents independently selected from halo, hydroxy, nitro, lower alkyl, lower heteroalkyl, C₁₋₄alkoxy, amino, cyano and [0423] Ar² is selected from

##STR00042## ##STR00043##

and is optionally substituted with one or more substituents independently selected from halo, hydroxy, nitro, lower alkyl, lower heteroalkyl, C₁₋₄alkoxy, amino, cyano and halomethyl, wherein the #—N(R⁴)—R¹³—Z^{2b}— substituent of formula (IIb) is attached to Ar² at any Ar² atom capable of being substituted; [0424] Z¹ is selected from N, CH, C-halo and C—CN; [0425] Z^{2a}, Z^{2b}, and Z^{2c} are each, independent from one another, selected from a bond, NR⁶, CR^{6a}R^{6b}, O, S, S(O), S(O)₂, NR⁶C(O), NR^{6a}C(O)NR^{6b}, and NR⁶C(O)O; [0426] R¹ is selected from hydrogen, methyl, halo, halomethyl, ethyl and cyano; [0427] R² is selected from hydrogen, methyl, halo, halomethyl and cyano; [0428] R³ is selected from hydrogen, lower alkyl and lower heteroalkyl; [0429] R⁴ is selected from hydrogen, lower alkyl, monocyclic cycloalkyl, monocyclic heterocyclyl, and lower heteroalkyl or is taken together with an atom of R¹³ to form a cycloalkyl or heterocyclyl ring having between 3 and 7 ring atoms, wherein the lower alkyl, monocyclic cycloalkyl, monocyclic heterocyclyl, and lower heteroalkyl are optionally substituted with one or more halo, cyano, hydroxy, C₁₋₄alkoxy, monocyclic cycloalkyl, monocyclic heterocyclyl, C(O)NR^{6a}R^{6b}, S(O)₂NR^{6a}R^{6b}, NHC(O)CHR^{6a}R^{6b}, NHS(O)CHR^{6a}R^{6b}, NHS(O)₂CHR^{6a}R^{6b}, S(O)₂CHR^{6a}R^{6b} or S(O)₂NH₂ groups; [0430] R⁶, R^{6a} and R^{6b} are each, independent from one another, selected from hydrogen, lower alkyl, lower heteroalkyl, optionally substituted monocyclic cycloalkyl and monocyclic heterocyclyl, or are taken together with an atom from R¹³ to form a cycloalkyl or heterocyclyl ring having between 3 and 7 ring atoms; [0431] R¹⁰ is selected from cyano, OR¹⁴, SR¹⁴, SOR¹⁴, SO₂R¹⁴, SO₂NR^{14a}R^{14b}, NR^{14a}R^{14b}, NHC(O)R¹⁴ and NHSO₂R¹⁴; [0432] R^{1a} and R^{1b} are each, independently of one another, selected from hydrogen, halo, methyl, ethyl, halomethyl, hydroxyl, methoxy, CN, and SCH₃; [0433] R¹² is selected from hydrogen, halo, cyano, lower alkyl, lower heteroalkyl, cycloalkyl, and heterocyclyl, wherein the alkyl, heteroalkyl, cycloalkyl, and heterocyclyl are optionally substituted with one or more halo, cyano, C₁₋₄alkoxy, monocyclic cycloalkyl, monocyclic heterocyclyl, NHC(O)CHR^{6a}R^{6b}, NHS(O)CHR^{6a}R^{6b}, NHS(O)₂CHR^{6a}R^{6b} or S(O)₂CHR^{6a}R^{6b} groups; [0434] R¹³ is selected from a bond, optionally substituted lower alkylene, optionally substituted lower heteroalkylene, optionally substituted cycloalkyl or optionally substituted heterocyclyl; [0435] R¹⁴ is selected from hydrogen, optionally substituted lower alkyl and optionally substituted lower heteroalkyl; [0436] R^{14a} and R^{14b} are each, independently of one another, selected from hydrogen, optionally substituted lower alkyl, and optionally substituted lower heteroalkyl, or

are taken together with the nitrogen atom to which they are bonded to form an optionally substituted monocyclic cycloalkyl or monocyclic heterocyclyl ring; [0437] R.sup.15 is selected from hydrogen, halo, C.sub.1-6 alkanyl, C.sub.2-4 alkenyl, C.sub.2-4 alkynyl, and C.sub.1-4 haloalkyl and C.sub.1-4 hydroxyalkyl, with the proviso that when R.sup.15 is present, R.sup.4 is not C.sub.1-4 alkyl, C.sub.2-4 alkenyl, C.sub.2-4 alkynyl, C.sub.1-4 haloalkyl or C.sub.1-4 hydroxyalkyl, wherein the R.sup.4 C.sub.1-6 alkanyl, C.sub.2-4 alkenyl, C.sub.2-4 alkynyl, C.sub.1-4 haloalkyl and C.sub.1-4 hydroxyalkyl are optionally substituted with one or more substituents independently selected from OCH.sub.3, OCH.sub.2CH.sub.2OCH.sub.3, and OCH.sub.2CH.sub.2NHCH.sub.3; and [0438] #represents a point of attachment to a linker or a hydrogen atom.

[0439] Another embodiment of Bcl-xL inhibitors that may be used in unconjugated form, or that may be included as part of an ADC include compounds according to structural formula (IIa) or (IIb):

##STR00044##

or salts thereof, wherein: [0440] Ar.sup.1 is selected from

##STR00045##

and is optionally substituted with one or more substituents independently selected from halo, hydroxy, nitro, lower alkyl, lower heteroalkyl, C.sub.1-4 alkoxy, amino, cyano and halomethyl;

[0441] Ar.sup.2 is selected from

##STR00046##

and is optionally substituted with one or more substituents independently selected from halo, hydroxy, nitro, lower alkyl, lower heteroalkyl, C.sub.1-4 alkoxy, amino, cyano and halomethyl, wherein the #—N(R.sup.4)—R.sup.13—Z.sup.2b— substituent of formula (IIb) is attached to Ar.sup.2 at any Ar.sup.2 atom capable of being substituted; [0442] Z.sup.1 is selected from N, CH, C-halo and C—CN; [0443] Z.sup.2a, Z.sup.2b, and Z.sup.2c are each, independent from one another, selected from a bond, NR.sup.6, CR.sup.6aR.sup.6b, O, S, S(O), S(O).sub.2, NR.sup.6C(O), NR.sup.6aC(O)NR.sup.6b, and NR.sup.6C(O)O; [0444] R.sup.1 is selected from hydrogen, methyl, halo, halomethyl, ethyl and cyano; [0445] R.sup.2 is selected from hydrogen, methyl, halo, halomethyl and cyano; [0446] R.sup.3 is selected from hydrogen, lower alkyl and lower heteroalkyl; [0447] R.sup.4 is selected from hydrogen, lower alkyl, monocyclic cycloalkyl, monocyclic heterocyclyl, lower heteroalkyl or is taken together with an atom of R.sup.13 to form a cycloalkyl or heterocyclyl ring having between 3 and 7 ring atoms, wherein the lower alkyl, monocyclic cycloalkyl, monocyclic heterocyclyl, lower heteroalkyl are optionally substituted with one or more halo, cyano, C.sub.1-4alkoxy, monocyclic cycloalkyl, monocyclic heterocyclyl, NHC(O)CR.sup.6aR.sup.6b, NHS(O)CR.sup.6aR.sup.6b, NHS(O).sub.2CR.sup.6aR.sup.6b, S(O).sub.2CR.sup.6aR.sup.6b or S(O).sub.2NH.sub.2 groups; [0448] R.sup.6, R.sup.6a and R.sup.6b are each, independent from one another, selected from hydrogen, lower alkyl, lower heteroalkyl, optionally substituted monocyclic cycloalkyl and monocyclic heterocyclyl, or are taken together with an atom from R.sup.13 to form a cycloalkyl or heterocyclyl ring having between 3 and 7 ring atoms; [0449] R.sup.10 is selected from cyano, OR.sup.14, SR.sup.14, SOR.sup.14, SO.sub.2R.sup.14, SO.sub.2NR.sup.14aR.sup.14b, NR.sup.14aR.sup.14b, NHC(O)R.sup.14 and NHSO.sub.2R.sup.14; [0450] R.sup.11a and R.sup.11b are each, independently of one another, selected from hydrogen, halo, methyl, ethyl, halomethyl, hydroxyl, methoxy, CN, and SCH.sub.3; [0451] R.sup.12 is selected from hydrogen, halo, cyano, lower alkyl, lower heteroalkyl, cycloalkyl, or heterocyclyl, wherein the alkyl, heteroalkyl, cycloalkyl, or heterocyclyl are optionally substituted with one or more halo, cyano, C.sub.1-4alkoxy, monocyclic cycloalkyl, monocyclic heterocyclyl, NHC(O)CR.sup.6aR.sup.6b, NHS(O)CR.sup.6aR.sup.6b, NHS(O).sub.2CR.sup.6aR.sup.6b or S(O).sub.2CR.sup.6aR.sup.6b groups; [0452] R.sup.13 is selected from a bond, optionally substituted lower alkylene, optionally substituted lower heteroalkylene, optionally substituted cycloalkyl or optionally substituted heterocyclyl; [0453]

R.sup.14 is selected from hydrogen, optionally substituted lower alkyl and optionally substituted lower heteroalkyl; [0454] R.sup.14a and R.sup.14b are each, independently of one another, selected from hydrogen, optionally substituted lower alkyl, optionally substituted lower heteroalkyl, or are taken together with the nitrogen atom to which they are bonded to form a monocyclic cycloalkyl or monocyclic heterocyclyl ring; [0455] R.sup.15 is selected from hydrogen, halo, C.sub.1-6 alkanyl, C.sub.2-4 alkenyl, C.sub.2-4 alkynyl, and C.sub.1-4 haloalkyl and C.sub.1-4 hydroxyalkyl, with the proviso that when R.sup.15 is present, R.sup.4 is not C.sub.1-4 alkyl, C.sub.2-4 alkenyl, C.sub.2-4 alkynyl, C.sub.1-4 haloalkyl or C.sub.1-4 hydroxyalkyl, wherein the R.sup.4 C.sub.1-6 alkanyl, C.sub.2-4 alkenyl, C.sub.2-4 alkynyl, C.sub.1-4 haloalkyl and C.sub.1-4 hydroxyalkyl are optionally substituted with one or more substituents independently selected from OCH.sub.3, OCH.sub.2CH.sub.2OCH.sub.3, and OCH.sub.2CH.sub.2NHCH.sub.3; and [0456] # represents a point of attachment to a linker or a hydrogen atom.

[0457] When a Bcl-xL inhibitor of structural formulae (IIa) and (IIb) is not a component of an ADC, # in formulae (IIa) and (IIb) represents the point of attachment to a hydrogen atom. When the Bcl-xL inhibitor is a component of an ADC, # in formulae (IIa) and (IIb) represents the point of attachment to a the linker. When a Bcl-xL inhibitor is a component of an ADC, the ADC may comprise one or more Bcl-xL inhibitors, which may be the same or different, but are typically the same.

[0458] In certain embodiments, Ar.sup.1 of formula (IIa) or (IIb) is selected from, and
##STR00047##

and is optionally substituted with one or more substituents independently selected from halo, cyano, methyl, and halomethyl. In particular embodiments, Ar.sup.1 is
##STR00048##

In particular embodiments, Ar.sup.1 is unsubstituted.

[0459] In all embodiments, the #—N(R.sup.4)—R.sup.13—Z.sup.2b— substituent of formula (IIb) is attached to Ar.sup.2 at any Ar.sup.2 atom capable of being substituted.

[0460] In certain embodiments, Ar.sup.2 of formula (IIa) or (IIb) is
##STR00049##

which is optionally substituted at the 5-position with a group selected from hydroxyl, C.sub.1-4 alkoxy, and cyano; or Ar.sup.2 is
##STR00050##

or [0461] Ar.sup.2 is
##STR00051##

or [0462] Ar.sup.2 is
##STR00052##

[0463] In certain embodiments, Ar.sup.2 of formula (IIa) or (IIb) is
##STR00053##

[0464] In certain embodiments, Ar.sup.2 of formula (IIa) or (IIb) is
##STR00054##

[0465] In certain embodiments, Ar.sup.2 of formula (IIa) or (IIb) is
##STR00055##

[0466] In certain embodiments, Ar.sup.2 of formula (IIa) or (IIb) is
##STR00056##

[0467] In certain embodiments, Ar.sup.2 of formula (IIa) or (IIb) is
##STR00057##

[0468] In certain embodiments, Ar.sup.2 of formula (IIa) or (IIb) is
##STR00058##

[0469] In certain embodiments, Ar.sup.2 of formula (IIa) or (IIb) is
##STR00059##

[0470] In certain embodiments, Ar.sup.2 of formula (IIa) or (IIb) is

##STR00060##

[0471] In certain embodiments, Ar.sup.2 of formula (IIa) or (IIb) is

##STR00061##

[0472] In certain embodiments, Ar.sup.2 of formula (IIa) or (IIb) is

##STR00062##

[0473] In certain embodiments, Ar.sup.2 of formula (IIa) or (IIb) is

##STR00063##

[0474] In certain embodiments, Ar.sup.2 of formula (IIa) or (IIb) is

##STR00064##

[0475] In certain embodiments, Ar.sup.2 of formula (IIa) or (IIb) is

##STR00065##

[0476] In certain embodiments, Ar.sup.2 of formula (IIa) or (IIb) is

##STR00066##

[0477] In certain embodiments, Ar.sup.2 of formula (IIa) or (IIb) is

##STR00067##

[0478] In certain embodiments, Ar.sup.2 of formula (IIa) or (IIb) is

##STR00068##

[0479] In certain embodiments, Ar.sup.2 of formula (IIa) or (IIb) is

##STR00069##

[0480] In certain embodiments, Ar.sup.2 of formula (IIa) or (IIb) is

##STR00070##

[0481] In certain embodiments, Ar.sup.2 of formula (IIa) or (IIb) is.

##STR00071##

[0482] In certain embodiments, Ar.sup.2 of formula (IIa) or (IIb) is

##STR00072##

[0483] In certain embodiments, Ar.sup.2 of formula (IIa) or (IIb) is

##STR00073##

[0484] In certain embodiments, Ar.sup.2 of formula (IIa) or (IIb) is

##STR00074##

[0485] In certain embodiments, Ar.sup.2 of formula (IIa) or (IIb) is

##STR00075##

[0486] In certain embodiments, Ar.sup.2 of formula (IIa) or (IIb) is

##STR00076##

[0487] In certain embodiments, Ar.sup.2 of formula (IIa) or (IIb) is

##STR00077##

In certain embodiments, Ar.sup.2 of formula (IIa) is unsubstituted.

[0488] In certain embodiments, Ar.sup.2 of formula (IIa) or (IIb) is

##STR00078##

which is substituted at the 5-position with a group selected from hydroxyl, C.sub.1-4alkoxy, and cyano.

[0489] In certain embodiments, Z.sup.1 of formula (IIa) or (IIb) is N.

[0490] In certain embodiments, R.sup.1 of formula (IIa) or (IIb) is selected from methyl and chloro.

[0491] In certain embodiments, R.sup.2 of formula (IIa) or (IIb) is selected from hydrogen and methyl. In particular embodiments, R.sup.2 is hydrogen.

[0492] In certain embodiments, R.sup.4 of formula (IIa) or (IIb) is methyl.

[0493] In certain embodiments, R.sup.4 of formula (IIa) or (IIb) is (CH.sub.2).sub.2OCH.sub.3.

[0494] In certain embodiments, R.sup.4 of formula (IIa) or (IIb) is hydrogen.

[0495] In certain embodiments, R.sup.4 of formula (IIa) or (IIb) is monocyclic heterocyclyl, wherein the monocyclic heterocycloalkyl is substituted with one S(O).sub.2CH.sub.3.

[0496] In certain embodiments, R^{sup.4} of formula (IIa) or (IIb) is hydrogen or lower alkyl, wherein the lower alkyl is optionally substituted with C_{sub.1-4} alkoxy or C(O)NR^{sup.6a}R^{sup.b}.

[0497] In certain embodiments, R^{sup.4} of formula (IIa) or (IIb) is lower alkyl, wherein the lower alkyl is substituted with C(O)NH_{sub.2}.

[0498] In certain embodiments, R^{sup.4} of formula (IIa) or (IIb) is lower alkyl, wherein the lower alkyl is substituted with S(O)_{sub.2}NH_{sub.2}.

[0499] In certain embodiments, R^{sup.4} of formula (IIa) or (IIb) is lower alkyl, wherein the lower alkyl is substituted with hydroxy.

[0500] In certain embodiments, R^{sup.4} of formula (IIa) or (IIb) is lower alkyl, wherein the lower alkyl is substituted with C(O)N(CH_{sub.3})_{sub.2}.

[0501] In certain embodiments, R^{sup.4} of formula (IIa) or (IIb) is lower alkyl, wherein the lower alkyl is substituted with C(O)NHCH_{sub.3}.

[0502] In certain embodiments, R^{sup.11a} and R^{sup.11b} of formula (IIa) or (IIb) are the same. In a particular embodiment, R^{sup.11a} and R^{sup.11b} are each methyl. In another embodiment, R^{sup.11a} and R^{sup.11b} are each ethyl. In another embodiment, R^{sup.11a} and R^{sup.11b} are each methoxy.

[0503] In certain embodiments, R^{sup.11a} and R^{sup.11b} of formula (IIa) or (IIb) are independently selected from F, Br and Cl.

[0504] In certain embodiments, Z is N, Z^{sup.2a} is O, R^{sup.1} is methyl or chloro, R^{sup.2} is hydrogen, and Ar^{sup.2} is
 ##STR00079##
 wherein the
 ##STR00080##
 is optionally substituted at the 5-position with a group selected from hydroxyl, C_{sub.1-4} alkoxy, and cyano.

[0505] Certain embodiments pertain to a compound of formula (IIa). In certain embodiments, Z^{sup.2a} of formula (IIa) is O.

[0506] In certain embodiments, Z^{sup.2a} of formula (IIa) is CH_{sub.2} or O.

[0507] In certain embodiments, Z^{sup.2a} of formula (IIa) is S.

[0508] In certain embodiments, Z^{sup.2a} of formula (IIa) is CH_{sub.2}.

[0509] In certain embodiments, Z^{sup.2a} of formula (IIa) is NR^{sup.6}. In some such embodiments R^{sup.6} is methyl.

[0510] In certain embodiments, Z^{sup.2a} of formula (IIa) is NR^{sup.6}C(O). In some such embodiments R^{sup.6} is hydrogen.

[0511] In certain embodiments, Z^{sup.2a} of formula (IIa) is O, R^{sup.13} is ethylene, and R^{sup.4} is lower alkyl.

[0512] In certain embodiments, Z^{sup.2a} of formula (IIa) is O, R^{sup.13} is ethylene, and R^{sup.4} is hydrogen or lower alkyl optionally substituted with C_{sub.1-4} alkoxy or C(O)NR^{sup.6a}R^{sup.6b}.

[0513] In certain embodiments, Z^{sup.2a} of formula (IIa) is O, R^{sup.13} is ethylene, and R^{sup.4} is methyl.

[0514] In certain embodiments, Z^{sup.2a} of formula (IIa) is O, R^{sup.13} is ethylene, and R^{sup.4} is hydrogen.

[0515] In certain embodiments, Z^{sup.2a} of formula (IIa) is NR^{sup.6}C(O), R^{sup.6} is hydrogen, R^{sup.13} is methylene, and R^{sup.4} is hydrogen.

[0516] In certain embodiments, Z^{sup.2a} of formula (IIa) is S, R^{sup.13} is ethylene, and R^{sup.4} is hydrogen.

[0517] In certain embodiments, Z^{sup.2a} of formula (IIa) is CH_{sub.2}, R^{sup.13} is ethylene, and R^{sup.4} is hydrogen.

[0518] In certain embodiments, the group R^{sup.13} in formula (IIa) is ethylene. In some such embodiments Z^{sup.2a} is O.

[0519] In certain embodiments, the group R¹³ in formula (IIa) is propylene. In some such embodiments Z^{2a} is O.

[0520] In certain embodiments, the group R¹³ in formula (IIa) is selected from lower alkylene or lower heteroalkylene.

[0521] In certain embodiments, the group R¹³ in formula (IIa) is selected from (CH₂)₂O(CH₂)₂, (CH₂)₂SO(CH₂)₂, (CH₂)₂O(CH₂)₃ and (CH₂)₂SO(CH₂)₃. In some such embodiments Z^{2a} is O.

[0522] In certain embodiments, the group R¹³ in formula (IIa) is selected from (CH₂)₂(SO)(CH₂)₂, (CH₂)₂SO(CH₂)₂, (CH₂)₂(SO)(CH₂)₃ and (CH₂)₂SO(CH₂)₃. In some such embodiments Z^{2a} is O.

[0523] In certain embodiments, the group R¹³ in formula (IIa) is selected from (CH₂)₂(SO)(CH₂)₂, (CH₂)₂(SO)(CH₂)₃, (CH₂)₃(SO)(CH₂)₂ and (CH₂)₃(SO)(CH₂)₃. In some such embodiments Z^{2a} is O.

[0524] In certain embodiments, the group R¹³ in formula (IIa) is selected from (CH₂)₂S(CH₂)₂, (CH₂)₂S(CH₂)₃, (CH₂)₃S(CH₂)₂ and (CH₂)₃S(CH₂)₃. In some such embodiments Z^{2a} is O.

[0525] In certain embodiments, the group

##STR00081##

in formula (IIa) is

##STR00082##

[0526] In certain embodiments, the group

##STR00083##

in formula (IIa) is

##STR00084##

[0527] In certain embodiments, the group I

##STR00085##

in formula (IIa) is

##STR00086##

[0528] In certain embodiments, the group

##STR00087##

in formula (IIa) is

##STR00088##

[0529] In certain embodiments, the group

##STR00089##

is selected from

##STR00090##

[0530] In certain embodiments, the group

##STR00091##

in formula (IIa) is

##STR00092##

[0531] In certain embodiments, the group

##STR00093##

in formula (IIa) is selected from

##STR00094##

[0532] In certain embodiments, the group

##STR00095##

in formula (IIa) is

##STR00096##

[0533] In certain embodiments, the group

##STR00097##

in formula (IIa) is

##STR00098##

[0534] In certain embodiments, the group

##STR00099##

in formula (IIa) is

##STR00100##

[0535] In certain embodiments, the group

##STR00101##

in formula (IIa) is

##STR00102##

[0536] In certain embodiments, the group

##STR00103##

in formula (IIa) is

##STR00104##

[0537] In certain embodiments, the group

##STR00105##

in formula (IIa) is

##STR00106##

[0538] In certain embodiments, the group

##STR00107##

in formula (IIa) is

##STR00108##

[0539] In certain embodiments, the group

##STR00109##

in formula (IIa) is

##STR00110##

[0540] Certain embodiments pertain to a compound of formula (IIb).

[0541] In certain embodiments, the group Z^{sup.2b} in formula (IIb) is a bond, 0, or NR^{sup.6}, or and R^{sup.13} is ethylene or optionally substituted heterocyclyl.

[0542] In certain embodiments, the group Z^{sup.2b} in formula (IIb) is NR^{sup.6}. In some such embodiments R^{sup.6} is methyl.

[0543] In certain embodiments, the group Z^{sup.2b} in formula (IIb) is NR^{sup.6} and R^{sup.13} is ethylene. In some such embodiments R^{sup.6} is methyl.

[0544] In certain embodiments, the group Z^{sup.2b} in formula (IIb) is 0 and R^{sup.13} is ethylene. In some such embodiments R^{sup.4} is methyl.

[0545] In certain embodiments, the group Z^{sup.2b} in formula (IIb) is NR^{sup.6}, wherein the R^{sup.6} group is taken together with an atom of R^{sup.13} to form a ring having between 4 and 6 atoms. In some such embodiments the ring is a five membered ring.

[0546] In certain embodiments, the group Z^{sup.2b} in formula (IIb) is methylene and the group R^{sup.13} is methylene.

[0547] In certain embodiments, the group Z^{sup.2b} in formula (IIb) is methylene and the group R^{sup.13} is a bond.

[0548] In certain embodiments, the group Z^{sup.2b} in formula (IIb) is oxygen and the group R^{sup.13} is selected from (CH_{sub.2})_{sub.2}O(CH_{sub.2})_{sub.2}, (CH_{sub.2})_{sub.3}O(CH_{sub.2})_{sub.2}, (CH_{sub.2})_{sub.2}O(CH_{sub.2})_{sub.3} and (CH_{sub.2})_{sub.3}O(CH_{sub.2})_{sub.3}. In some such embodiments R^{sup.4} is methyl.

[0549] In certain embodiments, the group Z.sup.2c in formula (IIb) is a bond and R.sup.12 is OH.

[0550] In certain embodiments, the group Z.sup.2c in formula (IIb) is a bond and R.sup.12 is selected from F, Cl, Br and I.

[0551] In certain embodiments, the group Z.sup.2c in formula (IIb) is a bond and R.sup.12 is lower alkyl. In some such embodiments R.sup.12 is methyl.

[0552] In certain embodiments, the group Z.sup.2c in formula (IIb) is 0 and R.sup.12 is a lower heteroalkyl. In some such embodiments R.sup.12 is O(CH.sub.2).sub.2OCH.sub.3.

[0553] In certain embodiments, the group Z.sup.2c in formula (IIb) is 0 and R.sup.12 is lower alkyl optionally substituted with one or more halo or C.sub.1-4 alkoxy.

[0554] In certain embodiments, the group Z.sup.2c in formula (IIb) is O and R.sup.12 is a lower alkyl. In particular embodiments R.sup.12 is methyl.

[0555] In certain embodiments, the group Z.sup.2c in formula (IIb) is S and R.sup.12 is a lower alkyl. In some such embodiments R.sup.12 is methyl.

[0556] Exemplary Bcl-xL inhibitors according to structural formulae (IIa)-(IIb) that may be used in the methods described herein in unconjugated form and/or included in the ADCs described herein include the following compounds, and/or a pharmaceutically acceptable salt thereof:

TABLE-US-00019 Appln Bcl-xL Inhibitory Ex. No. Compound 1.1 W3.01 1.2 W3.02 1.3 W3.03 1.4 W3.04 1.5 W3.05 1.6 W3.06 1.7 W3.07 1.8 W3.08 1.9 W3.09 1.10 W3.10 1.11 W3.11 1.12 W3.12 1.13 W3.13 1.14 W3.14 1.15 W3.15 1.16 W3.16 1.17 W3.17 1.18 W3.18 1.19 W3.19 1.20 W3.20 1.21 W3.21 1.22 W3.22 1.23 W3.23 1.24 W3.24 1.25 W3.25 1.26 W3.26 1.27 W3.27 1.28 W3.28 1.29 W3.29 1.30 W3.30 1.31 W3.31 1.32 W3.32 1.33 W3.33 1.34 W3.34 1.35 W3.35 1.36 W3.36 1.37 W3.37 1.38 W3.38 1.39 W3.39 1.40 W3.40 1.41 W3.41 1.42 W3.42 1.43 W3.43 1.44 (Control) W3.44

[0557] Notably, when the Bcl-xL inhibitor of the present application is in conjugated form, the hydrogen corresponding to the #position of structural formula (IIa) or (IIb) is not present, forming a monoradical. For example, compound W3.01 (Example 1.1) is 6-[1-(1,3-benzothiazol-2-ylcarbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]Idec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid.

[0558] When it is in unconjugated form, it has the following structure:

##STR00111##

[0559] When the same compound is included in the ADCs as shown in structural formula (IIa) or (IIb), the hydrogen corresponding to the #position is not present, forming a monoradical.

##STR00112##

[0560] In certain embodiments, the Bcl-xL inhibitor is selected from the group consisting of W3.01, W3.02, W3.03, W3.04, W3.05, W3.06, W3.07, W3.08, W3.09, W3.10, W3.11, W3.12, W3.13, W3.14, W3.15, W3.16, W3.17, W3.18, W3.19, W3.20, W3.21, W3.22, W3.23, W3.24, W3.25, W3.26, W3.27, W3.28, W3.29, W3.30, W3.31, W3.32, W3.33, W3.34, W3.35, W3.36, W3.37, W3.38, W3.39, W3.40, W3.41, W3.42, W3.43, and pharmaceutically acceptable salts thereof (see Example 1 for compounds).

[0561] In certain embodiments, the ADC, or a pharmaceutically acceptable salt thereof, comprises a drug linked to an antibody by way of a linker, wherein the drug is a Bcl-xL inhibitor selected from the group consisting of W3.01, W3.02, W3.03, W3.04, W3.05, W3.06, W3.07, W3.08, W3.09, W3.10, W3.11, W3.12, W3.13, W3.14, W3.15, W3.16, W3.17, W3.18, W3.19, W3.20, W3.21, W3.22, W3.23, W3.24, W3.25, W3.26, W3.27, W3.28, W3.29, W3.30, W3.31, W3.32, W3.33, W3.34, W3.35, W3.36, W3.37, W3.38, W3.39, W3.40, W3.41, W3.42, W3.43.

[0562] In certain embodiments, the ADC, or a pharmaceutically acceptable salt thereof, the Bcl-xL inhibitor is selected from the group consisting of the following compounds modified in that the hydrogen corresponding to the #position of structural formula (IIa) or (IIb) is not present forming a monoradical: [0563] 6-[1-(1,3-benzothiazol-2-ylcarbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl]-3-[1-

({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid; [0564] 6-[4-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydro-2H-1,4-benzoxazin-6-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid; [0565] 6-[4-(1,3-benzothiazol-2-ylcarbamoyl)-1-methyl-1,2,3,4-tetrahydroquinoxalin-6-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid; [0566] 3-(1-({3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl)-6-[1-(1,3-benzothiazol-2-ylcarbamoyl)-5,6-dihydroimidazo[1,5-a]pyrazin-7(8H)-yl]pyridine-2-carboxylic acid; [0567] 3-(1-({3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1.sup.3']dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-hydroxy-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid; [0568] 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)naphthalen-2-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid; [0569] 3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]-6-[8-([1,3]thiazolo[5,4-b]pyridin-2-ylcarbamoyl)naphthalen-2-yl]pyridine-2-carboxylic acid; [0570] 3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]-6-[8-([1,3]thiazolo[4,5-b]pyridin-2-ylcarbamoyl)naphthalen-2-yl]pyridine-2-carboxylic acid; [0571] 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.13'.sup.2]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid; [0572] 6-[5-(1,3-benzothiazol-2-ylcarbamoyl)quinolin-3-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid; [0573] 6-[4-(1,3-benzothiazol-2-ylcarbamoyl)quinolin-6-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid; [0574] 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{2-[(2-methoxyethyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid; [0575] 3-(1-({3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-cyano-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid; [0576] 6-[1-(1,3-benzothiazol-2-ylcarbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl]-3-{1-[(3-{2-[(2-methoxyethyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid; [0577] 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)naphthalen-2-yl]-3-{1-[(3-{2-[(2-methoxyethyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid; [0578] 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-[1-({3,5-dimethyl-7-[2-(oxetan-3-ylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid; [0579] 6-[6-(3-aminopyrrolidin-1-yl)-8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-({3-(2-methoxyethoxy)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid; [0580] 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3,5-dimethyl-7-{2-[(2-sulfamoyl)ethyl]amino]ethoxy}tricyclo[3.3.1.13]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid; [0581] 3-(1-({3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1.sup.3']dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl)-6-[3-(1,3-benzothiazol-2-ylcarbamoyl)-6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl]pyridine-2-carboxylic acid; [0582] 3-(1-({3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl)-6-[1-(1,3-benzothiazol-2-ylcarbamoyl)-3-(trifluoromethyl)-5,6-

dihydroimidazo[1,5-a]pyrazin-7(8H)-yl]pyridine-2-carboxylic acid; [0583] 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-6-{methyl[2-(methylamino)ethyl]amino}-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[3-(2-methoxyethoxy)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid; [0584] 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-6-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid; [0585] 3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[4-(1,3-benzothiazol-2-ylcarbamoyl)quinolin-6-yl]pyridine-2-carboxylic acid; [0586] 6-[5-amino-8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid; [0587] 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-6-[3-(methylamino)prop-1-yn-1-yl]-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[3-(2-methoxyethoxy)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid; [0588] 6-[4-(1,3-benzothiazol-2-ylcarbamoyl)isoquinolin-6-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid; [0589] 6-[7-(1,3-benzothiazol-2-ylcarbamoyl)-1H-indol-2-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid; [0590] 3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[7-(1,3-benzothiazol-2-ylcarbamoyl)-1H-indol-2-yl]pyridine-2-carboxylic acid; [0591] 6-[7-(1,3-benzothiazol-2-ylcarbamoyl)-3-methyl-1H-indol-2-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid; [0592] 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[3,5-dimethyl-7-(2-{[1-(methylsulfonyl)piperidin-4-yl]amino}ethoxy)tricyclo[3.3.1.1.sup.3,7]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid; [0593] 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[3,5-dimethyl-7-(2-{[1-(methylsulfonyl)azetidin-3-yl]amino}ethoxy)tricyclo[3.3.1.1.sup.3,7]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid; [0594] 3-{1-[(3-{2-[(3-amino-3-oxopropyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid; [0595] 6-[3-(1,3-benzothiazol-2-ylcarbamoyl)-1H-indazol-5-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid; [0596] 6-[3-(1,3-benzothiazol-2-ylcarbamoyl)-1H-indol-5-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid; [0597] 6-[3-(1,3-benzothiazol-2-ylcarbamoyl)-1H-pyrrolo[2,3-b]pyridin-5-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid; [0598] 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-((3-(2-((2-(N,N-dimethylsulfamoyl)ethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid; [0599] 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)naphthalen-2-yl]-3-{1-[(3-{2-[(3-hydroxypropyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid; [0600] 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[3-(2-{[3-(dimethylamino)-3-oxopropyl]amino}ethoxy)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid; [0601] 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[3,5-dimethyl-7-(2-{[3-(methylamino)-3-oxopropyl]amino}ethoxy)tricyclo[3.3.1.1.sup.3,7]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid; [0602] 3-(1-{[3-(2-aminoacetamido)-5,7-

dimethyltricyclo[3.3.1.1.sup.3,7]decan-1-yl)methyl}-5-methyl-1H-pyrazol-4-yl)-6-{8-[(1,3-benzothiazol-2-yl)carbamoyl]-3,4-dihydroisoquinolin-2(1H)-yl}pyridine-2-carboxylic acid; [0603] 3-[1-({3-[(2-aminoethyl)sulfanyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-yl)carbamoyl]-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid; [0604] 3-(1-{[3-(3-aminopropyl)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-yl)carbamoyl]-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid; and [0605] 3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1.sup.3']decan-1-yl)methyl}-5-methyl-1H-pyrazol-4-yl)-6-{5-[(1,3-benzothiazol-2-yl)carbamoyl]quinolin-3-yl}pyridine-2-carboxylic acid.

[0606] The Bcl-xL inhibitors bind to and inhibit anti-apoptotic Bcl-xL proteins, inducing apoptosis.

[0607] The ability of specific Bcl-xL inhibitors according to structural formulae (IIa)-(IIb) to bind to and inhibit Bcl-xL activity may be confirmed in standard binding and activity assays, including, for example, the TR-FRET Bcl-xL binding assays described in Tao et al., 2014, ACS Med. Chem. Lett., 5:1088-1093. A specific TR-FRET Bcl-xL binding assay that can be used to confirm Bcl-xL binding is provided in Example 4, below. Typically, Bcl-xL inhibitors useful as inhibitors per se and in the ADCs described herein will exhibit a $K_{sub.1}$ in the binding assay of Example 5 of less than about 1 nM, but may exhibit a significantly lower $K_{sub.1}$, for example a $K_{sub.1}$ of less than about 1, 0.1, or even 0.01.

[0608] Bcl-xL inhibitory activity may also be confirmed in standard cell-based cytotoxicity assays, such as the FL5.12 cellular and Molt-4 cytotoxicity assays described in Tao et al., 2014, ACS Med. Chem. Lett., 5:1088-1093.

[0609] The process of mitochondrial outer-membrane permeabilization (MOMP) is controlled by the Bel-2 family proteins. Specifically, MOMP is promoted by the pro-apoptotic Bel-2 family proteins Bax and Bak which, upon activation oligomerize on the outer mitochondrial membrane and form pores, leading to release of cytochrome c (cyt c). The release of cyt c triggers formation of the apoptosome which, in turn, results in caspase activation and other events that commit the cell to undergo programmed cell death (see, Goldstein et al., 2005, *Cell Death and Differentiation* 12:453-462). The oligomerization action of Bax and Bak is antagonized by the anti-apoptotic Bel-2 family members, including Bel-2 and Bcl-xL. Bcl-xL inhibitors, in cells that depend upon Bcl-xL for survival, can cause activation of Bax and/or Bak, MOMP, release of cyt c and downstream events leading to apoptosis. The process of cyt c release can be assessed via western blot of both mitochondrial and cytosolic fractions of cytochrome c in cells and used as a proxy measurement of apoptosis in cells.

[0610] As a means of detecting Bcl-xL inhibitory activity and consequent release of cyt c, the cells can be treated with an agent that causes selective pore formation in the plasma, but not mitochondrial, membrane. Specifically, the cholesterol/phospholipid ratio is much higher in the plasma membrane than the mitochondrial membrane. As a result, short incubation with low concentrations of the cholesterol-directed detergent digitonin selectively permeabilizes the plasma membrane without significantly affecting the mitochondrial membrane. This agent forms insoluble complexes with cholesterol leading to the segregation of cholesterol from its normal phospholipid binding sites. This action, in turn, leads to the formation of holes about 40-50 Å wide in the lipid bilayer. Once the plasma membrane is permeabilized, cytosolic components able to pass over digitonin-formed holes can be washed out, including the cytochrome C that was released from mitochondria to cytosol in the apoptotic cells (Campos, 2006, *Cytometry A* 69(6):515-523).

[0611] Although many of the Bcl-xL inhibitors of structural formulae (IIa)-(IIb) selectively or specifically inhibit Bcl-xL over other anti-apoptotic Bel-2 family proteins, selective and/or specific inhibition of Bcl-xL is not necessary. The Bcl-xL inhibitors and ADCs comprising the compounds may also, in addition to inhibiting Bcl-xL, inhibit one or more other anti-apoptotic Bel-2 family proteins, such as, for example, Bel-2. In some embodiments, the Bcl-xL inhibitors and/or ADCs are

selective and/or specific for Bcl-xL. By specific or selective is meant that the particular Bcl-xL inhibitor and/or ADC binds or inhibits Bcl-xL to a greater extent than Bel-2 under equivalent assay conditions. In specific embodiments, the Bcl-xL inhibitors and/or ADCs exhibit in the range of about 10-fold, 100-fold, or even greater specificity or selectivity for Bcl-xL than Bel-2 in binding assays.

3.2. Linkers

[0612] In the ADCs described herein, the Bcl-xL inhibitors (described in Section 3.1) are linked to the anti-EGFR antibody by way of linkers. The linker linking a Bcl-xL inhibitor to the anti-EGFR antibody of an ADC may be short, long, hydrophobic, hydrophilic, flexible or rigid, or may be composed of segments that each independently has one or more of the above-mentioned properties such that the linker may include segments having different properties. The linkers may be polyvalent such that they covalently link more than one Bcl-xL inhibitor to a single site on the antibody, or monovalent such that covalently they link a single Bcl-xL inhibitor to a single site on the antibody.

[0613] As will be appreciated by skilled artisans, the linkers link the Bcl-xL inhibitors to the anti-EGFR antibody by forming a covalent linkage to the Bcl-xL inhibitor at one location and a covalent linkage to antibody at another. The covalent linkages are formed by reaction between functional groups on the linker and functional groups on the inhibitors and antibody. As used herein, the expression “linker” is intended to include (i) unconjugated forms of the linker that include a functional group capable of covalently linking the linker to a Bcl-xL inhibitor and a functional group capable of covalently linking the linker to an anti-EGFR antibody; (ii) partially conjugated forms of the linker that include a functional group capable of covalently linking the linker to an anti-EGFR antibody and that is covalently linked to a Bcl-xL inhibitor, or vice versa; and (iii) fully conjugated forms of the linker that is covalently linked to both a Bcl-xL inhibitor and an anti-EGFR antibody. In some specific embodiments of intermediate synthons and ADCs described herein, moieties comprising the functional groups on the linker and covalent linkages formed between the linker and antibody are specifically illustrated as RX and LK, respectively. One embodiment pertains to an ADC formed by contacting an antibody that binds a cell surface receptor or tumor associated antigen expressed on a tumor cell with a synthon described herein under conditions in which the synthon covalently links to the anti-EGFR antibody. One embodiment pertains to a method of making an ADC formed by contacting a synthon described herein under conditions in which the synthon covalently links to the anti-EGFR antibody. One embodiment pertains to a method of inhibiting Bcl-xL activity in a cell that expresses Bcl-xL, comprising contacting the cell with an ADC described herein that is capable of binding the cell, under conditions in which the ADC binds the cell.

[0614] Exemplary polyvalent linkers that may be used to link many Bcl-xL inhibitors to an antibody are described, for example, in U.S. Pat. No. 8,399,512; U.S. Published Application No. 2010/0152725; U.S. Pat. Nos. 8,524,214; 8,349,308; U.S. Published Application No. 2013/189218; U.S. Published Application No. 2014/017265; WO 2014/093379; WO 2014/093394; WO 2014/093640, the contents of which are incorporated herein by reference in their entireties. For example, the Fleximer® linker technology developed by Mersana et al. has the potential to enable high-DAR ADCs with good physicochemical properties. As shown below, the Fleximer® linker technology is based on incorporating drug molecules into a solubilizing poly-acetal backbone via a sequence of ester bonds. The methodology renders highly-loaded ADCs (DAR up to 20) whilst maintaining good physicochemical properties. This methodology could be utilized with Bcl-xL inhibitors as shown in the Scheme below.

##STR00113##

[0615] To utilize the Fleximer® linker technology depicted in the scheme above, an aliphatic alcohol can be present or introduced into the Bcl-xL inhibitor. The alcohol moiety is then conjugated to an alanine moiety, which is then synthetically incorporated into the Fleximer® linker.

Liposomal processing of the ADC in vitro releases the parent alcohol-containing drug.
[0616] Additional examples of dendritic type linkers can be found in US 2006/116422; US 2005/271615; de Groot et al., (2003) *Angew. Chem. Int. Ed.* 42:4490-4494; Amir et al., (2003) *Angew. Chem. Int. Ed.* 42:4494-4499; Shamis et al., (2004) *J. Am. Chem. Soc.* 126:1726-1731; Sun et al., (2002) *Bioorganic & Medicinal Chemistry Letters* 12:2213-2215; Sun et al., (2003) *Bioorganic & Medicinal Chemistry* 11:1761-1768; King et al., (2002) *Tetrahedron Letters* 43:1987-1990.

[0617] Exemplary monovalent linkers that may be used are described, for example, in Nolting, 2013, *Antibody-Drug Conjugates, Methods in Molecular Biology* 1045:71-100; Kitson et al., 2013, *CROs/CMOs—Chemica Oggi—Chemistry Today* 31(4): 30-36; Ducry et al., 2010, *Bioconjugate Chem.* 21:5-13; Zhao et al., 2011, *J. Med. Chem.* 54:3606-3623; U.S. Pat. Nos. 7,223,837; 8,568,728; 8,535,678; and WO2004010957, the content of each of which is incorporated herein by reference in their entirety.

[0618] By way of example and not limitation, some cleavable and noncleavable linkers that may be included in the ADCs described herein are described below.

3.2.1 Cleavable Linkers

[0619] In certain embodiments, the linker selected is cleavable in vitro and in vivo. Cleavable linkers may include chemically or enzymatically unstable or degradable linkages. Cleavable linkers generally rely on processes inside the cell to liberate the drug, such as reduction in the cytoplasm, exposure to acidic conditions in the lysosome, or cleavage by specific proteases or other enzymes within the cell. Cleavable linkers generally incorporate one or more chemical bonds that are either chemically or enzymatically cleavable while the remainder of the linker is noncleavable.

[0620] In certain embodiments, a linker comprises a chemically labile group such as hydrazone and/or disulfide groups. Linkers comprising chemically labile groups exploit differential properties between the plasma and some cytoplasmic compartments. The intracellular conditions to facilitate drug release for hydrazone containing linkers are the acidic environment of endosomes and lysosomes, while the disulfide containing linkers are reduced in the cytosol, which contains high thiol concentrations, e.g., glutathione. In certain embodiments, the plasma stability of a linker comprising a chemically labile group may be increased by introducing steric hindrance using substituents near the chemically labile group.

[0621] Acid-labile groups, such as hydrazone, remain intact during systemic circulation in the blood's neutral pH environment (pH 7.3-7.5) and undergo hydrolysis and release the drug once the ADC is internalized into mildly acidic endosomal (pH 5.0-6.5) and lysosomal (pH 4.5-5.0) compartments of the cell. This pH dependent release mechanism has been associated with nonspecific release of the drug. To increase the stability of the hydrazone group of the linker, the linker may be varied by chemical modification, e.g., substitution, allowing tuning to achieve more efficient release in the lysosome with a minimized loss in circulation.

[0622] Hydrazone-containing linkers may contain additional cleavage sites, such as additional acid-labile cleavage sites and/or enzymatically labile cleavage sites. ADCs including exemplary hydrazone-containing linkers include the following structures:

##STR00114##

wherein D and Ab represent the drug and Ab, respectively, and n represents the number of drug-linkers linked to the anti-EGFR antibody. In certain linkers such as linker (Ig), the linker comprises two cleavable groups—a disulfide and a hydrazone moiety. For such linkers, effective release of the unmodified free drug requires acidic pH or disulfide reduction and acidic pH. Linkers such as (Ih) and (Ii) have been shown to be effective with a single hydrazone cleavage site.

[0623] Other acid-labile groups that may be included in linkers include cis-aconityl-containing linkers. cis-Aconityl chemistry uses a carboxylic acid juxtaposed to an amide bond to accelerate amide hydrolysis under acidic conditions.

[0624] Cleavable linkers may also include a disulfide group. Disulfides are thermodynamically

stable at physiological pH and are designed to release the drug upon internalization inside cells, wherein the cytosol provides a significantly more reducing environment compared to the extracellular environment. Scission of disulfide bonds generally requires the presence of a cytoplasmic thiol cofactor, such as (reduced) glutathione (GSH), such that disulfide-containing linkers are reasonable stable in circulation, selectively releasing the drug in the cytosol. The intracellular enzyme protein disulfide isomerase, or similar enzymes capable of cleaving disulfide bonds, may also contribute to the preferential cleavage of disulfide bonds inside cells. GSH is reported to be present in cells in the concentration range of 0.5-10 mM compared with a significantly lower concentration of GSH or cysteine, the most abundant low-molecular weight thiol, in circulation at approximately 5 μ M. Tumor cells, where irregular blood flow leads to a hypoxic state, result in enhanced activity of reductive enzymes and therefore even higher glutathione concentrations. In certain embodiments, the in vivo stability of a disulfide-containing linker may be enhanced by chemical modification of the linker, e.g., use of steric hindrance adjacent to the disulfide bond.

[0625] ADCs including exemplary disulfide-containing linkers include the following structures:
##STR00115##

wherein D and Ab represent the drug and antibody, respectively, n represents the number of drug-linkers linked to the anti-EGFR antibody and R is independently selected at each occurrence from hydrogen or alkyl, for example. In certain embodiments, increasing steric hindrance adjacent to the disulfide bond increases the stability of the linker. Structures such as (Ij) and (II) show increased in vivo stability when one or more R groups is selected from a lower alkyl such as methyl.

[0626] Another type of linker that may be used is a linker that is specifically cleaved by an enzyme. In one embodiment, the linker is cleavable by a lysosomal enzyme. Such linkers are typically peptide-based or include peptidic regions that act as substrates for enzymes. Peptide based linkers tend to be more stable in plasma and extracellular milieu than chemically labile linkers. Peptide bonds generally have good serum stability, as lysosomal proteolytic enzymes have very low activity in blood due to endogenous inhibitors and the unfavorably high pH value of blood compared to lysosomes. Release of a drug from an anti-EGFR antibody occurs specifically due to the action of lysosomal proteases, e.g., cathepsin and plasmin. These proteases may be present at elevated levels in certain tumor tissues. In certain embodiments, the linker is cleavable by a lysosomal enzyme. In certain embodiments, the linker is cleavable by a lysosomal enzyme, and the lysosomal enzyme is Cathepsin B. In certain embodiments, the linker is cleavable by a lysosomal enzyme, and the lysosomal enzyme is β -glucuronidase or β -galactosidase. In certain embodiments, the linker is cleavable by a lysosomal enzyme, and the lysosomal enzyme is β -glucuronidase. In certain embodiments, the linker is cleavable by a lysosomal enzyme, and the lysosomal enzyme is β -galactosidase.

[0627] In exemplary embodiments, the cleavable peptide is selected from tetrapeptides such as Gly-Phe-Leu-Gly, Ala-Leu-Ala-Leu or dipeptides such as Val-Cit, Val-Ala, and Phe-Lys. In certain embodiments, dipeptides are preferred over longer polypeptides due to hydrophobicity of the longer peptides.

[0628] A variety of dipeptide-based cleavable linkers useful for linking drugs such as doxorubicin, mitomycin, camptothecin, tallsomycin and auristatin/auristatin family members to antibodies have been described (see, Dubowchik et al., 1998, *J. Org. Chem.* 67:1866-1872; Dubowchik et al., 1998, *Bioorg. Med. Chem. Lett.* 8:3341-3346; Walker et al., 2002, *Bioorg. Med. Chem. Lett.* 12:217-219; Walker et al., 2004, *Bioorg. Med. Chem. Lett.* 14:4323-4327; and Francisco et al., 2003, *Blood* 102:1458-1465, the contents of each of which are incorporated herein by reference). All of these dipeptide linkers, or modified versions of these dipeptide linkers, may be used in the ADCs described herein. Other dipeptide linkers that may be used include those found in ADCs such as Seattle Genetics' Brentuximab Vendotin SGN-35 (AdcetrisTM), Seattle Genetics SGN-75 (anti-CD-70, MC-monomethyl auristatin F(MMAF), Celldex Therapeutics glembatumumab (CDX-011)

(anti-NMB, Val-Cit-monomethyl auristatin E(MMAE), and Cytogen PSMA-ADC (PSMA-ADC-1301) (anti-PSMA, Val-Cit-MMAE).

[0629] Enzymatically cleavable linkers may include a self-immolative spacer to spatially separate the drug from the site of enzymatic cleavage. The direct attachment of a drug to a peptide linker can result in proteolytic release of an amino acid adduct of the drug, thereby impairing its activity. The use of a self-immolative spacer allows for the elimination of the fully active, chemically unmodified drug upon amide bond hydrolysis.

[0630] One self-immolative spacer is the bifunctional para-aminobenzyl alcohol group, which is linked to the peptide through the amino group, forming an amide bond, while amine containing drugs may be attached through carbamate functionalities to the benzylic hydroxyl group of the linker (to give a p-amidobenzylcarbamate, PABC). The resulting prodrugs are activated upon protease-mediated cleavage, leading to a 1,6-elimination reaction releasing the unmodified drug, carbon dioxide, and remnants of the linker group. The following scheme depicts the fragmentation of p-amidobenzyl carbamate and release of the drug:

##STR00116## [0631] wherein X-D represents the unmodified drug.

Heterocyclic variants of this self-immolative group have also been described. See U.S. Pat. No. 7,989,434.

[0632] In certain embodiments, the enzymatically cleavable linker is a B-glucuronic acid-based linker. Facile release of the drug may be realized through cleavage of the B-glucuronide glycosidic bond by the lysosomal enzyme B-glucuronidase. This enzyme is present abundantly within lysosomes and is overexpressed in some tumor types, while the enzyme activity outside cells is low. B-Glucuronic acid-based linkers may be used to circumvent the tendency of an ADC to undergo aggregation due to the hydrophilic nature of B-glucuronides. In certain embodiments, B-glucuronic acid-based linkers are preferred as linkers for ADCs linked to hydrophobic drugs. The following scheme depicts the release of the drug from an ADC containing a B-glucuronic acid-based linker:

##STR00117##

[0633] A variety of cleavable B-glucuronic acid-based linkers useful for linking drugs such as auristatins, camptothecin and doxorubicin analogues, CBI minor-groove binders, and psymberin to antibodies have been described (see, Jeffrey et al., 2006, *Bioconjug. Chem.* 17:831-840; Jeffrey et al., 2007, *Bioorg. Med. Chem. Lett.* 17:2278-2280; and Jiang et al., 2005, *J. Am. Chem. Soc.* 127:11254-11255, the contents of each of which are incorporated herein by reference). All of these β -glucuronic acid-based linkers may be used in the ADCs described herein. In certain embodiments, the enzymatically cleavable linker is a B-galactoside-based linker. B-Galactoside is present abundantly within lysosomes, while the enzyme activity outside cells is low.

[0634] Additionally, Bcl-xL inhibitors containing a phenol group can be covalently bonded to a linker through the phenolic oxygen. One such linker, described in U.S. Patent App. No. 2009/0318668, relies on a methodology in which a diamino-ethane "SpaceLink" is used in conjunction with traditional "PABO"-based self-immolative groups to deliver phenols. The cleavage of the linker is depicted schematically below using a Bcl-xL inhibitor of the disclosure.

##STR00118##

[0635] Cleavable linkers may include noncleavable portions or segments, and/or cleavable segments or portions may be included in an otherwise non-cleavable linker to render it cleavable. By way of example only, polyethylene glycol (PEG) and related polymers may include cleavable groups in the polymer backbone. For example, a polyethylene glycol or polymer linker may include one or more cleavable groups such as a disulfide, a hydrazone or a dipeptide.


[0636] Other degradable linkages that may be included in linkers include ester linkages formed by the reaction of PEG carboxylic acids or activated PEG carboxylic acids with alcohol groups on a biologically active agent, wherein such ester groups generally hydrolyze under physiological conditions to release the biologically active agent. Hydrolytically degradable linkages include, but

are not limited to, carbonate linkages; imine linkages resulting from reaction of an amine and an aldehyde; phosphate ester linkages formed by reacting an alcohol with a phosphate group; acetal linkages that are the reaction product of an aldehyde and an alcohol; orthoester linkages that are the reaction product of a formate and an alcohol; and oligonucleotide linkages formed by a phosphoramidite group, including but not limited to, at the end of a polymer, and a 5' hydroxyl group of an oligonucleotide.

[0637] In certain embodiments, the linker comprises an enzymatically cleavable peptide moiety, for example, a linker comprising structural formula (IVa), (IVb), (IVc), or (IVd):

##STR00119##

or a pharmaceutically acceptable salt thereof, wherein: [0638] peptide represents a peptide (illustrated N—C, wherein peptide includes the amino and carboxy “termini”) a cleavable by a lysosomal enzyme; [0639] T represents a polymer comprising one or more ethylene glycol units or an alkylene chain, or combinations thereof; [0640] R^{sup.a} is selected from hydrogen, C_{sub.1-6}alkyl, SO_{sub.3}H and CH_{sub.2}SO_{sub.3}H; [0641] R^{sup.y} is hydrogen or C_{sub.1-4} alkyl-(O)_{sub.r}—(C_{sub.1-4} alkylene)_{sub.s}-G^{sup.1} or C_{sub.1-4} alkyl-(N)—[(C_{sub.1-4} alkylene)-G^{sup.1}]_{sub.2}; [0642] R^{sup.z} is C_{sub.1-4} alkyl-(O)_{sub.r}—(C_{sub.1-4} alkylene)_{sub.s}-G^{sup.2}; [0643] G^{sup.1} is SO_{sub.3}H, CO_{sub.2}H, PEG 4-32, or sugar moiety; [0644] G^{sup.2} is SO_{sub.3}H, CO_{sub.2}H, or PEG 4-32 moiety; [0645] r is 0 or 1; [0646] s is 0 or 1; [0647] p is an integer ranging from 0 to 5; [0648] q is 0 or 1; [0649] x is 0 or 1; [0650] y is 0 or 1; [0651]

 custom-character represents the point of attachment of the linker to the Bcl-xL inhibitor; and [0652] * represents the point of attachment to the remainder of the linker.

[0653] In certain embodiments, the linker comprises an enzymatically cleavable peptide moiety, for example, a linker comprising structural formula (IVa), (IVb), (IVc), (IVd) or a pharmaceutically acceptable salt thereof.

[0654] In certain embodiments, linker L comprises a segment according to structural formula IVa or IVb or a pharmaceutically acceptable salt thereof.

[0655] In certain embodiments, the peptide is selected from a tripeptide or a dipeptide. In particular embodiments, the dipeptide is selected from: Val-Cit; Cit-Val; Ala-Ala; Ala-Cit; Cit-Ala; Asn-Cit; Cit-Asn; Cit-Cit; Val-Glu; Glu-Val; Ser-Cit; Cit-Ser; Lys-Cit; Cit-Lys; Asp-Cit; Cit-Asp; Ala-Val; Val-Ala; Phe-Lys; Lys-Phe; Val-Lys; Lys-Val; Ala-Lys; Lys-Ala; Phe-Cit; Cit-Phe; Leu-Cit; Cit-Leu; Ile-Cit; Cit-Ile; Phe-Arg; Arg-Phe; Cit-Trp; and Trp-Cit, or a pharmaceutically acceptable salt thereof.

[0656] Exemplary embodiments of linkers according to structural formula (IVa) that may be included in the ADCs described herein include the linkers illustrated below (as illustrated, the linkers include a group suitable for covalently linking the linker to an antibody):


##STR00120## ##STR00121##

[0657] Exemplary embodiments of linkers according to structural formula (IVb), (IVc), or (IVd) that may be included in the ADCs described herein include the linkers illustrated below (as illustrated, the linkers include a group suitable for covalently linking the linker to an antibody):

##STR00122## ##STR00123## ##STR00124## ##STR00125## ##STR00126## ##STR00127##
##STR00128## ##STR00129##

[0658] In certain embodiments, the linker comprises an enzymatically cleavable sugar moiety, for example, a linker comprising structural formula (Va), (Vb), (Vc), (Vd), or (Ve):

##STR00130## ##STR00131##

or a pharmaceutically acceptable salt thereof, wherein: [0659] q is 0 or 1; [0660] r is 0 or 1; [0661] X^{sup.1} is CH_{sub.2}, O or NH; [0662]  custom-character represents the point of attachment of the linker to the drug; and [0663] * represents the point of attachment to the remainder of the linker.

[0664] Exemplary embodiments of linkers according to structural formula (Va) that may be included in the ADCs described herein include the linkers illustrated below (as illustrated, the linkers include a group suitable for covalently linking the linker to an anti-EGFR antibody):

##STR00132## ##STR00133## ##STR00134##

[0665] Exemplary embodiments of linkers according to structural formula (Vb) that may be included in the ADCs described herein include the linkers illustrated below (as illustrated, the linkers include a group suitable for covalently linking the linker to an anti-EGFR antibody):

##STR00135## ##STR00136## ##STR00137##

[0666] Exemplary embodiments of linkers according to structural formula (Vc) that may be included in the ADCs described herein include the linkers illustrated below (as illustrated, the linkers include a group suitable for covalently linking the linker to an anti-EGFR antibody):

##STR00138## ##STR00139## ##STR00140##

[0667] Exemplary embodiments of linkers according to structural formula (Vd) that may be included in the ADCs described herein include the linkers illustrated below (as illustrated, the linkers include a group suitable for covalently linking the linker to an anti-EGFR antibody):

##STR00141## ##STR00142##

[0668] Exemplary embodiments of linkers according to structural formula (Ve) that may be included in the ADCs described herein include the linkers illustrated below (as illustrated, the linkers include a group suitable for covalently linking the linker to an anti-EGFR antibody):

##STR00143##


3.2.2 Non-Cleavable Linkers


[0669] Although cleavable linkers may provide certain advantages, the linkers comprising the ADC described herein need not be cleavable. For noncleavable linkers, the drug release does not depend on the differential properties between the plasma and some cytoplasmic compartments. The release of the drug is postulated to occur after internalization of the ADC via antigen-mediated endocytosis and delivery to lysosomal compartment, where the anti-EGFR antibody is degraded to the level of amino acids through intracellular proteolytic degradation. This process releases a drug derivative, which is formed by the drug, the linker, and the amino acid residue to which the linker was covalently attached. The amino-acid drug metabolites from conjugates with noncleavable linkers are more hydrophilic and generally less membrane permeable, which leads to less bystander effects and less nonspecific toxicities compared to conjugates with a cleavable linker. In general, ADCs with noncleavable linkers have greater stability in circulation than ADCs with cleavable linkers. Non-cleavable linkers may be alkylene chains, or maybe polymeric in natures, such as, for example, based upon polyalkylene glycol polymers, amide polymers, or may include segments of alkylene chains, polyalkylene glycols and/or amide polymers. In certain embodiments, the linker comprises a polyethylene glycol segment having from 1 to 6 ethylene glycol units.

[0670] A variety of non-cleavable linkers used to link drugs to antibodies have been described. (See, Jeffrey et al., 2006, *Bioconjug. Chem.* 17:831-840; Jeffrey et al., 2007, *Bioorg. Med. Chem. Lett.* 17:2278-2280; and Jiang et al., 2005, *J. Am. Chem. Soc.* 127:11254-11255, the contents of which are incorporated herein by reference). All of these linkers may be included in the ADCs described herein.

[0671] In certain embodiments, the linker is non-cleavable in vivo, for example a linker according to structural formula (VIa), (VIb), (VIc) or (VId) (as illustrated, the linkers include a group suitable for covalently linking the linker to an anti-EGFR antibody):

##STR00144##

or a pharmaceutically acceptable salt thereof, wherein: [0672] R_{sup.a} is selected from hydrogen, alkyl, sulfonate and methyl sulfonate; [0673] R_{sup.x} is a moiety including a functional group capable of covalently linking the linker to an antibody; and [0674]  custom-character represents the point of attachment of the linker to the Bcl-xL inhibitor.

[0675] Exemplary embodiments of linkers according to structural formula (VIa)-(VId) that may be included in the ADCs described herein include the linkers illustrated below (as illustrated, the linkers include a group suitable for covalently linking the linker to an anti-EGFR antibody, and “ custom-character” represents the point of attachment to a Bcl-xL inhibitor):

##STR00145##

3.2.3 Groups Used to Attach Linkers to Anti-EGFR Antibodies

[0676] Attachment groups can be electrophilic in nature and include: maleimide groups, activated disulfides, active esters such as NHS esters and HOBt esters, haloformates, acid halides, alkyl and benzyl halides such as haloacetamides. As discussed below, there are also emerging technologies related to “self-stabilizing” maleimides and “bridging disulfides” that can be used in accordance with the disclosure.

[0677] One example of a “self-stabilizing” maleimide group that hydrolyzes spontaneously under antibody conjugation conditions to give an ADC species with improved stability is depicted in the schematic below. See U.S. Published Application No. 2013/0309256, International Application Publication No. WO 2013/173337, Tumey et al., 2014, *Bioconjugate Chem.* 25: 1871-1880, and Lyon et al., 2014, *Nat. Biotechnol.* 32: 1059-1062. Thus, the maleimide attachment group is reacted with a sulfhydryl of an antibody to give an intermediate succinimide ring. The hydrolyzed form of the attachment group is resistant to deconjugation in the presence of plasma proteins.

##STR00146## ##STR00147##

[0678] As shown above, the maleimide ring of a linker may react with an antibody Ab, forming a covalent attachment as either a succinimide (closed form) or succinamide (open form).

[0679] Polytherics has disclosed a method for bridging a pair of sulfhydryl groups derived from reduction of a native hinge disulfide bond. See, Badescu et al., 2014, *Bioconjugate Chem.* 25:1124-1136. The reaction is depicted in the schematic below. An advantage of this methodology is the ability to synthesize homogenous DAR4 ADCs by full reduction of IgGs (to give 4 pairs of sulfhydryls) followed by reaction with 4 equivalents of the alkylating agent. ADCs containing “bridged disulfides” are also claimed to have increased stability.

##STR00148##

[0680] Similarly, as depicted below, a maleimide derivative that is capable of bridging a pair of sulfhydryl groups has been developed. See U.S. Published Application No. 2013/0224228.

##STR00149##



[0681] In certain embodiments the attachment moiety comprises the structural formulae (VIIa), (VIIb), or (VIIc):

##STR00150##

or a pharmaceutically acceptable salt thereof, wherein: [0682] R^{sup.q} is H or —O—(CH_{sub.2}CH_{sub.2}O)_{sub.11}—CH_{sub.3}; [0683] x is 0 or 1; [0684] y is 0 or 1; [0685] G^{sup.3} is —CH_{sub.2}CH_{sub.2}CH_{sub.2}SO_{sub.3}H or —CH_{sub.2}CH_{sub.2}O—(CH_{sub.2}CH_{sub.2}O)_{sub.11}—CH_{sub.3}; [0686] R^{sup.w} is —O—CH_{sub.2}CH_{sub.2}SO_{sub.3}H or —NH(CO)—CH_{sub.2}CH_{sub.2}O—(CH_{sub.2}CH_{sub.2}O)_{sub.12}—CH_{sub.3}; and [0687] * represents the point of attachment to the remainder of the linker.

[0688] In certain embodiments, the linker comprises a segment according to structural formulae (VIIa), (VIIb), or (VIIc):

##STR00151## ##STR00152##

or a hydrolyzed derivative or a pharmaceutically acceptable salt thereof, wherein: [0689] R^{sup.q} is H or —O—(CH_{sub.2}CH_{sub.2}O)_{sub.11}—CH_{sub.3}; [0690] x is 0 or 1; [0691] y is 0 or 1; [0692] G^{sup.3} is —CH_{sub.2}CH_{sub.2}CH_{sub.2}SO_{sub.3}H or —CH_{sub.2}CH_{sub.2}O—(CH_{sub.2}CH_{sub.2}O)_{sub.11}—CH_{sub.3}; [0693] R^{sup.w} is —O—CH_{sub.2}CH_{sub.2}SO_{sub.3}H or —NH(CO)—CH_{sub.2}CH_{sub.2}O—(CH_{sub.2}CH_{sub.2}O)_{sub.12}—CH_{sub.3}; [0694] * represents the point of attachment to the remainder of the linker; and [0695]  represents the point of attachment of the linker to the antibody, wherein when in the hydrolyzed form,  custom-character can be either at the α-position or β-position of the carboxylic acid next to it.

[0696] Exemplary embodiments of linkers according to structural formula (VIIa) and (VIIb) that may be included in the ADCs described herein include the linkers illustrated below (as illustrated,

the linkers include a group suitable for covalently linking the linker to an antibody):

##STR00153## ##STR00154## ##STR00155## ##STR00156## ##STR00157##

##STR00158##


[0697] Exemplary embodiments of linkers according to structural formula (VIIc) that may be included in the ADCs described herein include the linkers illustrated below (as illustrated, the linkers include a group suitable for covalently linking the linker to an antibody):

##STR00159## ##STR00160##

[0698] In certain embodiments, L is selected from the group consisting of IVa.1-IVa.8, IVb.1-IVb.19, IVc.1-IVc.7, IVd.1-IVd.4, Va.1-Va.12, Vb.1-Vb.10, Vc.1-Vc.11, Vd.1-Vd.6, Ve.1-Ve.2, VIa.1, VIc.1-VIc.2, VID.1-VID.4, VIIa.1-VIIa.4, VIIb.1-VIIb.8, VIIc.1-VIIc.6 in either the closed or open form.

[0699] In certain embodiments, L is selected from the group consisting of IVb.2, IVc.5, IVc.6, IVc.7, IVd.4, Vb.9, Vc.11, VIIa.1, VIIa.3, VIIc.1, VIIc.4, and VIIc.5, wherein the maleimide of each linker has reacted with the antibody Ab, forming a covalent attachment as either a succinimide (closed form) or succinamide (open form).

[0700] In certain embodiments, linker L is selected from the group consisting of IVb.2, IVc.5, IVc.6, IVd.4, Vc.11, VIIa.1, VIIa.3, VIIc.1, VIIc.4, VIIc.5, wherein the maleimide of each linker has reacted with the antibody Ab, forming a covalent attachment as either a succinimide (closed form) or succinamide (open form).

[0701] In certain embodiments, linker L is selected from the group consisting of IVb.2, Vc.11, VIIa.3, IVc.6, and VIIc.1, wherein  is the attachment point to drug D and @ is the attachment point to the LK, wherein when the linker is in the open form as shown below, @ can be either at the α -position or β -position of the carboxylic acid next to it:

##STR00161## ##STR00162## ##STR00163##

3.2.3 Linker Selection Considerations

[0702] As is known by skilled artisans, the linker selected for a particular ADC may be influenced by a variety of factors, including but not limited to, the site of attachment to the antibody (e.g., lys, cys or other amino acid residues), structural constraints of the drug pharmacophore and the lipophilicity of the drug. The specific linker selected for an ADC should seek to balance these different factors for the specific antibody/drug combination. For a review of the factors that are influenced by choice of linkers in ADCs, see Nolting, Chapter 5 “Linker Technology in Antibody-Drug Conjugates,” *In: Antibody-Drug Conjugates: Methods in Molecular Biology*, vol. 1045, pp. 71-100, Laurent Ducry (Ed.), Springer Science & Business Media, LLC, 2013.

[0703] For example, ADCs have been observed to effect killing of bystander antigen-negative cells present in the vicinity of the antigen-positive tumor cells. The mechanism of bystander cell killing by ADCs has indicated that metabolic products formed during intracellular processing of the ADCs may play a role. Neutral cytotoxic metabolites generated by metabolism of the ADCs in antigen-positive cells appear to play a role in bystander cell killing while charged metabolites may be prevented from diffusing across the membrane into the medium and therefore cannot affect bystander killing. In certain embodiments, the linker is selected to attenuate the bystander killing effect caused by cellular metabolites of the ADC. In certain embodiments, the linker is selected to increase the bystander killing effect.

[0704] The properties of the linker may also impact aggregation of the ADC under conditions of use and/or storage. Typically, ADCs reported in the literature contain no more than 3-4 drug molecules per antibody molecule (see, e.g., Chari, 2008, *Acc Chem Res* 41:98-107). Attempts to obtain higher drug-to-antibody ratios (“DAR”) often failed, particularly if both the drug and the linker were hydrophobic, due to aggregation of the ADC (King et al., 2002, *J Med Chem* 45:4336-4343; Hollander et al., 2008, *Bioconjugate Chem* 19:358-361; Burke et al., 2009 *Bioconjugate Chem* 20:1242-1250). In many instances, DARs higher than 3-4 could be beneficial as a means of increasing potency. In instances where the Bcl-xL inhibitor is hydrophobic in nature, it may be

desirable to select linkers that are relatively hydrophilic as a means of reducing ADC aggregation, especially in instances where DARS greater than 3-4 are desired. Thus, in certain embodiments, the linker incorporates chemical moieties that reduce aggregation of the ADCs during storage and/or use. A linker may incorporate polar or hydrophilic groups such as charged groups or groups that become charged under physiological pH to reduce the aggregation of the ADCs. For example, a linker may incorporate charged groups such as salts or groups that deprotonate, e.g., carboxylates, or protonate, e.g., amines, at physiological pH.

[0705] Exemplary polyvalent linkers that have been reported to yield DARs as high as 20 that may be used to link numerous Bcl-xL inhibitors to an antibody are described in U.S. Pat. No. 8,399,512; U.S. Published Application No. 2010/0152725; U.S. Pat. Nos. 8,524,214; 8,349,308; U.S. Published Application No. 2013/189218; U.S. Published Application No. 2014/017265; WO 2014/093379; WO 2014/093394; WO 2014/093640, the content of which are incorporated herein by reference in their entireties.

[0706] In particular embodiments, the aggregation of the ADCs during storage or use is less than about 40% as determined by size-exclusion chromatography (SEC). In particular embodiments, the aggregation of the ADCs during storage or use is less than 35%, such as less than about 30%, such as less than about 25%, such as less than about 20%, such as less than about 15%, such as less than about 10%, such as less than about 5%, such as less than about 4%, or even less, as determined by size-exclusion chromatography (SEC).

4. ADC Synthons

[0707] Antibody-Drug Conjugate synthons are synthetic intermediates used to form ADCs. The synthons are generally compounds according to structural formula (III):

D-L-R.sup.x (III) [0708] or salts thereof, wherein D is a Bcl-xL inhibitor as previously described, L is a linker as previously described, and R is a reactive group suitable for linking the synthon to an antibody. In specific embodiments, the ADC synthons are compounds according to structural formulae (IIIa) and (IIIb), or salts thereof, where the various substituents are as previously defined for structural formulae (IIa) and (IIb), respectively, and L and R are as defined for structural formula (III):

##STR00164##

[0709] To synthesize an ADC, an intermediate synthon according to structural formula (III), or a salt thereof, is contacted with an antibody of interest under conditions in which functional group R.sup.x reacts with a "complementary" functional group on the antibody, F.sup.x, to form a covalent linkage.

##STR00165##

[0710] The identities of groups R.sup.x and F.sup.x will depend upon the chemistry used to link the synthon to the antibody. Generally, the chemistry used should not alter the integrity of the antibody, for example its ability to bind its target. Preferably, the binding properties of the conjugated antibody will closely resemble those of the unconjugated antibody. A variety of chemistries and techniques for conjugating molecules to biological molecules such as antibodies are known in the art and in particular to antibodies, are well-known. See, e.g., Amon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy," in: *Monoclonal Antibodies And Cancer Therapy*, Reisfeld et al., Eds., Alan R. Liss, Inc., 1985; Hellstrom et al., "Antibodies For Drug Delivery," in: *Controlled Drug Delivery*, Robinson et al., Eds., Marcel Dekker, Inc., 2nd Ed. 1987; Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review," in: *Monoclonal Antibodies '84: Biological And Clinical Applications*, Pinchera et al., Eds., 1985; "Analysis, Results, and Future Prospective of the Therapeutic Use of Radiolabeled Antibody In Cancer Therapy," in: *Monoclonal Antibodies For Cancer Detection And Therapy*, Baldwin et al., Eds., Academic Press, 1985; Thorpe et al., 1982, Immunol. Rev. 62:119-58; PCT publication WO 89/12624. Any of these chemistries may be used to link the synthons to an antibody.

[0711] In one embodiment, RX comprises a functional group capable of linking the synthon to an amino group on an antibody. In another embodiment, RX comprises an NHS-ester or an isothiocyanate. In another embodiment, RX comprises a functional group capable of linking the synthon to a sulfhydryl group on an antibody. In another embodiment, RX comprises a haloacetyl or a maleimide. In another embodiment, L is selected from IVa or IVb and salts thereof; and RX comprises a functional group selected from the group consisting of NHS-ester, isothiocyanate, haloacetyl and maleimide.

[0712] Typically, the synthons are linked to the side chains of amino acid residues of the antibody, including, for example, the primary amino group of accessible lysine residues or the sulfhydryl group of accessible cysteine residues. Free sulfhydryl groups may be obtained by reducing interchain disulfide bonds.

[0713] In one embodiment, LK is a linkage formed with an amino group on the anti-hEGFR antibody Ab. In another embodiment, LK is an amide or a thiourea. In another embodiment, LK is a linkage formed with a sulfhydryl group on the anti-hEGFR antibody Ab. In another embodiment, LK is a thioether.

[0714] In one embodiment, LK is selected from the group consisting of amide, thiourea and thioether; and m is an integer ranging from 1 to 8.

[0715] A number of functional groups RX and chemistries useful for linking synthons to accessible lysine residues are known, and include by way of example and not limitation NHS-esters and isothiocyanates.

[0716] A number of functional groups RX and chemistries useful for linking synthons to accessible free sulfhydryl groups of cysteine residues are known, and include by way of example and not limitation haloacetyls and maleimides.

[0717] However, conjugation chemistries are not limited to available side chain groups. Side chains such as amines may be converted to other useful groups, such as hydroxyls, by linking an appropriate small molecule to the amine. This strategy can be used to increase the number of available linking sites on the antibody by conjugating multifunctional small molecules to side chains of accessible amino acid residues of the antibody. Functional groups RX suitable for covalently linking the synthons to these “converted” functional groups are then included in the synthons.

[0718] The antibody may also be engineered to include amino acid residues for conjugation. An approach for engineering antibodies to include non-genetically encoded amino acid residues useful for conjugating drugs in the context of ADCs is described in Axup et al., 2003, *Proc Natl Acad Sci* 109:16101-16106 and Tian et al., 2014, *Proc Natl Acad Sci* 111:1776-1771, as are chemistries and functional group useful for linking synthons to the non-encoded amino acids.

[0719] Exemplary synthons useful for making ADCs described herein include, but are not limited to, the following synthons listed below in Table 5.

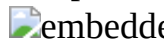
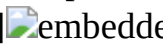
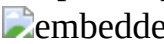
TABLE-US-00020 TABLE 5 Appln Ex. No. Synthon Synthon Structure 2.1 BS [00166]

 DK [00167]  DQ [00168] 

DJ [00169]  DO [00170]  DP [00171]

 HO [00172]  IT [00173] 

KA [00174]  KB [00175]  KT [00176]

 KU [00177]  KV [00178] 



KW [00179]  DC [00180]  KZ [00181]

 LW [00182]  LY [00183] 



LZ [00184]  MB [00185]  MC [00186]

 ME [00187]  MF [00188] 

MH [00189]  MI [00190]  NJ [00191]

 NK [00192]  NL [00193] 

NM [00194]  NR [00195]  EB [00196]

 embedded image 2.34 OG [00197]  embedded image 2.35 OH [00198]  embedded image 2.36 ON [00199]  embedded image 2.37 OT [00200]  embedded image 2.38 OP [00201]  embedded image 2.39 OU [00202]  embedded image 2.40 OO [00203]  embedded image 2.41 OQ [00204]  embedded image 2.42 OR [00205]  embedded image 2.43 OS [00206]  embedded image 2.44 OX [00207]  embedded image 2.45 OZ [00208]  embedded image 2.46 PA [00209]  embedded image 2.47 QL [00210]  embedded image 2.48 QM [00211]  embedded image 2.49 QN [00212]  embedded image 2.50 QT [00213]  embedded image 2.51 RF [00214]  embedded image 2.52 RG [00215]  embedded image 2.53 SF [00216]  embedded image 2.54 SR [00217]  embedded image 2.55 YZ [00218]  embedded image 2.56 QR [00219]  embedded image 2.57 SE [00220]  embedded image 2.58 UH [00221]  embedded image 2.59 UI [00222]  embedded image 2.60 US [00223]  embedded image 2.61 UY [00224]  embedded image 2.62 UX [00225]  embedded image 2.63 WZ [00226]  embedded image 2.64 XO [00227]  embedded image 2.65 XW [00228]  embedded image 2.66 YG [00229]  embedded image 2.67 ZT [00230]  embedded image 2.68 AAN [00231]  embedded image 2.69 AAO [00232]  embedded image 2.70 AAP [00233]  embedded image 2.71 ABF [00234]  embedded image 2.72 ZZ [00235]  embedded image 2.73 (control) CZ [00236]  embedded image 2.74 (control) TX [00237]  embedded image 2.75 (control) LB [00238]  embedded image 2.76 (control) WD [00239]  embedded image 2.77 (control) TV [00240]  embedded image 2.78 (control) YY [00241]  embedded image 2.79 (control) AAA [00242]  embedded image 2.80 (control) AAD [00243]  embedded image

[0720] In certain embodiments, the synthon is selected from the group consisting of synthon examples 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 2.10, 2.11, 2.12, 2.13, 2.14, 2.15, 2.16, 2.17, 2.18, 2.19, 2.20, 2.21, 2.22, 2.23, 2.24, 2.25, 2.26, 2.27, 2.28, 2.29, 2.30, 2.31, 2.34, 2.35, 2.36, 2.37, 2.38, 2.39, 2.40, 2.41, 2.42, 2.43, 2.44, 2.45, 2.46, 2.47, 2.48, 2.49, 2.50, 2.51, 2.52, 2.53, 2.54, 2.55, 2.56, 2.57, 2.58, 2.59, 2.60, 2.61, 2.62, 2.63, 2.64, 2.65, 2.66, 2.67, 2.68, 2.69, 2.70, 2.71, 2.72, and pharmaceutically acceptable salts thereof. The corresponding compound names of these synthons are provided below: [0721] N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[(2-((3-((4-((6-[1-(1,3-benzothiazol-2-yl)carbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl]-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl)(methyl)carbamoyl}oxy)methyl]phenyl}-N.sup.5-carbamoyl-L-ornithinamide; [0722] N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[(2-((3-((4-((6-[4-(1,3-benzothiazol-2-yl)carbamoyl)-3,4-dihydro-2H-1,4-benzoxazin-6-yl]-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl)(methyl)carbamoyl}oxy)methyl]phenyl}-N.sup.5-carbamoyl-L-ornithinamide; [0723] N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[(2-((3-((4-((6-[4-(1,3-benzothiazol-2-yl)carbamoyl)-1-methyl-1,2,3,4-tetrahydroquinoxalin-6-yl]-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl)(methyl)carbamoyl}oxy)methyl]phenyl}-N.sup.5-carbamoyl-L-ornithinamide; [0724] 4-[(1E)-3-((2-((3-((4-((6-[1-(1,3-benzothiazol-2-yl)carbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl]-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl)(methyl)carbamoyl}oxy)prop-1-en-yl]-2-((N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid; [0725] 4-[(1E)-3-((2-((3-((4-((6-[4-(1,3-benzothiazol-2-yl)carbamoyl)-1-methyl-1,2,3,4-tetrahydroquinoxalin-6-yl]-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl)(methyl)carbamoyl}oxy)prop-1-en-yl]-2-((N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid; [0726] 4-[(1E)-3-((2-((3-((4-((6-[4-(1,3-benzothiazol-2-yl)carbamoyl)-3,4-dihydro-2H-1,4-benzoxazin-6-yl]-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl)(methyl)carbamoyl}oxy)prop-1-en-yl]-2-((N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-

1-yl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid; [0727] 4-[(1E)-3-
({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)naphthalen-2-yl]-2-carboxypyridin-3-yl}-5-
methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl]
(methyl)carbamoyl}oxy)prop-1-en-yl]-2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-
beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid; [0728] 4-[(1E)-3-({[2-({3-[(4-{6-[8-
(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-
methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1-3,7-]dec-1-yl}oxy)ethyl](oxetan-3-
yl)carbamoyl}oxy)prop-1-en-1-yl]-2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-
beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid; [0729] 4-[(1E)-3-({[2-({3-[(4-{6-[8-
(1,3-benzothiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-
3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl]
(2-methoxyethyl)carbamoyl}oxy)prop-1-en-1-yl]-2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-
yl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid; [0730] 4-[(1E)-3-({[2-
({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-2-
carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-
yl}oxy)ethyl](2-methoxyethyl)carbamoyl}oxy)prop-1-en-1-yl]-2-({N-[(2,5-dioxo-2,5-dihydro-1H-
pyrrol-1-yl)acetyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid; [0731] 4-[(1E)-3-({[2-
({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-2-
carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-
1-yl}oxy)ethyl](2-methoxyethyl)carbamoyl}oxy)methyl]-3-[2-(2-{[3-(2,5-dioxo-2,5-dihydro-1H-
pyrrol-1-yl)propanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid; [0732] 6-
[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[3-(2-
{[(2E)-3-[4-{[(2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl]oxy}-3-
({3-[(2E)-3-(4-{1[(2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-
yl]oxy}-3-{[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-
yl)propanoyl]amino}propanoyl]amino)phenyl]prop-2-en-1-
yl}oxy}carbonyl)amino]propanoyl}amino)phenyl]prop-2-en-1-yl}oxy)carbonyl](2-
methoxyethyl)amino}ethoxy)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl]methyl}-5-methyl-1H-
pyrazol-4-yl]pyridine-2-carboxylic; [0733] 4-[(1E)-3-({[2-(2-{2-[(1E)-3-[(4-{6-[8-(1,3-benzothiazol-2-
ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-
pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl](2-
methoxyethyl)carbamoyl}oxy)methyl]-5-(beta-D-
glucopyranuronosyloxy)phenoxy}ethoxy)ethyl]carbamoyl}oxy)methyl]-3-[2-(2-{[3-(2,5-dioxo-
2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic
acid; [0734] 4-[(1E)-3-({[2-(2-{3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-methoxy-3,4-
dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-
dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl](2-methoxyethyl)carbamoyl}oxy)methyl]-3-
[2-(2-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}ethoxy)ethoxy]phenyl beta-D-
glucopyranosiduronic acid; [0735] 6-[1-(1,3-benzothiazol-2-ylcarbamoyl)-1,2,3,4-
tetrahydroquinolin-7-yl]-3-{1-[(3-{[34-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3-methyl-4,32-
dioxo-7,10,13,16,19,22,25,28-octa-3,31-diazatetratricont-1-yl]oxy}-5,7-
dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic
acid; [0736] 4-[(1E)-3-({[2-(2-{3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-cyano-3,4-
dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-
dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl]carbamoyl}oxy)methyl]-3-[2-(2-{[3-(2,5-
dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}ethoxy)ethoxy]phenyl beta-D-
glucopyranosiduronic acid; [0737] 4-[(1E)-3-({[2-(2-{3-[(4-{6-[8-(1,3-benzothiazol-2-
ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-
pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl](2-
methoxyethyl)carbamoyl}oxy)prop-1-en-1-yl]-2-({N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-

yl)propanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid; [0738] N-[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-3-sulfo-L-alanyl-N-{5-[(1E)-3-({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl](2-methoxyethyl)carbamoyl}oxy)prop-1-en-1-yl]-2-(beta-D-glucopyranuronosyloxy)phenyl}-beta-alaninamide; [0739] N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-3-sulfo-L-alanyl-N-{5-[(1E)-3-({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl](2-methoxyethyl)carbamoyl}oxy)prop-1-en-1-yl]-2-(beta-D-glucopyranuronosyloxy)phenyl}-beta-alaninamide; [0740] N-[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-beta-alanyl-N-{5-[(1E)-3-({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl](2-methoxyethyl)carbamoyl}oxy)prop-1-en-1-yl]-2-(beta-D-glucopyranuronosyloxy)phenyl}-beta-alaninamide; [0741] N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-beta-alanyl-N-{5-[(1E)-3-({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl](2-methoxyethyl)carbamoyl}oxy)prop-1-en-1-yl]-2-(beta-D-glucopyranuronosyloxy)phenyl}-beta-alaninamide; [0742] 4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl](2-methoxyethyl)carbamoyl}oxy)methyl]-3-{2-[2-({N-[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-3-sulfo-L-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid; [0743] 4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl](2-methoxyethyl)carbamoyl}oxy)methyl]-3-{2-[2-({N-[(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-3-sulfo-L-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid; [0744] 4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl](2-methoxyethyl)carbamoyl}oxy)methyl]-3-{2-[2-({N-[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-beta-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid; [0745] 4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl](2-methoxyethyl)carbamoyl}oxy)methyl]-3-{2-[2-({N-[(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-beta-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid; [0746] 2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl](2-methoxyethyl)carbamoyl}oxy)methyl]-5-{2-[2-({N-[(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-3-sulfo-L-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid; [0747] 2-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl](2-methoxyethyl)carbamoyl}oxy)methyl]-5-{2-[2-({N-[(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid; [0748] 4-[({[1[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl](2-methoxyethyl)carbamoyl}oxy)methyl]-3-[3-({N-[(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-

alanyl}amino)propoxy]phenyl beta-D-glucopyranosiduronic acid; [0749] 4-[(1[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-[3-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)propoxy]phenyl beta-D-glucopyranosiduronic acid; [0750] N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[(1[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-2-[6-carboxy-5-(1-{3-(2-methoxyethoxy)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl]-1,2,3,4-tetrahydroisoquinolin-6-yl}pyrrolidin-3-yl]carbamoyl}oxy)methyl]phenyl}-L-alaninamide; [0751] N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[(1[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl](2-sulfamoyl)ethyl)carbamoyl}oxy)methyl]phenyl}-N.sup.5-carbamoyl-L-ornithinamide; [0752] 4-[(1[2-({3-[(4-{6-[1-(1,3-benzothiazol-2-ylcarbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl](2-methoxyethyl)carbamoyl}oxy)methyl]-3-{2-[2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid; [0753] 2-[(1[2-({3-[(4-{6-[5-(1,3-benzothiazol-2-ylcarbamoyl)quinolin-3-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-{2-[2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid; [0754] 2-[(1[2-({3-[(4-{6-[1-(1,3-benzothiazol-2-ylcarbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-{2-[2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid; [0755] 4-[(1[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)naphthalen-2-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-{2-[2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid; [0756] 2-[(1[2-({3-[(4-{6-[4-(1,3-benzothiazol-2-ylcarbamoyl)quinolin-6-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-{2-[2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid; [0757] 4-[(1[2-({3-[(4-{6-[1-(1,3-benzothiazol-2-ylcarbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-{2-[2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid; [0758] 4-[(1[2-({3-[(4-{6-[1-(1,3-benzothiazol-2-ylcarbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-(3-{1[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]amino}propoxy)phenyl beta-D-glucopyranosiduronic acid; [0759] 4-[(1[2-({3-[(4-{6-[1-(1,3-benzothiazol-2-ylcarbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-[3-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)propoxy]phenyl beta-D-glucopyranosiduronic acid; [0760] 2-[(1[2-({3-[(4-{6-[1-(1,3-benzothiazol-2-ylcarbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-{2-[2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-

yl)hexanoyl]-3-sulfo-L-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid; [0761] 4-[(2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)naphthalen-2-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl](2-methoxyethyl)carbamoyl}oxy)methyl]-3-[2-2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid; [0762] N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-[4-({2-({8-(1,3-benzothiazol-2-ylcarbamoyl)-2-[6-carboxy-5-(1-{[3-(2-methoxyethoxy)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl]-1,2,3,4-tetrahydroisoquinolin-6-yl}(methyl)amino)ethyl}(methyl)carbamoyl}oxy)methyl)phenyl]-L-alaninamide; [0763] N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[(2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-6-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]phenyl}-L-alaninamide; [0764] 2-[(2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)naphthalen-2-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl)(2-methoxyethyl)carbamoyl}oxy)methyl]-5-[2-2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid; [0765] 2-[(1[2-({3-[(4-{6-[5-(1,3-benzothiazol-2-ylcarbamoyl)quinolin-3-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-[2-2-({6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid; [0766] 4-[(1[2-({3-[(4-{6-[5-(1,3-benzothiazol-2-ylcarbamoyl)quinolin-3-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-[2-2-({6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid; [0767] 6-[5-(1,3-benzothiazol-2-ylcarbamoyl)quinolin-3-yl]-3-(1-{[3-(2-({6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl)(methyl)amino}ethoxy)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid; [0768] 4-[(1[2-({3-[(4-{6-[7-(1,3-benzothiazol-2-ylcarbamoyl)-1H-indol-2-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-2-({N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid; [0769] 4-[(1[2-({3-[(4-{6-[7-(1,3-benzothiazol-2-ylcarbamoyl)-1H-indol-2-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-[2-2-({1[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid; [0770] 4-[(1[2-({3-[(4-{6-[7-(1,3-benzothiazol-2-ylcarbamoyl)-1H-indol-2-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-[2-2-({N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-3-sulfo-L-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid; [0771] 4-[(2-({3-[(4-{6-[7-(1,3-benzothiazol-2-ylcarbamoyl)-3-methyl-1H-indol-2-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-[2-2-({3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid; [0772] N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[(2-({3-[(4-{6-[4-(1,3-benzothiazol-2-ylcarbamoyl)isoquinolin-6-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]phenyl}-N.sup.5-carbamoyl-L-ornithinamide; [0773] 4-[(2-({3-[(4-{6-[1-(1,3-benzothiazol-2-ylcarbamoyl)-5,6-dihydroimidazo[1,5-a]pyrazin-7(8H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-

yl}oxy)ethyl]carbamoyl}oxy)methyl]-3-[2-(2-[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino)ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid; [0774] 2-[(2-[(3-[(4-{6-[5-(1,3-benzothiazol-2-ylcarbamoyl)quinolin-3-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl]carbamoyl}oxy)methyl]-4-[19-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-14-oxo-4,7,10-trioxa-13-azanonadec-1-yl]phenyl beta-D-glucopyranosiduronic acid; [0775] 4-[(2-[(3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)naphthalen-2-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-[4-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)butyl]phenyl beta-D-glucopyranosiduronic acid; [0776] 2-{6-[2-[(3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl]-2-methyl-3,3-dioxido-7-oxo-8-oxa-3lambda.sup.6-thia-2,6-diazanonan-9-yl}-5-(4-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}butyl)phenyl beta-D-glucopyranosiduronic acid; [0777] 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[3-(2-[(2-[(2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl]oxy}-4-(4-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}butyl)benzyl]oxy}carbonyl)[3-(dimethylamino)-3-oxopropyl]amino}ethoxy)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid; [0778] 2-[(2-[(3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl](2-sulfamoyl)ethyl)carbamoyl}oxy)methyl]-5-(4-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}butyl)phenyl beta-D-glucopyranosiduronic acid; [0779] 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[3-(2-[(2-[(2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl]oxy}-4-(4-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}butyl)benzyl]oxy}carbonyl)[3-(methylamino)-3-oxopropyl]amino}ethoxy)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid; [0780] 3-{1-[(3-{2-[(3-amino-3-oxopropyl)](2-[(2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl]oxy}-4-(4-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}butyl)benzyl]oxy}carbonyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid; [0781] 2-[(2-[(3-[(4-{6-[3-(1,3-benzothiazol-2-ylcarbamoyl)-1H-indol-5-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-(4-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}butyl)phenyl beta-D-glucopyranosiduronic acid; [0782] 2-[(2-[(3-[(4-{6-[1-(1,3-benzothiazol-2-ylcarbamoyl)-5,6-dihydroimidazo[1,5-a]pyrazin-7(8H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl]carbamoyl}oxy)methyl]-5-(4-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}butyl)phenyl beta-D-glucopyranosiduronic acid; [0783] (6S)-2,6-anhydro-6-(2-{2-[(2-[(3-[(4-{6-[1-(1,3-benzothiazol-2-ylcarbamoyl)-5,6-dihydroimidazo[1,5-a]pyrazin-7(8H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl]carbamoyl}oxy)methyl]-5-({N-[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-L-valyl-L-alanyl}amino)phenyl)ethyl)-L-gulonic acid; [0784] (6S)-2,6-anhydro-6-[2-(2-[(2-[(3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl](2-methoxyethyl)carbamoyl}oxy)methyl]-5-{[N-[(3S,5S)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-oxo-5-(2-sulfoethoxy)methyl]pyrrolidin-1-yl]acetyl)-L-valyl-L-alanyl]amino}phenyl)ethyl]-L-gulonic acid; [0785] 8-[2-[(3-amino-3-oxopropyl)]2-[(3-{4-(6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-

dihydroisoquinolin-2(1H)-yl}-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl}-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]decan-1-yl)oxy}ethyl}carbamoyl}oxy}methyl)-5-{{[(2S)-2-((2S)-2-[2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetamido]-3-methylbutanoyl}amino)propanoyl]amino}phenyl]-2,6-anhydro-7,8-dideoxy-L-glycero-L-gulo-octonic acid; [0786] 4-{{[(2-[(3-{{[4-(6-{8-[(1,3-benzothiazol-2-yl)carbamoyl]-3,4-dihydroisoquinolin-2(1H)-yl}-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl}-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]decan-1-yl)oxy}ethyl}{3-(methylamino)-3-oxopropyl}carbamoyl)oxy}methyl}-3-{3-[2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetamido]propoxy}phenyl beta-D-glucopyranosiduronic acid; [0787] 2,6-anhydro-8-(2-{{[(2-[(3-{{[4-(6-{8-[(1,3-benzothiazol-2-yl)carbamoyl]-3,4-dihydroisoquinolin-2(1H)-yl}-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl}-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]decan-1-yl)oxy}ethyl}{3-(methylamino)-3-oxopropyl}carbamoyl)oxy}methyl}-5-{{[(2S)-2-((2S)-2-[2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetamido]-3-methylbutanoyl}amino)propanoyl]amino}phenyl]-7,8-dideoxy-L-glycero-L-gulo-octonic acid; [0788] 2,6-anhydro-8-(2-{{[(2-[(3-{{[4-(6-{8-[(1,3-benzothiazol-2-yl)carbamoyl]-3,4-dihydroisoquinolin-2(1H)-yl}-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl}-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]decan-1-yl)oxy}ethyl}{3-(methylamino)-3-oxopropyl}carbamoyl)oxy}methyl}-5-{{[(2S)-2-((2S)-2-(2-{{(3S,5S)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-oxo-5-[(2-sulfoethoxy)methyl]pyrrolidin-1-yl}acetamido)-3-methylbutanoyl]amino}propanoyl]amino}phenyl)-7,8-dideoxy-L-glycero-L-gulo-octonic acid; [0789] 6-{8-[(1,3-benzothiazol-2-yl)carbamoyl]-3,4-dihydroisoquinolin-2(1H)-yl}-3-[1-((3-[2-((4-{{[(2S)-5-(carbamoylamino)-2-{{[(2S)-2-{{1[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]amino}-3-methylbutanoyl]amino}pentanoyl]amino}phenyl)methoxy]carbonyl}amino)acetamido]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]decan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid; and [0790] 8-[2-((3-amino-3-oxopropyl){2-[(3-{{[4-(6-{8-[(1,3-benzothiazol-2-yl)carbamoyl]-3,4-dihydroisoquinolin-2(1H)-yl}-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl}-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]decan-1-yl)oxy}ethyl}carbamoyl}oxy}methyl)-5-{{1[(2S)-2-{{1[(2S)-2-(2-{{(3S,5S)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-oxo-5-[(2-sulfoethoxy)methyl]pyrrolidin-1-yl}acetamido)-3-methylbutanoyl]amino}propanoyl]amino}phenyl]-2,6-anhydro-7,8-dideoxy-L-glycero-L-gulo-octonic acid.

[0791] In certain embodiments, the ADC, or a pharmaceutically acceptable salt thereof, comprises [0792] D is the Bcl-xL inhibitor selected from the group consisting of the following compounds modified in that the hydrogen corresponding to the #position is not present, forming a monoradical: [0793] W3.01, W3.02, W3.03, W3.04, W3.05, W3.06, W3.07, W3.08, W3.09, W3.10, W3.11, W3.12, W3.13, W3.14, W3.15, W3.16, W3.17, W3.18, W3.19, W3.20, W3.21, W3.22, W3.23, W3.24, W3.25, W3.26, W3.27, W3.28, W3.29, W3.30, W3.31, W3.32, W3.33, W3.34, W3.35, W3.36, W3.37, W3.38, W3.39, W3.40, W3.41, W3.42, and W3.43 and pharmaceutically acceptable salts thereof; [0794] L is selected from the group consisting of linkers IVa.1-IVa.8, IVb.1-IVb.19, IVc.1-IVc.7, IVd.1-IVd.4, Va.1-Va.12, Vb.1-Vb.10, Vc.1-Vc.11, Vd.1-Vd.6, Ve.1-Ve.2, VIa.1, VIc.1-VIc.2, VId.1-VId.4, VIIa.1-VIIa.4, VIIb.1-VIIb.8, and VIIc.1-VIIc.6, wherein each linker has reacted with the antibody, Ab, forming a covalent attachment; [0795] LK is thioether; and [0796] m is an integer ranging from 1 to 8.

[0797] In certain embodiments, the ADC, or a pharmaceutically acceptable salt thereof, [0798] D is the Bcl-xL inhibitor selected from the group consisting of the following compounds modified in that the hydrogen corresponding to the #position is not present, forming a monoradical: [0799] 3-(1-{{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl}-5-methyl-1H-pyrazol-4-yl)-6-[1-(1,3-benzothiazol-2-ylcarbamoyl)-5,6-dihydroimidazo[1,5-a]pyrazin-7(8H)-yl]pyridine-2-carboxylic acid; [0800] 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)naphthalen-2-yl]-3-[1-

({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid; [0801] 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{2-[(2-methoxyethyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid; [0802] 3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-cyano-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid; [0803] 6-[4-(1,3-benzothiazol-2-ylcarbamoyl)isoquinolin-6-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid; [0804] 3-{1-[(3-{2-[(3-amino-3-oxopropyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid; [0805] and pharmaceutically acceptable salts thereof; [0806] L is selected from the group consisting of linkers IVb.2, IVc.5, IVc.6, IVc.7, Vc.11, IVd.4, Vb.9, VIIa.1, VIIa.3, VIIc.1, VIIc.4, and VIIc.5 in either closed or open forms, and pharmaceutically acceptable salts thereof; [0807] LK is thioether; and [0808] m is an integer ranging from 2 to 4.

[0809] To form an ADC, the maleimide ring of a synthon (for example, the synthons listed in Table 5) may react with an antibody Ab, forming a covalent attachment as either a succinimide (closed form) or succinamide (open form). Similarly, other functional groups, e.g. acetyl halide or vinyl sulfone may react with an antibody, Ab, forming a covalent attachment.

[0810] In certain embodiments, the ADC, or a pharmaceutically acceptable salt thereof, is selected from the group consisting of AbA-ZT, AbA-ZZ, AbA-XW, AbA-SE, AbA-SR, AbA-YG, AbA-KZ, AbB-ZT, AbB-ZZ, AbB-XW, AbB-SE, AbB-SR, AbB-YG, AbB-KZ, AbG-ZT, AbG-ZZ, AbG-XW, AbG-SE, AbG-SR, AbG-YG, AbG-KZ, AbK-ZT, AbK-ZZ, AbK-XW, AbK-SE, AbK-SR, AbK-YG, and AbK-KZ, wherein KZ, SR, SE, XW, YG, ZT and ZZ are synthons disclosed in Table 5, and where in the synthons are either in open or closed form. In a specific embodiment, the ADC is AbA-ZT, AbA-ZZ, AbA-SE, AbA-SR, AbB-ZT, AbB-ZZ, AbB-SE, AbB-SR, AbG-ZT, AbG-ZZ, AbG-SE, AbG-SR, AbK-ZT, AbK-ZZ, AbK-SE, AbK-SR, wherein AbA, AbB, AbG, and AbK are the anti-hEGFR antibodies and KZ, SR, SE, XW, YG, ZT and ZZ are synthons disclosed in Table 5, and wherein the synthons are either in open or closed form.

[0811] In certain embodiments, the ADC, or a pharmaceutically acceptable salt thereof, is
##STR00244## ##STR00245## ##STR00246## ##STR00247## ##STR00248## ##STR00249##
##STR00250##

wherein m is an integer from 1 to 6. In a specific embodiment, m is an integer from 2 to 6.

[0812] In one embodiment, the ADC, or a pharmaceutically acceptable salt thereof, is
##STR00251##

wherein m is 2, Ab is an hEGFR antibody, wherein the hEGFR antibody comprises a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 12, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 11, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 10; and a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 8, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 7, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 6; optionally wherein the hEGFR antibody comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 9, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 5; optionally, wherein the hEGFR antibody comprises a heavy chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 41 and/or a light chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 43; optionally, wherein the hEGFR antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 15, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 13; optionally, wherein the hEGFR antibody comprises a heavy chain comprising the amino

acid sequence set forth in SEQ ID NO: 102, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 13.

[0813] In one embodiment, the ADC, or a pharmaceutically acceptable salt thereof, is

##STR00252##

wherein m is 2, Ab is an hEGFR antibody, wherein the hEGFR antibody comprises a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 18, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 17, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 16; and a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 25, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 24, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 23; optionally, wherein the hEGFR antibody comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 72, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 73; optionally, wherein the hEGFR antibody comprises a heavy chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 41 and/or a light chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 43; optionally, wherein the hEGFR antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 93, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 95; optionally, wherein the hEGFR antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 94, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 95.

[0814] In one embodiment, the ADC, or a pharmaceutically acceptable salt thereof, is

##STR00253##

wherein m is 2, Ab is an hEGFR antibody, wherein the hEGFR antibody comprises a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 12, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 11, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 10; and a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 8, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 7, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 6; optionally wherein the hEGFR antibody comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 9, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 5; optionally, wherein the hEGFR antibody comprises a heavy chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 41 and/or a light chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 43; optionally, wherein the hEGFR antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 15, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 13; optionally, wherein the hEGFR antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 102, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 13.

[0815] In one embodiment, the ADC, or a pharmaceutically acceptable salt thereof, is

##STR00254##

wherein m is 2, Ab is an hEGFR antibody, wherein the hEGFR antibody comprises a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 18, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 17, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 16; and a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 25, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 24, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 23; optionally, wherein the hEGFR antibody comprises a heavy chain variable region comprising the amino acid

sequence set forth in SEQ ID NO: 72, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 73; optionally, wherein the hEGFR antibody comprises a heavy chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 41 and/or a light chain constant region comprising the amino acid sequence set forth in SEQ ID NO: 43; optionally, wherein the hEGFR antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 93, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 95; optionally, wherein the hEGFR antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 94, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 95.

[0816] Bcl-xL inhibitors, including warheads and synthons, and methods of making the same are described in US 2016-0158377 (AbbVie Inc.), which is incorporated by reference herein.

5. Methods of Synthesis of ADCs

[0817] The Bcl-xL inhibitors and synthons described herein may be synthesized using standard, known techniques of organic chemistry. General schemes for synthesizing Bcl-xL inhibitors and synthons that may be used as-is or modified to synthesize the full scope of Bcl-xL inhibitors and synthons described herein are provided below. Specific methods for synthesizing exemplary Bcl-xL inhibitors and synthons that may be useful for guidance are provided in the Examples section.

[0818] ADCs may likewise be prepared by standard methods, such as methods analogous to those described in Hamblett et al., 2004, "Effects of Drug Loading on the Antitumor Activity of a Monoclonal Antibody Drug Conjugate", *Clin. Cancer Res.* 10:7063-7070; Doronina et al., 2003, "Development of potent and highly efficacious monoclonal antibody auristatin conjugates for cancer therapy," *Nat. Biotechnol.* 21(7):778-784; and Francisco et al., 2003, "cACIO-vcMMAE., an anti-CD30-monomethylauristatin E conjugate with potent and selective antitumor activity," *Blood* 102:1458-1465. For example, ADCs with four drugs per antibody may be prepared by partial reduction of the antibody with an excess of a reducing reagent such as DTT or TCEP at 37° C. for 30 min, then the buffer exchanged by elution through SEPHADEX® G-25 resin with 1 mM DTPA in DPBS. The eluent is diluted with further DPBS, and the thiol concentration of the antibody may be measured using 5,5'-dithiobis(2-nitrobenzoic acid) [Ellman's reagent]. An excess, for example 5-fold, of a linker-drug synthon is added at 4° C. for 1 hour, and the conjugation reaction may be quenched by addition of a substantial excess, for example 20-fold, of cysteine. The resulting ADC mixture may be purified on SEPHADEX G-25 equilibrated in PBS to remove unreacted synthons, desalted if desired, and purified by size-exclusion chromatography. The resulting ADC may then be then sterile-filtered, for example, through a 0.2 μ m filter, and lyophilized if desired for storage. In certain embodiments, all of the interchain cysteine disulfide bonds are replaced by linker-drug conjugates. One embodiment pertains to a method of making an ADC, comprising contacting a synthon described herein with an antibody under conditions in which the synthon covalently links to the antibody.

[0819] Specific methods for synthesizing exemplary ADCs that may be used to synthesize the full range of ADCs described herein are provided in the Examples section.

5.1 General Methods for Synthesizing Bcl-xL Inhibitors

[0820] In the schemes below, the various substituents Ar.sup.1, Ar.sup.2, Z.sup.1, R.sup.4, R.sup.10, R.sup.11a and R.sup.11b are as defined in the Detailed Description section.

5.1.1 Synthesis of Compound (9)

##STR00255## ##STR00256##

[0821] The synthesis of compound (9) is described in Scheme 1. Compound (1) can be treated with BH.sub.3.Math.rTHF to afford compound (2). The reaction is typically performed at ambient temperature in a solvent, such as, but not limited to, tetrahydrofuran. Compound (3) can be prepared by treating compound (2) with

##STR00257##

in the presence of cyanomethylenetriethylphosphorane. The reaction is typically performed at an

elevated temperature in a solvent such as, but not limited to, toluene. Compound (3) can be treated with ethane-1,2-diol in the presence of a base such as, but not limited to, triethylamine, to provide compound (4). The reaction is typically performed at an elevated temperature, and the reaction may be performed under microwave conditions. Compound (4) can be treated with a strong base, such as, but not limited to, n-butyllithium, followed by the addition of iodomethane, to provide compound (5). The addition and reaction is typically performed in a solvent such as, but not limited to, tetrahydrofuran, at a reduced temperature before warming up to ambient temperature for work up. Compound (5) can be treated with N-iodosuccinimide to provide compound (6). The reaction is typically performed at ambient temperature in a solvent such as, but not limited to, N,N-dimethylformamide. Compound (7) can be prepared by reacting compound (6) with methanesulfonyl chloride, in the presence of a base such as, but not limited to, triethylamine, followed by the addition of NHR.sup.4. The reaction with methanesulfonyl chloride is typically performed at low temperature, before increasing the temperature for the reaction with NHR.sup.4, and the reaction is typically performed in a solvent such as, but not limited to tetrahydrofuran. Compound (7) can be reacted with di-tert-butyl dicarbonate in the presence of 4-dimethylaminopyridine to provide compound (8). The reaction is typically performed at ambient temperature in a solvent such as, but not limited to tetrahydrofuran. The borylation of compound (8) to provide compound (9) can be performed under conditions described herein and readily available in the literature.

5.1.2. Synthesis of Compound (12)

##STR00258##

[0822] The synthesis of intermediate (12) is described in Scheme 2. Compound (3) can be treated with tri-n-butyl-allylstannane in the presence of ZnCl.sub.2.Math.Et.sub.2O or N, N'-azoisobutyronitrile (AIBN) to provide compound (10) (Yamamoto et al., 1998, *Heterocycles* 47:765-780). The reaction is typically performed at -78° C. in a solvent, such as, but not limited to dichloromethane. Compound (10) can be treated under standard conditions known in the art for hydroboration/oxidation to provide compound (11). For example, treatment of compound (10) with a reagent such as BH.sub.3.Math.THF in a solvent such as, but not limited to, tetrahydrofuran followed by treatment of the intermediate alkylborane adduct with an oxidant such as, but not limited to, hydrogen peroxide in the presence of a base such as, but not limited to, sodium hydroxide would provide compound (11) (Brown et al., 1968, *J. Am. Chem. Soc.*, 86:397). Typically the addition of BH.sub.3.Math.THF is performed at low temperature before warming to ambient temperature, which is followed by the addition of hydrogen peroxide and sodium hydroxide to generate the alcohol product. Compound (12) can be generated according to Scheme 1, as previously described for compound (9).

5.1.3. Synthesis of Compound (15)

##STR00259##

[0823] The synthesis of intermediate (15), is described in Scheme 3. Compound (3) can be reacted with thiourea in a solvent mixture of acetic acid and 48% aqueous HBr solution at 100° C. to yield an intermediate that can be subsequently treated with sodium hydroxide in a solvent mixture such as, but not limited to, 20% v/v ethanol in water to provide compound (13). Compound (13) can be reacted with 2-chloroethanol in the presence of a base such as, but not limited to, sodium ethoxide to provide compound (14). The reaction is typically performed at ambient or elevated temperatures in a solvent such as, but not limited to, ethanol. Compound (15) can be generated according to Scheme 1, as previously described for compound (9).

5.1.4. Synthesis of Compound (22)

##STR00260## ##STR00261##

[0824] The synthesis of compound (22) is described in Scheme 4. Compound (16) can be reacted with iodomethane in the presence of a base such as, but not limited to, potassium carbonate to provide compound (17). The reaction is typically conducted at ambient or elevated temperature in a

solvent such as, but not limited to, acetone or N,N-dimethylformamide. Compound (17) can be reacted under photochemical conditions with tosyl cyanide in the presence of benzophenone to provide compound (18) (see Kamijo et al., *Org. Lett.*, 2011, 13:5928-5931). The reaction is typically run at ambient temperature in a solvent such as, but not limited to, acetonitrile or benzene using a Riko 100W medium pressure mercury lamp as the light source. Compound (18) can be reacted with lithium hydroxide in a solvent system such as, but not limited to, mixtures of water and tetrahydrofuran or water and methanol to provide compound (19). Compound (19) can be treated with BH.sub.3.Math.rTHF to provide compound (20). The reaction is typically performed at ambient temperature in a solvent, such as, but not limited to, tetrahydrofuran. Compound (21) can be prepared by treating compound (20) with

##STR00262##

in the presence of cyanomethylenetriethylphosphorane. The reaction is typically performed at an elevated temperature in a solvent such as, but not limited to, toluene. Compound (21) can be treated with N-iodosuccinimide to provide compound (22). The reaction is typically performed at ambient temperature in a solvent such as, but not limited to, N,N-dimethylformamide.

5.1.5. Synthesis of Compound (24)

##STR00263##

[0825] The synthesis of compound (24) is described in Scheme 5. Compound (22) can be treated with a reducing agent such as, but not limited to, lithium aluminum hydride in a solvent such as, but not limited to, diethyl ether or tetrahydrofuran to provide compound (23). Typically the reaction is performed at 0° C. before warming to ambient or elevated temperature. Compound (23) can be reacted with di-tert-butyl dicarbonate under standard conditions described herein or in the literature to provide compound (24).

5.1.6. Synthesis of Compound (24a)

##STR00264##

[0826] The synthesis of intermediate (24a) is described in Scheme 6. Compound (22a) can be hydrolyzed using conditions described in the literature to provide compound (23a). Typically the reaction is run in the presence of potassium hydroxide in a solvent such as, but not limited to, ethylene glycol at elevated temperatures (see Roberts et al., 1994, *J. Org. Chem.*, 1994, 59:6464-6469; Yang et al, 2013, *Org. Lett.*, 15:690-693). Compound (24a) can be made from compound (23a) by Curtius rearrangement using conditions described in the literature. For example, compound (23a) can be reacted with sodium azide in the presence of tetrabutylammonium bromide, zinc(II) triflate and di-tert-butyl dicarbonate to provide compound (24a) (see Lebel et al., *Org. Lett.*, 2005, 7:4107-4110). Typically the reaction is run at elevated temperatures, preferably from 40-50° C., in a solvent such as, but not limited to, tetrahydrofuran.

5.1.7. Synthesis of Compound (29)

##STR00265##

[0827] Scheme 7 describes a functionalization of the adamantane ring substituent. Dimethyl sulfoxide can be reacted with oxalyl chloride, followed by the addition of compound (25), in the presence of a base such as, but not limited to triethylamine, to provide compound (26). The reaction is typically performed at low temperature in a solvent such as, but not limited to, dichloromethane. Compound (27) can be reacted with compound (26), followed by treatment with sodium borohydride, to provide compound (28). The reaction is typically performed at ambient temperature in a solvent such as, but not limited to, dichloromethane, methanol, or mixtures thereof. Compound (29) can be prepared by reacting compound (28) with di-tert-butyl dicarbonate, in the presence of N,N-dimethylpyridin-4-amine. The reaction is typically performed at ambient temperature in a solvent such as, but not limited to, tetrahydrofuran.

5.1.8. Synthesis of Compound (35)

##STR00266##

[0828] As shown in Scheme 8, compound (30), can be reacted with compound (31) under Suzuki

coupling conditions described herein and readily available in the literature, to provide compound (32). Compound (34) can be prepared by reacting compound (32) with compound (33) under conditions described herein, and readily available in the literature. Compound (35) can be prepared by treating compound (34) with an acid such as, but not limited to, trifluoroacetic acid. The reaction is typically performed at ambient temperature in a solvent such as, but not limited to, dichloromethane.

5.1.9. Synthesis of Compound (43)

##STR00267##

[0829] Scheme 9 describes the synthesis of substituted 1,2,3,4-tetrahydroisoquinoline intermediates. Trimethylsilanecarbonitrile can be treated with tetrabutylammonium fluoride and then reacted with compound (36), wherein X is Br or I, to provide compound (37). The additions are typically performed at ambient temperature before heating to an elevated temperature, in a solvent such as, but not limited to, tetrahydrofuran, acetonitrile, or mixtures thereof. Compound (37) can be treated with borane to provide compound (38). The reaction is typically performed at ambient temperature in a solvent such as, but not limited to, tetrahydrofuran. Compound (39) can be prepared by treating compound (38) with trifluoroacetic anhydride, in the presence of a base such as, but not limited to, triethylamine. The reaction is initially performed at low temperature before warming to ambient temperature in a solvent such as, but not limited to, dichloromethane. Compound (39) can be treated with paraformaldehyde in the presence of sulfuric acid to provide compound (40). The reaction is typically performed at ambient temperature. Compound (41) can be prepared by reacting compound (40) with dicyanozinc in the presence of a catalyst such as, but not limited to, tetrakis(triphenylphosphine)palladium(0). The reaction is typically performed at an elevated temperature under a nitrogen atmosphere in a solvent such as, but not limited to, N,N-dimethylformamide. Compound (41) can be treated with potassium carbonate to provide compound (42). The reaction is typically performed at ambient temperature in a solvent such as, but not limited to, methanol, tetrahydrofuran, water, or mixtures thereof.

5.1.10 Synthesis of Compound (47)

##STR00268##

[0830] As shown in Scheme 10, compound (45) can be prepared by reacting compound (43), with tert-butyl 3-bromo-6-fluoropicolinate (44) in the presence of a base, such as, but not limited to, N,N-diisopropylethylamine or triethylamine. The reaction is typically performed under an inert atmosphere at an elevated temperature, in a solvent, such as, but not limited to, dimethyl sulfoxide. Compound (45) can be reacted with 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (46), under borylation conditions described herein or in the literature to provide compound (47).

5.1.11. Synthesis of Compound (53)

##STR00269##

[0831] Scheme 11 describes the synthesis of optionally substituted 1,2,3,4-tetrahydroisoquinoline Bcl-xL inhibitors. Compound (47) can be prepared by reacting compound (45) with pinacolborane, in the presence of a base such as but not limited to triethylamine, and a catalyst such as but not limited to [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II). The reaction is typically performed at an elevated temperature in a solvent such as, but not limited to acetonitrile.

Compound (50) can be prepared by reacting compound (47) with compound (8) under Suzuki coupling conditions described herein and readily available in the literature. Compound (50) can be treated with lithium hydroxide to provide compound (51). The reaction is typically performed at ambient temperature in a solvent such as, but not limited to, tetrahydrofuran, methanol, water, or mixtures thereof. Compound (51) can be reacted with compound (33) under amidation conditions described herein and readily available in the literature to provide compound (52). Compound (53) can be prepared by treating compound (52) with an acid such as, but not limited to, trifluoroacetic acid. The reaction is typically performed at ambient temperature in a solvent such as, but not limited to, dichloromethane.

5.1.12. Synthesis of Compound (66)

##STR00270## ##STR00271##

[0832] Scheme 12 describes the synthesis of 5-methoxy 1,2,3,4-tetrahydroisoquinoline Bcl-xL inhibitors. tert-Butyl 8-bromo-5-hydroxy-3,4-dihydroisoquinoline-2(1H)-carboxylate (54) can be prepared by treating tert-butyl 5-hydroxy-3,4-dihydroisoquinoline-2(1H)-carboxylate with N-bromosuccinimide. The reaction is typically performed at ambient temperature in a solvent such as, but not limited to N,N-dimethylformamide. Butyl 8-bromo-5-hydroxy-3,4-dihydroisoquinoline-2(1H)-carboxylate (54) can be reacted with benzyl bromide (55) in the presence of a base such as, but not limited to, potassium carbonate to provide tert-butyl 5-(benzyloxy)-8-bromo-3,4-dihydroisoquinoline-2(1H)-carboxylate (56). The reaction is typically performed at an elevated temperature in a solvent such as, but not limited to, acetone. tert-Butyl 5-(benzyloxy)-8-bromo-3,4-dihydroisoquinoline-2(1H)-carboxylate (56) can be treated with carbon monoxide in the presence of methanol and a base such as, but not limited to, triethylamine, and a catalyst such as but not limited to [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II), to provide 2-tert-butyl 8-methyl 5-(benzyloxy)-3,4-dihydroisoquinoline-2,8(1H)-dicarboxylate (57). The reaction is typically performed at an elevated temperature. Methyl 5-(benzyloxy)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate (58) can be prepared by treating 2-tert-butyl 8-methyl 5-(benzyloxy)-3,4-dihydroisoquinoline-2,8(1H)-dicarboxylate (57) with hydrochloric acid. The reaction is typically performed at ambient temperature, in a solvent such as, but not limited to, tetrahydrofuran, dioxane, or mixtures thereof.

[0833] Methyl 5-(benzyloxy)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate (58) can be reacted with tert-butyl 3-bromo-6-fluoropicolinate (44) in the presence of a base such as, but not limited to, triethylamine, to provide methyl 5-(benzyloxy)-2-(5-bromo-6-(tert-butoxycarbonyl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate (59). The reaction is typically performed at elevated temperature in a solvent such as, but not limited to, dimethyl sulfoxide. Methyl 5-(benzyloxy)-2-(5-bromo-6-(tert-butoxycarbonyl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate (59) can be reacted with compound (60), wherein Ad is a methyladamantane moiety of the compounds of the disclosure (e.g., the compounds of formula (IIa) and (IIb)) under Suzuki coupling conditions described herein and readily available in the literature, to provide compound (61). Compound (61) can be treated with hydrogen gas in the presence of palladium hydroxide to provide compound (62). The reaction is typically performed at elevated temperature in a solvent such as, but not limited to, tetrahydrofuran. Compound (63) can be prepared by reacting compound (62) with (trimethylsilyl)diazomethane. The reaction is typically performed at ambient temperature, in a solvent such as, but not limited to, dichloromethane, methanol, diethyl ether, or mixtures thereof. Compound (63) can be treated with lithium hydroxide to provide compound (64). The reaction is typically performed at ambient temperature in a solvent such as, but not limited to, tetrahydrofuran, methanol, water, or mixtures thereof. Compound (64) can be reacted with compound (33) under amidation conditions described herein and readily available in the literature to provide compound (65). Compound (66) can be prepared by treating compound (65) with hydrochloric acid. The reaction is typically performed at ambient temperature in a solvent such as, but not limited to, dioxane.

5.2 General Methods for Synthesizing Synthons

[0834] In the schemes below, the various substituents Ar.sup.1, Ar.sup.2, Z.sup.1, R.sup.4, R.sup.11a and R.sup.11b are as defined in the Detailed Description section.

5.2.1. Synthesis of Compound (89)

##STR00272##

[0835] As shown in scheme 13, compounds of formula (77), wherein PG is an appropriate base labile protecting group and AA(2) is Cit, Ala, or Lys, can be reacted with 4-(aminophenyl)methanol (78), under amidation conditions described herein or readily available in the literature to provide compound (79). Compound (80) can be prepared by reacting compound (79) with a base such as,

but not limited to, diethylamine. The reaction is typically performed at ambient temperature in a solvent such as but not limited to N,N-dimethylformamide. Compound (81), wherein PG is an appropriate base or acid labile protecting group and AA(1) is Val or Phe, can be reacted with compound (80), under amidation conditions described herein or readily available in the literature to provide compound (82). Compound (83) can be prepared by treating compound (82) with diethylamine or trifluoroacetic acid, as appropriate. The reaction is typically performed at ambient temperature in a solvent such as but not limited to dichloromethane. Compound (84), wherein Sp is a spacer, can be reacted with compound (83) to provide compound (85). The reaction is typically performed at ambient temperature in a solvent such as but not limited to N,N-dimethylformamide. Compound (85) can be reacted with bis(4-nitrophenyl) carbonate (86) in the presence of a base such as, but not limited to N,N-diisopropylethylamine, to provide compounds (87). The reaction is typically performed at ambient temperature in a solvent such as but not limited to N,N-dimethylformamide. Compounds (87) can be reacted with compounds of formula (88) in the presence of a base such as, but not limited to, N,N-diisopropylethylamine, to provide compound (89). The reaction is typically performed at ambient temperature in a solvent such as, but not limited to, N,N-dimethylformamide.

5.2.2. Synthesis of Compounds (94) and (96)

##STR00273##

[0836] Scheme 14 describes the installment of alternative mAb-linker attachments to dipeptide synthons. Compound (88), wherein can be reacted with compound (90) in the presence of a base such as, but not limited to, N-ethyl-N-isopropylpropan-2-amine, to provide compound (91). The reaction is typically performed at ambient temperature in a solvent such as but not limited to N,N-dimethylformamide. Compound (92) can be prepared by reacting compound (91) with diethylamine. The reaction is typically performed at ambient temperature in a solvent such as but not limited to N,N-dimethylformamide. Compound (93), wherein X^{sup.1} is Cl, Br, or I, can be reacted with compound (92), under amidation conditions described herein or readily available in the literature to provide compound (94). Compound (92) can be reacted with compounds of formula (95) under amidation conditions described herein or readily available in the literature to provide compound (96).

5.2.3. Synthesis of Compound (106)

##STR00274##

[0837] Scheme 15 describes the synthesis of vinyl glucuronide linker intermediates and synthons. (2R,3R,4S,5S,6S)-2-Bromo-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (97) can be treated with silver oxide, followed by 4-bromo-2-nitrophenol (98) to provide (2S,3R,4S,5S,6S)-2-(4-bromo-2-nitrophenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (99). The reaction is typically performed at ambient temperature in a solvent, such as, but not limited to, acetonitrile. (2S,3R,4S,5S,6S)-2-(4-Bromo-2-nitrophenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (99) can be reacted with (E)-tert-butylldimethyl((3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)allyl)oxy)silane (100) in the presence of a base such as, but not limited to, sodium carbonate, and a catalyst such as but not limited to tris(dibenzylideneacetone)dipalladium (Pd₂(dba)₃), to provide (2S,3R,4S,5S,6S)-2-(4-((E)-3-((tert-butylldimethylsilyl)oxy)prop-1-en-1-yl)-2-nitrophenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (101). The reaction is typically performed at an elevated temperature in a solvent, such as, but not limited to, tetrahydrofuran. (2S,3R,4S,5S,6S)-2-(2-amino-4-((E)-3-hydroxyprop-1-en-1-yl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (102) can be prepared by reacting (2S,3R,4S,5S,6S)-2-(4-((E)-3-((tert-butylldimethylsilyl)oxy)prop-1-en-1-yl)-2-nitrophenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (101) with zinc in the presence of an acid such as, but not limited to, hydrochloric acid. The addition is typically performed at low temperature before warming to ambient temperature in a solvent such as, but not limited to,

tetrahydrofuran, water, or mixtures thereof. (2S,3R,4S,5S,6S)-2-(2-amino-4-((E)-3-hydroxyprop-1-en-1-yl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (102) can be reacted with (9H-fluoren-9-yl)methyl (3-chloro-3-oxopropyl)carbamate (103), in the presence of a base such as, but not limited to, N,N-diisopropylethylamine, to provide (2S,3R,4S,5S,6S)-2-(2-(3-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-((E)-3-hydroxyprop-1-en-1-yl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (104). The addition is typically performed at low temperature before warming to ambient temperature in a solvent such as, but not limited to, dichloromethane. Compound (88) can be reacted with (2S,3R,4S,5S,6S)-2-(2-(3-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-((E)-3-hydroxyprop-1-en-1-yl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (104) in the presence of a base such as, but not limited to, N-ethyl-N-isopropylpropan-2-amine, followed by work up and reaction with compound (105) in the presence of a base such as, but not limited to, N,N-diisopropylethylamine to provide compound (106). The reactions are typically performed at ambient temperature in a solvent such as, but not limited to N,N-dimethylformamide.

5.2.4. Synthesis of Compound (115)

##STR00275## ##STR00276##

[0838] Scheme 16 describes the synthesis of a representative 2-ether glucuronide linker intermediate and synthon. (2S,3R,4S,5S,6S)-2-Bromo-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (97) can be reacted with 2,4-dihydroxybenzaldehyde (107) in the presence of silver carbonate to provide (2S,3R,4S,5S,6S)-2-(4-formyl-3-hydroxyphenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (108). The reaction is typically performed at an elevated temperature in a solvent, such as, but not limited to, acetonitrile. (2S,3R,4S,5S,6S)-2-(4-Formyl-3-hydroxyphenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (108) can be treated with sodium borohydride to provide (2S,3R,4S,5S,6S)-2-(3-hydroxy-4-(hydroxymethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (109). The addition is typically performed at low temperature before warming to ambient temperature in a solvent such as but not limited to tetrahydrofuran, methanol, or mixtures thereof. (2S,3R,4S,5S,6S)-2-(4-(((tert-butyldimethylsilyl)oxy)methyl)-3-hydroxyphenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (110) can be prepared by reacting (2S,3R,4S,5S,6S)-2-(3-hydroxy-4-(hydroxymethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (109) with tert-butyldimethylsilyl chloride in the presence of imidazole. The reaction is typically performed at low temperature in a solvent, such as, but not limited to, dichloromethane. (2S,3R,4S,5S,6S)-2-(3-(2-(2-(((9H-Fluoren-9-yl)methoxy)carbonyl)amino)ethoxy)ethoxy)-4-(((tert-butyldimethylsilyl)oxy)methyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (111) can be prepared by reacting (2S,3R,4S,5S,6S)-2-(4-(((tert-butyldimethylsilyl)oxy)methyl)-3-hydroxyphenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (110) with (9H-fluoren-9-yl)methyl (2-(2-hydroxyethoxy)ethyl)carbamate in the presence of triphenylphosphine and a azodicarboxylate such as, but not limited to, di-tert-butyl diazene-1,2-dicarboxylate. The reaction is typically performed at ambient temperature in a solvent such as but not limited to toluene. (2S,3R,4S,5S,6S)-2-(3-(2-(2-(((9H-Fluoren-9-yl)methoxy)carbonyl)amino)ethoxy)ethoxy)-4-(((tert-butyldimethylsilyl)oxy)methyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (111) can be treated with acetic acid to provide (2S,3R,4S,5S,6S)-2-(3-(2-(2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethoxy)ethoxy)-4-(hydroxymethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (112). The reaction is typically performed at ambient temperature in a solvent such as but not limited to water, tetrahydrofuran, or mixtures thereof. (2S,3R,4S,5S,6S)-2-(3-(2-(2-(((9H-Fluoren-9-yl)methoxy)carbonyl)amino)ethoxy)ethoxy)-4-(((4-nitrophenoxy)carbonyl)oxy)methyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (113) can be prepared by reacting (2S,3R,4S,5S,6S)-2-(3-(2-(2-(((9H-fluoren-9-

yl)methoxy)carbonyl)amino)ethoxy)ethoxy)-4-(hydroxymethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (91) with bis(4-nitrophenyl) carbonate in the presence of a base such as but not limited to N-ethyl-N-isopropylpropan-2-amine. The reaction is typically performed at ambient temperature in a solvent such as but not limited to N,N-dimethylformamide. (2S,3R,4S,5S,6S)-2-(3-(2-(2-(((9H-Fluoren-9-yl)methoxy)carbonyl)amino)ethoxy)ethoxy)-4-(((4-nitrophenoxy)carbonyl)oxy)methyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (113) can be treated with compound (88) in the presence of a base such as but not limited to N-ethyl-N-isopropylpropan-2-amine, followed by treatment with lithium hydroxide to provide a compound (114). The reaction is typically performed at ambient temperature in a solvent such as but not limited to N,N-dimethylformamide, tetrahydrofuran, methanol, or mixtures thereof. Compound (115) can be prepared by reacting compound (114) with compound (84) in the presence of a base such as but not limited to N-ethyl-N-isopropylpropan-2-amine. The reaction is typically performed at ambient temperature in a solvent such as but not limited to N,N-dimethylformamide.

5.2.5. Synthesis of Compound (119)

##STR00277##

[0839] Scheme 17 describes the introduction of a second solubilizing group to a sugar linker. Compound (116) can be reacted with (R)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-sulfopropanoic acid (117), under amidation conditions described herein or readily available in the literature, followed by treatment with a base such as but not limited to diethylamine, to provide compound (118). Compound (118) can be reacted with compound (84), wherein Sp is a spacer, under amidation conditions described herein or readily available in the literature, to provide compound (119).

5.2.6. Synthesis of Compound (129)

##STR00278## ##STR00279##

[0840] Scheme 18 describes the synthesis of 4-ether glucuronide linker intermediates and synthons. 4-(2-(2-Bromoethoxy)ethoxy)-2-hydroxybenzaldehyde (122) can be prepared by reacting 2,4-dihydroxybenzaldehyde (120) with 1-bromo-2-(2-bromoethoxy)ethane (121) in the presence of a base such as, but not limited to, potassium carbonate. The reaction is typically performed at an elevated temperature in a solvent such as but not limited to acetonitrile. 4-(2-(2-Bromoethoxy)ethoxy)-2-hydroxybenzaldehyde (122) can be treated with sodium azide to provide 4-(2-(2-azidoethoxy)ethoxy)-2-hydroxybenzaldehyde (123). The reaction is typically performed at ambient temperature in a solvent such as but not limited to N,N-dimethylformamide.

(2S,3R,4S,5S,6S)-2-(5-(2-(2-Azidoethoxy)ethoxy)-2-formylphenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (125) can be prepared by reacting 4-(2-(2-azidoethoxy)ethoxy)-2-hydroxybenzaldehyde (123) with (3R,4S,5S,6S)-2-bromo-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (124) in the presence of silver oxide. The reaction is typically performed at ambient temperature in a solvent such as, but not limited to, acetonitrile. Hydrogenation of (2S,3R,4S,5S,6S)-2-(5-(2-(2-azidoethoxy)ethoxy)-2-formylphenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (125) in the presence of Pd/C will provide (2S,3R,4S,5S,6S)-2-(5-(2-(2-aminoethoxy)ethoxy)-2-(hydroxymethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (126). The reaction is typically performed at ambient temperature in a solvent such as, but not limited to, tetrahydrofuran. (2S,3R,4S,5S,6S)-2-(5-(2-(2-(((9H-Fluoren-9-yl)methoxy)carbonyl)amino)ethoxy)ethoxy)-2-(hydroxymethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (127) can be prepared by treating (2S,3R,4S,5S,6S)-2-(5-(2-(2-aminoethoxy)ethoxy)-2-(hydroxymethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (126) with (9H-fluoren-9-yl)methyl carbonochloridate in the presence of a base, such as, but not limited to, N-ethyl-N-isopropylpropan-2-amine. The reaction is typically performed at low temperature in a solvent such as, but not

limited to, dichloromethane. Compound (88) can be reacted with (2S,3R,4S,5S,6S)-2-(5-(2-(2-(((9H-Fluoren-9-yl)methoxy)carbonyl)amino)ethoxy)ethoxy)-2-(hydroxymethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (127) in the presence of a base, such as, but not limited to, N-ethyl-N-isopropylpropan-2-amine, followed by treatment with lithium hydroxide to provide compound (128). The reaction is typically performed at low temperature in a solvent such as, but not limited to, N,N-dimethylformamide. Compound (129) can be prepared by reacting compound (128) with compound (84) in the presence of a base such as, but not limited to, N-ethyl-N-isopropylpropan-2-amine. The reaction is typically performed at ambient temperature in a solvent such as but not limited to N,N-dimethylformamide.

5.2.7. Synthesis of Compound (139)

##STR00280## ##STR00281##

[0841] Scheme 19 describes the synthesis of carbamate glucuronide intermediates and synthons. 2-Amino-5-(hydroxymethyl)phenol (130) can be treated with sodium hydride and then reacted with 2-(2-azidoethoxy)ethyl 4-methylbenzenesulfonate (131) to provide (4-amino-3-(2-(2-azidoethoxy)ethoxy)phenyl)methanol (132). The reaction is typically performed at an elevated temperature in a solvent such as, but not limited to N,N-dimethylformamide. 2-(2-(2-Azidoethoxy)ethoxy)-4-(((tert-butyldimethylsilyl)oxy)methyl)aniline (133) can be prepared by reacting (4-amino-3-(2-(2-azidoethoxy)ethoxy)phenyl)methanol (132) with tert-butyldimethylchlorosilane in the presence of imidazole. The reaction is typically performed at ambient temperature in a solvent such as, but not limited to tetrahydrofuran. 2-(2-(2-Azidoethoxy)ethoxy)-4-(((tert-butyldimethylsilyl)oxy)methyl)aniline (133) can be treated with phosgene, in the presence of a base such as but not limited to triethylamine, followed by reaction with (3R,4S,5S,6S)-2-hydroxy-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (134) in the presence of a base such as but not limited to triethylamine, to provide 2S,3R,4S,5S,6S)-2-(((2-(2-(2-azidoethoxy)ethoxy)-4-(((tert-butyldimethylsilyl)oxy)methyl)phenyl)carbamoyl)oxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (135). The reaction is typically performed in a solvent such as, but not limited to, toluene, and the additions are typically performed at low temperature, before warming up to ambient temperature after the phosgene addition and heating at an elevated temperature after the (3R,4S,5S,6S)-2-hydroxy-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (134) addition. (2S,3R,4S,5S,6S)-2-(((2-(2-(2-Azidoethoxy)ethoxy)-4-(hydroxymethyl)phenyl)carbamoyl)oxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (136) can be prepared by reacting 2S,3R,4S,5S,6S)-2-(((2-(2-(2-azidoethoxy)ethoxy)-4-(((tert-butyldimethylsilyl)oxy)methyl)phenyl)carbamoyl)oxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (135) with p-toluenesulfonic acid monohydrate. The reaction is typically performed at ambient temperature in a solvent such as, but not limited to methanol. (2S,3R,4S,5S,6S)-2-(((2-(2-(2-Azidoethoxy)ethoxy)-4-(hydroxymethyl)phenyl)carbamoyl)oxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (136) can be reacted with bis(4-nitrophenyl)carbonate in the presence of a base such as, but not limited to, N,N-diisopropylethylamine, to provide (2S,3R,4S,5S,6S)-2-(((2-(2-(2-azidoethoxy)ethoxy)-4-(((4-nitrophenoxy)carbonyl)oxy)methyl)phenyl)carbamoyl)oxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (137). The reaction is typically performed at ambient temperature in a solvent such as, but not limited to, N,N-dimethylformamide. (2S,3R,4S,5S,6S)-2-(((2-(2-(2-Azidoethoxy)ethoxy)-4-(((4-nitrophenoxy)carbonyl)oxy)methyl)phenyl)carbamoyl)oxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (137) can be reacted with compound in the presence of a base such as, but not limited to, N,N-diisopropylethylamine, followed by treatment with aqueous lithium hydroxide, to provide compound (138). The first step is typically conducted at ambient temperature in a solvent such as, but not limited to N,N-dimethylformamide, and the second step is typically conducted at low temperature in a solvent such as but not limited to methanol. Compound (138) can be treated with tris(2-carboxyethyl)phosphine hydrochloride,

followed by reaction with compound (84) in the presence of a base such as, but not limited to, N,N-diisopropylethylamine, to provide compound (139). The reaction with tris(2-carboxyethyl)phosphine hydrochloride is typically performed at ambient temperature in a solvent such as, but not limited to, tetrahydrofuran, water, or mixtures thereof, and the reaction with N-succinimidyl 6-maleimidohexanoate is typically performed at ambient temperature in a solvent such as, but not limited to, N,N-dimethylformamide.

5.2.8. Synthesis of Compound (149)

##STR00282## ##STR00283##

[0842] Scheme 20 describes the synthesis of galactoside linker intermediates and synthons.

(2S,3R,4S,5S,6R)-6-(Acetoxymethyl)tetrahydro-2H-pyran-2,3,4,5-tetraol tetraacetate (140) can be treated with HBr in acetic acid to provide (2R,3S,4S,5R,6S)-2-(acetoxymethyl)-6-bromotetrahydro-2H-pyran-3,4,5-triyl triacetate (141). The reaction is typically performed at ambient temperature under a nitrogen atmosphere. (2R,3S,4S,5R,6S)-2-(Acetoxymethyl)-6-(4-formyl-2-nitrophenoxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (143) can be prepared by treating (2R,3S,4S,5R,6S)-2-(acetoxymethyl)-6-bromotetrahydro-2H-pyran-3,4,5-triyl triacetate (141) with silver(I) oxide in the presence of 4-hydroxy-3-nitrobenzaldehyde (142). The reaction is typically performed at ambient temperature in a solvent such as, but not limited to, acetonitrile.

(2R,3S,4S,5R,6S)-2-(Acetoxymethyl)-6-(4-formyl-2-nitrophenoxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (143) can be treated with sodium borohydride to provide (2R,3S,4S,5R,6S)-2-(acetoxymethyl)-6-(4-(hydroxymethyl)-2-nitrophenoxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (144). The reaction is typically performed at low temperature in a solvent such as but not limited to tetrahydrofuran, methanol, or mixtures thereof. (2R,3S,4S,5R,6S)-2-(Acetoxymethyl)-6-(2-amino-4-(hydroxymethyl)phenoxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (145) can be prepared by treating (2R,3S,4S,5R,6S)-2-(acetoxymethyl)-6-(4-(hydroxymethyl)-2-nitrophenoxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (144) with zinc in the presence of hydrochloric acid. The reaction is typically performed at low temperature, under a nitrogen atmosphere, in a solvent such as, but not limited to, tetrahydrofuran. (2S,3R,4S,5S,6R)-2-(2-(3-(((9H-Fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-(hydroxymethyl)phenoxy)-6-

(acetoxymethyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (146) can be prepared by reacting (2R,3S,4S,5R,6S)-2-(acetoxymethyl)-6-(2-amino-4-(hydroxymethyl)phenoxy)tetrahydro-2H-pyran-3,4,5-triyl triacetate (145) with (9H-fluoren-9-yl)methyl (3-chloro-3-oxopropyl)carbamate (103) in the presence of a base such as, but not limited to, N,N-diisopropylethylamine. The reaction is typically performed at low temperature, in a solvent such as, but not limited to, dichloromethane.

(2S,3R,4S,5S,6R)-2-(2-(3-(((9H-Fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-(hydroxymethyl)phenoxy)-6-(acetoxymethyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (146) can be reacted with bis(4-nitrophenyl)carbonate in the presence of a base such as, but not limited to, N,N-diisopropylethylamine, to provide (2S,3R,4S,5S,6R)-2-(2-(3-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-(((4-nitrophenoxy)carbonyl)oxy)methyl)phenoxy)-6-(acetoxymethyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (147). The reaction is typically performed at low temperature, in a solvent such as, but not limited to, N,N-dimethylformamide.

(2S,3R,4S,5S,6R)-2-(2-(3-(((9H-Fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-(((4-nitrophenoxy)carbonyl)oxy)methyl)phenoxy)-6-(acetoxymethyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (147) can be reacted with compound (88) in the presence of a base such as, but not limited to N,N-diisopropylethylamine, followed by treatment with lithium hydroxide, to provide compound (148). The first step is typically performed at low temperature, in a solvent such as, but not limited to, N,N-dimethylformamide, and the second step is typically performed at ambient temperature, in a solvent such as, but not limited to, methanol. Compound (148) can be treated with compound (84), wherein Sp is a spacer, in the presence of a base, such as, but not limited to N,N-diisopropylethylamine, to provide compound (149). The reaction is typically performed at ambient temperature, in a solvent such as, but not limited to, N,N-dimethylformamide.

5.3 General Methods for Synthesizing Anti-EGFR ADCs

[0843] The present invention also discloses a process to prepare an anti-EGFR ADC according to structural formula (I):

##STR00284##

wherein D, L, LK, Ab and m are as defined in the Detailed Description section. The process comprises: [0844] treating an antibody in an aqueous solution with an effective amount of a disulfide reducing agent at 30-40° C. for at least 15 minutes, and then cooling the antibody solution to 20-27° C.; [0845] adding to the reduced antibody solution a solution of water/dimethyl sulfoxide comprising a synthon selected from the group of 2.1 to 2.31 and 2.34 to 2.72 (Table 5); [0846] adjusting the pH of the solution to a pH of 7.5 to 8.5; and [0847] allowing the reaction to run for 48 to 80 hours to form the ADC; [0848] wherein the mass is shifted by 18 ± 2 amu for each hydrolysis of a succinimide to a succinamide as measured by electron spray mass spectrometry; and [0849] wherein the ADC is optionally purified by hydrophobic interaction chromatography.

[0850] In certain embodiments, Ab is an anti-hEGFR antibody comprises the heavy and light chain CDRs of the anti-hEGFR antibodies disclosed herein, including, AbA, AbB, AbG, and AbK;

[0851] The present invention is also directed to an anti-EGFR ADC prepared by the above-described process.

[0852] In one embodiment, the anti-EGFR ADC disclosed in the present application is formed by contacting an antibody that binds an hEGFR cell surface receptor or tumor associated antigen expressed on a tumor cell with a drug-linker synthon under conditions in which the drug-linker synthon covalently links to the antibody through a maleimide moiety as shown in formula (II_d) or (II_e),

##STR00285##

wherein D is the Bcl-xL inhibitor drug according to structural formula (II_a) or (II_b) as described above and L_{sup.1} is the portion of the linker not formed from the maleimide upon attachment of the synthon to the antibody; and wherein the drug-linker synthon is selected from the group consisting of synthon examples 2.1 to 2.31 and 2.34 to 2.72 (Table 5), or a pharmaceutically acceptable salt thereof.

[0853] In certain embodiments, the contacting step is carried out under conditions such that the anti-EGFR ADC has a DAR of 2, 3 or 4.

6. Purification of Anti-EGFR ADCs

[0854] Purification of the ADCs may be achieved in such a way that ADCs having certain DARs are collected. For example, HIC resin may be used to separate high drug loaded ADCs from ADCs having optimal drug to antibody ratios (DARs), e.g. a DAR of 4 or less. In one embodiment, a hydrophobic resin is added to an ADC mixture such that undesired ADCs, i.e., higher drug loaded ADCs, bind the resin and can be selectively removed from the mixture. In certain embodiments, separation of the ADCs may be achieved by contacting an ADC mixture (e.g., a mixture comprising a drug loaded species of ADC of 4 or less and a drug loaded species of ADC of 6 or more) with a hydrophobic resin, wherein the amount of resin is sufficient to allow binding of the drug loaded species which is being removed from the ADC mixture. The resin and ADC mixture are mixed together, such that the ADC species being removed (e.g., a drug loaded species of 6 or more) binds to the resin and can be separated from the other ADC species in the ADC mixture. The amount of resin used in the method is based on a weight ratio between the species to be removed and the resin, where the amount of resin used does not allow for significant binding of the drug loaded species that is desired. Thus, methods may be used to reduce the average DAR to less than 4. Further, the purification methods described herein may be used to isolate ADCs having any desired range of drug loaded species, e.g., a drug loaded species of 4 or less, a drug loaded species of 3 or less, a drug loaded species of 2 or less, a drug loaded species of 1 or less.

[0855] Certain species of molecule(s) binds to a surface based on hydrophobic interactions between the species and a hydrophobic resin. In one embodiment, method of the invention refers to a

purification process that relies upon the intermixing of a hydrophobic resin and a mixture of ADCs, wherein the amount of resin added to the mixture determines which species (e.g., ADCs with a DAR of 6 or more) will bind. Following production and purification of an antibody from an expression system (e.g., a mammalian expression system), the antibody is reduced and coupled to a drug through a conjugation reaction. The resulting ADC mixture often contains ADCs having a range of DARs, e.g., 1 to 8. In one embodiment, the ADC mixture comprises a drug loaded species of 4 or less and a drug loaded species of 6 or more. According to the methods of the invention, the ADC mixture may be purified using a process, such as, but not limited to, a batch process, such that ADCs having a drug loaded species of 4 or less are selected and separated from ADCs having a higher drug load (e.g., ADCs having a drug loaded species of 6 or more). Notably, the purification methods described herein may be used to isolate ADCs having any desired range of DAR, e.g., a DAR of 4 or less, a DAR of 3 or less, and a DAR of 2 or less.

[0856] Thus, in one embodiment, an ADC mixture comprising a drug loaded species of 4 or less and a drug loaded species of 6 or more may be contacted with a hydrophobic resin to form a resin mixture, wherein the amount of hydrophobic resin contacted with the ADC mixture is sufficient to allow binding of the drug loaded species of 6 or more to the resin but does not allow significant binding of the drug load species of 4 or less; and removing the hydrophobic resin from the ADC mixture, such that the composition comprising ADCs is obtained, wherein the composition comprises less than 15% of the drug loaded species of 6 or more, and wherein the ADC comprises an antibody conjugated to a Bcl-xL inhibitor. In a separate embodiment, the method of the invention comprises contacting an ADC mixture comprising a drug loaded species of 4 or less and a drug loaded species of 6 or more with a hydrophobic resin to form a resin mixture, wherein the amount of hydrophobic resin contacted with the ADC mixture is sufficient to allow binding of the drug loaded species of 6 or more to the resin but does not allow significant binding of the drug load species of 4 or less; and removing the hydrophobic resin from the ADC mixture, such that the composition comprising ADCs is obtained, wherein the composition comprises less than 15% of the drug loaded species of 6 or more, and wherein the ADC comprises an antibody conjugated to a Bcl-xL inhibitor, wherein the hydrophobic resin weight is 3 to 12 times the weight of the drug loaded species of 6 or more in the ADC mixture.

[0857] The ADC separation method described herein method may be performed using a batch purification method. The batch purification process generally includes adding the ADC mixture to the hydrophobic resin in a vessel, mixing, and subsequently separating the resin from the supernatant. For example, in the context of batch purification, a hydrophobic resin may be prepared in or equilibrated to the desired equilibration buffer. A slurry of the hydrophobic resin may thus be obtained. The ADC mixture may then be contacted with the slurry to adsorb the specific species of ADC(s) to be separated by the hydrophobic resin. The solution comprising the desired ADCs that do not bind to the hydrophobic resin material may then be separated from the slurry, e.g., by filtration or by allowing the slurry to settle and removing the supernatant. The resulting slurry can be subjected to one or more washing steps. In order to elute bound ADCs, the salt concentration can be decreased. In one embodiment, the process used in the invention includes no more than 50 g of hydrophobic resin.

[0858] Thus, a batch method may be used to contact an ADC mixture comprising a drug loaded species of 4 or less and a drug loaded species of 6 or more with a hydrophobic resin to form a resin mixture, wherein the amount of hydrophobic resin contacted with the ADC mixture is sufficient to allow binding of the drug loaded species of 6 or more to the resin but does not allow significant binding of the drug load species of 4 or less; and removing the hydrophobic resin from the ADC mixture, such that the composition comprising ADCs is obtained, wherein the composition comprises less than 15% of the drug loaded species of 6 or more, and wherein the ADC comprises an antibody conjugated to a Bcl-xL inhibitor. In a separate embodiment, a batch method is used to contact an ADC mixture comprising a drug loaded species of 4 or less and a drug loaded species of

6 or more with a hydrophobic resin to form a resin mixture, wherein the amount of hydrophobic resin contacted with the ADC mixture is sufficient to allow binding of the drug loaded species of 6 or more to the resin but does not allow significant binding of the drug load species of 4 or less; and removing the hydrophobic resin from the ADC mixture, such that the composition comprising ADCs is obtained, wherein the composition comprises less than 15% of the drug loaded species of 6 or more, and wherein the ADC comprises an antibody conjugated to a Bcl-xL inhibitor, wherein the hydrophobic resin weight is 3 to 12 times the weight of the drug loaded species of 6 or more in the ADC mixture.

[0859] Alternatively, in a separate embodiment, purification may be performed using a circulation process, whereby the resin is packed in a container and the ADC mixture is passed over the hydrophobic resin bed until the specific species of ADC(s) to be separated have been removed. The supernatant (containing the desired ADC species) is then pumped from the container and the resin bed may be subjected to washing steps.

[0860] A circulation process may be used to contact an ADC mixture comprising a drug loaded species of 4 or less and a drug loaded species of 6 or more with a hydrophobic resin to form a resin mixture, wherein the amount of hydrophobic resin contacted with the ADC mixture is sufficient to allow binding of the drug loaded species of 6 or more to the resin but does not allow significant binding of the drug load species of 4 or less; and removing the hydrophobic resin from the ADC mixture, such that the composition comprising ADCs is obtained, wherein the composition comprises less than 15% of the drug loaded species of 6 or more, and wherein the ADC comprises an antibody conjugated to a Bcl-xL inhibitor. In a separate embodiment, a circulation process is used to contact an ADC mixture comprising a drug loaded species of 4 or less and a drug loaded species of 6 or more with a hydrophobic resin to form a resin mixture, wherein the amount of hydrophobic resin contacted with the ADC mixture is sufficient to allow binding of the drug loaded species of 6 or more to the resin but does not allow significant binding of the drug load species of 4 or less; and removing the hydrophobic resin from the ADC mixture, such that the composition comprising ADCs is obtained, wherein the composition comprises less than 15% of the drug loaded species of 6 or more, and wherein the ADC comprises an antibody conjugated to a Bcl-xL inhibitor, wherein the hydrophobic resin weight is 3 to 12 times the weight of the drug loaded species of 6 or more in the ADC mixture.

[0861] Alternatively, a flow through process may be used to purify an ADC mixture to arrive at a composition comprising a majority of ADCs having a certain desired DAR. In a flow through process, resin is packed in a container, e.g., a column, and the ADC mixture is passed over the packed resin such that the desired ADC species does not substantially bind to the resin and flows through the resin, and the undesired ADC species is bound to the resin. A flow through process may be performed in a single pass mode (where the ADC species of interest are obtained as a result of a single pass through the resin of the container) or in a multi-pass mode (where the ADC species of interest are obtained as a result of multiple passes through the resin of the container). The flow through process is performed such that the weight of resin selected binds to the undesired ADC population, and the desired ADCs (e.g., DAR 2-4) flow over the resin and are collected in the flow through after one or multiple passes.

[0862] A flow through process may be used to contact an ADC mixture comprising a drug loaded species of 4 or less and a drug loaded species of 6 or more with a hydrophobic resin, wherein the amount of hydrophobic resin contacted with the ADC mixture is sufficient to allow binding of the drug loaded species of 6 or more to the resin but does not allow significant binding of the drug load species of 4 or less, where the drug load species of 4 or less passes over the resin and is subsequently collected after one or multiple passes, such that the composition comprising the desired ADCs (e.g. DAR 2-4) is obtained, wherein the composition comprises less than 15% of the drug loaded species of 6 or more, and wherein the ADC comprises an antibody conjugated to a Bcl-xL inhibitor. In a separate embodiment, a flow through process is used to contact an ADC mixture

comprising a drug loaded species of 4 or less and a drug loaded species of 6 or more with a hydrophobic resin by passing the ADC mixture over the resin, wherein the amount of hydrophobic resin contacted with the ADC mixture is sufficient to allow binding of the drug loaded species of 6 or more to the resin but does not allow significant binding of the drug load species of 4 or less, where the drug load species of 4 or less passes over the resin and is subsequently collected, such that the composition comprising ADCs is obtained, wherein the composition comprises less than 15% of the drug loaded species of 6 or more, and wherein the ADC comprises an antibody conjugated to a Bcl-xL inhibitor, wherein the amount of hydrophobic resin weight is 3 to 12 times the weight of the drug loaded species of 6 or more in the ADC mixture.

[0863] Following a flow through process, the resin may be washed with a one or more washes following in order to further recover ADCs having the desired DAR range (found in the wash filtrate). For example, a plurality of washes having decreasing conductivity may be used to further recover ADCs having the DAR of interest. The elution material obtained from the washing of the resin may be subsequently combined with the filtrate resulting from the flow through process for improved recovery of ADCs having the DAR of interest.

[0864] The aforementioned batch, circulation, and flow through process purification methods are based on the use of a hydrophobic resin to separate high vs. low drug loaded species of ADC. Hydrophobic resin comprises hydrophobic groups which interact with the hydrophobic properties of the ADCs. Hydrophobic groups on the ADC interact with hydrophobic groups within the hydrophobic resin. The more hydrophobic a protein is the stronger it will interact with the hydrophobic resin.

[0865] Hydrophobic resin normally comprises a base matrix (e.g., cross-linked agarose or synthetic copolymer material) to which hydrophobic ligands (e.g., alkyl or aryl groups) are coupled. Many hydrophobic resins are available commercially. Examples include, but are not limited to, Phenyl Sepharose™ 6 Fast Flow with low or high substitution (Pharmacia LKB Biotechnology, AB, Sweden); Phenyl Sepharose™ High Performance (Pharmacia LKB Biotechnology, AB, Sweden); Octyl Sepharose™ High Performance (Pharmacia LKB Biotechnology, AB, Sweden); Fractogel™ EMD Propyl or Fractogel™ EMD Phenyl columns (E. Merck, Germany); Macro-Prep™ Methyl or Macro-Prep™. t-Butyl Supports (Bio-Rad, California); WP HI-Propyl (C.sub.3)™ (J. T. Baker, New Jersey); and Toyopearl™ ether, hexyl, phenyl or butyl (TosoHaas, PA). In one embodiment, the hydrophobic resin is a butyl hydrophobic resin. In another embodiment, the hydrophobic resin is a phenyl hydrophobic resin. In another embodiment, the hydrophobic resin is a hexyl hydrophobic resin, an octyl hydrophobic resin, or a decyl hydrophobic resin. In one embodiment, the hydrophobic resin is a methacrylic polymer having n-butyl ligands (e.g. TOYOPEARL Butyl-600M).

[0866] Further methods for purifying ADC mixtures to obtain a composition having a desired DAR are described in U.S. application Ser. No. 14/210,602 (U.S. Patent Appln. Publication No. US 2014/0286968), incorporated by reference in its entirety.

[0867] In certain embodiments of the invention, ADCs described herein having a DAR2 are purified from ADCs having higher or lower DARs. Such purified DAR2 ADCs are referred to herein as “E2”. Purification methods for achieving a composition having E2 anti-EGFR ADCs. In one embodiment, of the invention provides a composition comprising an ADC mixture, wherein at least 75% of the ADCs are anti-EGFR ADCs (like those described herein) having a DAR2. In another embodiment, the invention provides a composition comprising an ADC mixture, wherein at least 80% of the ADCs are anti-EGFR ADCs (like those described herein) having a DAR2. In another embodiment, the invention provides a composition comprising an ADC mixture, wherein at least 85% of the ADCs are anti-EGFR ADCs (like those described herein) having a DAR2. In another embodiment, the invention provides a composition comprising an ADC mixture, wherein at least 90% of the ADCs are anti-EGFR ADCs (like those described herein) having a DAR2.

7. Uses of Anti-EGFR ADCs

[0868] The Bcl-xL inhibitors included in the ADCs as well as the synthons delivered by the ADCs, inhibit Bcl-xL activity and induce apoptosis in cells expressing Bcl-xL. Accordingly, the Bcl-xL inhibitors and/or ADCs may be used in methods to inhibit Bcl-xL activity and/or induce apoptosis in cells.

[0869] For Bcl-xL inhibitors, the method generally involves contacting a cell whose survival depends, at least in part, upon Bcl-xL expression with an amount of a Bcl-xL inhibitor sufficient to inhibit Bcl-xL activity and/or induce apoptosis. For ADCs, the method generally involves contacting a cell whose survival depends, at least in part upon Bcl-xL expression, and that expresses a cell-surface antigen, i.e., EGFR, for the antibody of the ADC with an ADC under conditions in which the ADC binds the antigen.

[0870] In certain embodiments, the antibody of the ADC binds EGFR and facilitates internalization of the ADC into the cell, where the Bcl-xL inhibitory synthon is delivered. The method may be carried out in vitro in a cellular assay to inhibit Bcl-xL activity and/or inhibit apoptosis, or in vivo as a therapeutic approach towards treating diseases in which inhibition of apoptosis and/or induction of apoptosis would be desirable.

[0871] Dysregulated apoptosis has been implicated in a variety of diseases, including, for example, autoimmune disorders (e.g., systemic lupus erythematosus, rheumatoid arthritis, graft-versus-host disease, myasthenia gravis, or Sjogren's syndrome), chronic inflammatory conditions (e.g., psoriasis, asthma or Crohn's disease), hyperproliferative disorders (e.g., breast cancer, lung cancer), viral infections (e.g., herpes, papilloma, or HIV), and other conditions, such as osteoarthritis and atherosclerosis. The Bcl-xL inhibitor or ADCs described herein may be used to treat or ameliorate any of these diseases. Such treatments generally involve administering to a subject suffering from the disease an amount of a Bcl-xL inhibitor or ADC described herein sufficient to provide therapeutic benefit. For ADCs, identity of the antibody of the ADC administered will depend upon the disease being treated—to the antibody should bind a cell-surface antigen expressed in the cell type where inhibition of Bcl-xL activity would be beneficial. The therapeutic benefit achieved will also depend upon the specific disease being treated. In certain instances, the Bcl-xL inhibitor or ADC may treat or ameliorate the disease itself, or symptoms of the disease, when administered as monotherapy. In other instances, the Bcl-xL inhibitor or ADC may be part of an overall treatment regimen including other agents that, together with the inhibitor or ADC, treat or ameliorate the disease being treated, or symptoms of the disease. Agents useful to treat or ameliorate specific diseases that may be administered adjunctive to, or with, the Bcl-xL inhibitors and/or ADCs described herein will be apparent to those of skill in the art.

[0872] Although absolute cure is always desirable in any therapeutic regimen, achieving a cure is not required to provide therapeutic benefit. Therapeutic benefit may include halting or slowing the progression of the disease, regressing the disease without curing, and/or ameliorating or slowing the progression of symptoms of the disease. Prolonged survival as compared to statistical averages and/or improved quality of life may also be considered therapeutic benefit. One particular class of diseases that involve dysregulated apoptosis and that are significant health burden world-wide are cancers. In a specific embodiment, the Bcl-xL inhibitors and/or ADCs described herein may be used to treat cancers. The cancer may be, for example, solid tumors or hematological tumors. Cancers that may be treated with the ADCs described herein include, but are not limited to include, but are not limited to bladder cancer, brain cancer, breast cancer, bone marrow cancer, cervical cancer, chronic lymphocytic leukemia, colorectal cancer, esophageal cancer, hepatocellular cancer, lymphoblastic leukemia, follicular lymphoma, lymphoid malignancies of T-cell or B-cell origin, melanoma, myelogenous leukemia, myeloma, oral cancer, ovarian cancer, non-small cell lung cancer, chronic lymphocytic leukemia, myeloma, prostate cancer, or spleen cancer. ADCs may be especially beneficial in the treatment of cancers because the antibody can be used to target the Bcl-xL inhibitory synthon specifically to tumor cells, thereby potentially avoiding or ameliorating undesirable side-effects and/or toxicities that may be associated with systemic administration of

unconjugated inhibitors. One embodiment pertains to a method of treating a disease involving dysregulated intrinsic apoptosis, comprising administering to a subject having a disease involving dysregulated apoptosis an amount of an ADC described herein effective to provide therapeutic benefit, wherein the antibody of the ADC binds a cell surface receptor on a cell whose intrinsic apoptosis is dysregulated. One embodiment pertains to a method of treating cancer, comprising administering to a subject having cancer an ADC described herein that is capable of binding a cell surface receptor or a tumor associated antigen expressed on the surface of the cancer cells, in an amount effective to provide therapeutic benefit.

[0873] In the context of tumorigenic cancers, therapeutic benefit, in addition to including the effects discussed above, may also specifically include halting or slowing progression of tumor growth, regressing tumor growth, eradicating one or more tumors and/or increasing patient survival as compared to statistical averages for the type and stage of the cancer being treated. In one embodiment, the cancer being treated is a tumorigenic cancer.

[0874] The ADCs of the invention are capable of neutralizing human EGFR activity both in vivo and in vitro. Accordingly, such ADCs of the invention can be used to inhibit hEGFR activity, e.g., in a cell culture containing hEGFR, in human subjects or in other mammalian subjects having EGFR with which an antibody of the invention cross-reacts. In one embodiment, the invention provides a method for inhibiting hEGFR activity comprising contacting hEGFR with an antibody or antibody portion of the invention such that hEGFR activity is inhibited. For example, in a cell culture containing, or suspected of containing hEGFR, an antibody or antibody portion of the invention can be added to the culture medium to inhibit hEGFR activity in the culture.

[0875] In another embodiment, the invention features a method for reducing hEGFR activity in a subject, advantageously from a subject suffering from a disease or disorder in which EGFR activity is detrimental. The invention provides methods for reducing EGFR activity in a subject suffering from such a disease or disorder, which method comprises administering to the subject an ADC of the invention such that EGFR activity in the subject is reduced. Preferably, the EGFR is human EGFR, and the subject is a human subject. Alternatively, the subject can be a mammal expressing an EGFR to which ADCs of the invention are capable of binding. Still further the subject can be a mammal into which EGFR has been introduced (e.g., by administration of EGFR or by expression of an EGFR transgene). ADCs of the invention can be administered to a human subject for therapeutic purposes.

[0876] Moreover, ADCs of the invention can be administered to a non-human mammal expressing an EGFR with which the antibody is capable of binding for veterinary purposes or as an animal model of human disease. Regarding the latter, such animal models may be useful for evaluating the therapeutic efficacy of antibodies of the invention (e.g., testing of dosages and time courses of administration).

[0877] As used herein, the term “a disorder in which EGFR activity is detrimental” is intended to include diseases and other disorders in which the presence of EGFR in a subject suffering from the disorder has been shown to be or is suspected of being either responsible for the pathophysiology of the disorder or a factor that contributes to a worsening of the disorder. Accordingly, a disorder in which EGFR activity is detrimental is a disorder in which reduction of EGFR activity is expected to alleviate the symptoms and/or progression of the disorder. Such disorders may be evidenced, for example, by an increase in the concentration of EGFR in a biological fluid of a subject suffering from the disorder (e.g., an increase in the concentration of EGFR in a tumor, serum, plasma, synovial fluid, etc. of the subject), which can be detected, for example, using an anti-EGFR antibody as described above. Non-limiting examples of disorders that can be treated with the ADCs of the invention, for example, an ADC comprising AbA, include those disorders discussed below. For example, suitable disorders include, but are not limited to, a variety of cancers including, but not limited to, breast cancer, lung cancer, a glioma, prostate cancer, pancreatic cancer, colon cancer, head and neck cancer, and kidney cancer. Other examples of cancer that may be treated using the

compositions and methods disclosed herein include squamous cell carcinoma (e.g., squamous lung cancer or squamous head and neck cancer), triple negative breast cancer, non-small cell lung cancer, colorectal cancer, and mesothelioma. In one embodiment, the ADCs disclosed herein are used to treat a solid tumor, e.g., inhibit growth of or decrease size of a solid tumor, overexpressing EGFR or which is EGFR positive.

[0878] In one embodiment, the invention is directed to the treatment of EGFR amplified squamous lung cancer. In one embodiment, the ADCs disclosed herein are used to treat EGFR amplified squamous head and neck cancer. In another embodiment, the ADCs disclosed herein are used to treat triple negative breast cancer (TNBC). Diseases and disorders described herein may be treated by anti-EGFR ADCs of the invention, as well as pharmaceutical compositions comprising such anti-EGFR ADCs.

[0879] In certain embodiments, the ADCs disclosed herein are administered to a subject in need thereof in order to treat advanced solid tumor types likely to exhibit elevated levels of Epidermal Growth Factor Receptor (EGFR). Examples of such tumors include, but are not limited to, head and neck squamous cell carcinoma, non-small cell lung cancer, triple negative breast cancer, colorectal carcinoma, and glioblastoma multiforme.

[0880] In certain embodiments, the invention includes a method for inhibiting or decreasing solid tumor growth in a subject having a solid tumor, said method comprising administering an anti-EGFR ADC described herein, to the subject having the solid tumor, such that the solid tumor growth is inhibited or decreased. In certain embodiments, the solid tumor is a non-small cell lung carcinoma or a glioblastoma. In further embodiments, the solid tumor is an EGFRvIII positive tumor or an EGFR-expressing solid tumors. In further embodiments, the solid tumor is an EGFR amplified solid tumor or an EGFR overexpressing solid tumors. In certain embodiments the anti-EGFR ADCs described herein are administered to a subject having glioblastoma multiforme, alone or in combination with an additional agent, e.g., radiation and/or temozolomide.

[0881] In one embodiment, the ADCs of the invention may be used to treating cancer associated with an activating EGFR mutation. Examples of such mutations include, but are not limited to, an exon 19 deletion mutation, a single-point substitution mutation L858R in exon 21, a T790M point mutation, and combinations thereof.

[0882] In certain embodiments, the invention includes a method for inhibiting or decreasing solid tumor growth in a subject having a solid tumor which was identified as an EGFR expressing or EGFR overexpressing tumor (or an EGFRvIII expressing tumor), said method comprising administering an anti-EGFR ADC described herein, to the subject having the solid tumor, such that the solid tumor growth is inhibited or decreased. Methods for identifying EGFR expressing tumors (e.g., EGFR overexpressing tumors) are known in the art, and include FDA-approved tests and validation assays.

[0883] For example, the EGFR pharmDx™ assay (Dako North America, Inc.) is a qualitative immunohistochemical (IHC) kit system used to identify EGFR expression in normal and neoplastic tissues routinely-fixed for histological evaluation. EGFR pharmDx specifically detects the EGFR (HER1) protein in EGFR-expressing cells. In addition, PCR-based assays may also be used for identifying EGFR overexpressing tumors. For example, these assays may use primers that are specific for the variant EGFR gene (e.g., SEQ ID NO: 33) and/or cDNA and result in the amplification of the EGFR gene/cDNA, or a portion thereof. The amplified PCR products may be subsequently analyzed, for example, by gel electrophoresis using standard methods known in the art to determine the size of the PCR products. Such tests may be used to identify tumors that may be treated with the methods and compositions described herein.

[0884] Any of the methods for gene therapy available in the art can be used according to the invention. For general reviews of the methods of gene therapy, see Goldspiel et al., 1993, *Clinical Pharmacy* 12:488-505; Wu and Wu, 1991, *Biotherapy* 3:87-95; Tolstoshev, 1993, *Ann. Rev. Pharmacol. Toxicol.* 32:573-596; Mulligan, *Science* 260:926-932 (1993); and Morgan and

Anderson, 1993, *Ann. Rev. Biochem.* 62:191-217; May, 1993, *TIBTECH* 11(5):155-215. Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, NY (1993); and Kriegler, *Gene Transfer and Expression, A Laboratory Manual*, Stockton Press, NY (1990). Detailed description of various methods of gene therapy is provided in US20050042664 A1 which is incorporated herein by reference.

[0885] In another aspect, this application features a method of treating (e.g., curing, suppressing, ameliorating, delaying or preventing the onset of, or preventing recurrence or relapse of) or preventing a EGFR-associated disorder, in a subject. The method includes: administering to the subject an EGFR binding agent (particularly an antagonist), e.g., an anti-EGFR antibody or fragment thereof as described herein, in an amount sufficient to treat or prevent the EGFR-associated disorder. The EGFR antagonist, e.g., the anti-EGFR antibody or fragment thereof, can be administered to the subject, alone or in combination with other therapeutic modalities as described herein.

[0886] ADCs of the invention, or antigen binding portions thereof can be used alone or in combination to treat such diseases. It should be understood that the ADCs of the invention can be used alone or in combination with an additional agent, e.g., a therapeutic agent, said additional agent being selected by the skilled artisan for its intended purpose. For example, the additional agent can be a therapeutic agent art-recognized as being useful to treat the disease or condition being treated by the ADC of the invention. The additional agent also can be an agent that imparts a beneficial attribute to the therapeutic composition, e.g., an agent which affects the viscosity of the composition.

[0887] It should further be understood that the combinations which are to be included within this invention are those combinations useful for their intended purpose. The agents set forth below are illustrative for purposes and not intended to be limited. The combinations, which are part of this invention, can be the antibodies of the invention and at least one additional agent selected from the lists below. The combination can also include more than one additional agent, e.g., two or three additional agents if the combination is such that the formed composition can perform its intended function.

[0888] The combination therapy can include anti-EGFR antagonists ADCs of the invention formulated with, and/or co-administered with, one or more additional therapeutic agents, e.g., one or more cytokine and growth factor inhibitors, immunosuppressants, anti-inflammatory agents (e.g., systemic anti-inflammatory agents), anti-fibrotic agents, metabolic inhibitors, enzyme inhibitors, and/or cytotoxic or cytostatic agents, mitotic inhibitors, antitumor antibiotics, immunomodulating agents, vectors for gene therapy, alkylating agents, antiangiogenic agents, antimetabolites, boron-containing agents, chemoprotective agents, hormones, antihormone agents, corticosteroids, photoactive therapeutic agents, oligonucleotides, radionuclide agents, topoisomerase inhibitors, kinase inhibitors, or radiosensitizers, as described in more herein.

[0889] In a particular embodiment, the anti-EGFR ADCs described herein, are used in combination with an anti-cancer agent or an antineoplastic agent. The terms "anti-cancer agent" and "antineoplastic agent" refer to drugs used to treat malignancies, such as cancerous growths. Drug therapy may be used alone, or in combination with other treatments such as surgery or radiation therapy. Several classes of drugs may be used in cancer treatment, depending on the nature of the organ involved. For example, breast cancers are commonly stimulated by estrogens, and may be treated with drugs which inactive the sex hormones. Similarly, prostate cancer may be treated with drugs that inactivate androgens, the male sex hormone. Anti-cancer agents that may be used in conjunction with the anti-EGFR ADCs of the invention include, among others, the following agents:

TABLE-US-00021 Anti-Cancer Agent Comments Examples Antibodies Antibodies which bind IGF- A12 (fully humanized mAb) (a) antibodies other 1R (insulin-like growth 19D12 (fully

humanized mAb) than anti-EGFR factor type 1 receptor), Cp751-871 (fully humanized mAb) antibodies; and which is expressed on the H7C10 (humanized mAb) (b) anti-EGFR cell surface of most human alphaIR3 (mouse) antibodies which cancers ScFV/FC (mouse/human chimera) bind different Antibodies which bind EM/164 (mouse) epitopes EGFR (epidermal growth Matuzumab (EMD72000) factor receptor); Mutations Erbitux ®/Cetuximab (Imclone) affecting EGFR expression Vectibix ®/Panitumumab (Amgen) or activity could result in mAb 806 cancer Nimotuxumab (TheraCIM) Antibodies which bind AVEO (AV299) (AVEO) cMET (Mesenchymal AMG102 (Amgen) epithelial transition factor); 5D5 (OA-5d5) (Genentech) a member of the MET H244G11 (Pierre Fabre) family of receptor tyrosine Ab #14 (MM 121-14) kinases) Herceptin ® (Trastuzumab; Genentech) Anti-ErbB3 antibodies 1B4C3; 2D1D12 (U3 Pharma AG) which bind different epitopes Small Molecules Insulin-like growth factor NVP-AEW541-A Targeting IGF1R type 1 receptor which is BMS-536,924 (1H-benzoimidazol-2-yl)-1H- expressed on the cell pyridin-2-one) surface of many human BMS-554,417 cancers Cycloligan TAE226 PQ401 Small Molecules EGFR (epidermal growth Iressa ®/Gefitinib (AstraZeneca) Targeting EGFR factor receptor); CI-1033 (PD 183805) (Pfizer) Overexpression or Lapatinib (GW-572016) (GlaxoSmithKline) mutations affecting EGFR Tykerb ®/Lapatinib Ditosylate (Smith Kline expression or activity could Beecham) result in cancer Tarceva ®/Erlotinib HCL (OSI-774) (OSI Pharma) PKI-166 (Novartis) PD-158780 EKB-569 Tyrphostin AG 1478 (4-(3-Chloroanillino)- 6,7-dimethoxyquinazoline) Small Molecules cMET (Mesenchymal PHA665752 Targeting cMET epithelial transition factor); ARQ 197 a member of the MET family of receptor tyrosine kinases) Antimetabolites Fluorouracil (5-FU) Capecitabine/XELODA ® (HLR Roche) 5-Trifluoromethyl-2'-deoxyuridine Methotrexate sodium (Trexall) (Barr) Raltitrexed/Tomudex ® (AstraZeneca) Pemetrexed/Alimta ® (Lilly) Tegafur Cytosine Arabinoside (Cytarabine, Ara-C)/ Thioguanine ® (GlaxoSmithKline) 5-azacytidine 6-mercaptopurine (Mercaptopurine, 6-MP) Azathioprine/Azasan ® (AAIPHARMA LLC) 6-thioguanine (6-TG)/Purinethol ® (TEVA) Pentostatin/Nipent ® (Hospira Inc.) Fludarabine phosphate/Fludara ® (Bayer Health Care) Cladribine (2-CdA, 2- chlorodeoxyadenosine)/Leustatin ® (Ortho Biotech) Alkylating agents An alkylating antineoplastic Ribonucleotide Reductase Inhibitor (RNR) agent is an alkylating agent Cyclophosphamide/Cytosan (BMS) that attaches an alkyl group Neosar (TEVA) to DNA. Since cancer cells Ifosfamide/Mitoxana ® (ASTA Medica) generally proliferate Thiotepa (Bedford, Abraxis, Teva) unrestrictively more than do BCNU.fwdarw. 1,3-bis(2-chloroethyl)-1- healthy cells they are more nitrosourea sensitive to DNA damage, CCNU.fwdarw. 1, -(2-chloroethyl)-3-cyclohexyl-1- and alkylating agents are nitrosourea (methyl CCNU) used clinically to treat a Hexamethylmelamine (Altretamine, HMM) / variety of tumors. Hexalen ® (MGI Pharma Inc.) Busulfan/Myleran (GlaxoSmithKline) Procarbazine HCL/Matulane (Sigma Tau Pharmaceuticals, Inc.) Dacarbazine (DTIC) Chlorambucil/Leukara ® (SmithKline Beecham) Melphalan/Alkeran ® (GlaxoSmithKline) Cisplatin (Cisplatinum, CDDP)/Platinol (Bristol Myers) Carboplatin/Paraplatin (BMS) Oxaliplatin/Eloxitan ® (Sanofi-Aventis US) Topoisomerase Topoisomerase inhibitors Doxorubicin HCL/Doxil ® (Alza) inhibitors are chemotherapy agents Daunorubicin citrate/Daunoxome ® designed to interfere with (Gilead) Mitoxantrone HCL/Novantrone the action of topoisomerase (EMD Serono) enzymes (topoisomerase I Actinomycin D and II), which are enzymes Etoposide/Vepesid ® (BMS)/Etopophos ® that control the changes in (Hospira, Bedford, Teva Parenteral, Etc.) DNA structure by Topotecan HCL/Hycamtin ® catalyzing the breaking and (GlaxoSmithKline) rejoining of the Teniposide (VM-26)/Vumon ® (BMS) phosphodiester backbone of Irinotecan HCL(CPT-11)/Camptosar ® DNA strands during the (Pharmacia & Upjohn) normal cell cycle. Microtubule Microtubules are one of the Vincristine/Oncovin ® (Lilly) targeting agents components of the Vinblastine sulfate/Velban ®(discontinued) cytoskeleton. They have (Lilly) diameter of ~24 nm and Vinorelbine tartrate/Navelbine ® length varying from several (PierreFabre) micrometers to possibly Vindesine sulphate/Eldisine ® (Lilly) millimeters in axons of Paclitaxel/Taxol ® (BMS) nerve cells. Microtubules Docetaxel/Taxotere ® (Sanofi Aventis US) serve as structural

Nanoparticle paclitaxel (ABI-007)/ components within cells Abraxane® (Abraxis BioScience, Inc.) and are involved in many Ixabepilone/IXEMPRATM (BMS) cellular processes including mitosis, cytokinesis, and vesicular transport. Kinase inhibitors Kinases are enzymes that Imatinib mesylate/Gleevec (Novartis) catalyze the transfer of Sunitinib malate/Sutent® (Pfizer) phosphate groups from Sorafenib tosylate/Nexavar® (Bayer) high-energy, phosphate- Nilotinib hydrochloride monohydrate/ donating molecules to Tasigna® (Novartis), Osimertinib, specific substrates, and are Cobimetinib, Trametinib, Dabrafenib, utilized to transmit signals Dinaciclib and regulate complex processes in cells. Protein synthesis Induces cell apoptosis L-asparaginase/Elspar® (Merck & Co.) inhibitors Immunotherapeutic Induces cancer patients to Alpha interferon agents exhibit immune Angiogenesis Inhibitor/Avastin® responsiveness (Genentech) IL-2.fwdarw. Interleukin 2 (Aldesleukin)/ Proleukin® (Chiron) IL-12.fwdarw. Interleukin 12 Antibody/small molecule Anti-CTLA-4 and PR-1 therapies immune checkpoint Yervoy® (ipilimumab; Bristol-Myers modulators Squibb) Opdivo® (nivolumab; Bristol-Myers Squibb) Keytrada® (pembrolizumab; Merck) Hormones Hormone therapies Toremifene citrate/Fareston® (GTX, Inc.) associated with menopause Fulvestrant/Faslodex® (AstraZeneca) and aging seek to increase Raloxifene HCL/Evista® (Lilly) the amount of certain Anastrozole/Arimidex® (AstraZeneca) hormones in your body to Letrozole/Femara® (Novartis) compensate for age- or Fadrozole (CGS 16949A) disease-related hormonal Exemestane/Aromasin® (Pharmacia & declines. Hormone therapy Upjohn) as a cancer treatment either Leuprolide acetate/Eligard® (QTL USA) reduces the level of specific Lupron® (TAP Pharm) hormones or alters the Goserelin acetate/Zoladex® (AstraZeneca) cancer's ability to use these Triptorelin pamoate/Trelstar® (Watson hormones to grow and Labs) spread. Buserelin/Suprefact® (Sanofi Aventis) Nafarelin/Synarel® (Pfizer) Cetorelix/Cetrotide® (EMD Serono) Bicalutamide/Casodex® (AstraZeneca) Nilutamide/Nilandron® (Aventis Pharm.) Megestrol acetate/Megace® (BMS) Somatostatin Analogs (Octreotide acetate / Sandostatin® (Novartis) Glucocorticoids Anti-inflammatory drugs Prednisolone used to reduce swelling that Dexamethasone/Decadron® (Wyeth) causes cancer pain. Aromatase inhibitors Includes imidazoles Ketoconazole mTOR inhibitors The mTOR signaling Sirolimus (Rapamycin)/Rapamune® pathway was originally (Wyeth) discovered during studies of Temsirolimus (CCI-779)/Torisel® (Wyeth) the immunosuppressive Deforolimus (AP23573)/(Ariad Pharm.) agent rapamycin. This Everolimus (RAD001)/Certican® highly conserved pathway (Novartis) regulates cell proliferation and metabolism in response to environmental factors, linking cell growth factor receptor signaling via phosphoinositide-3-kinase (PI-3K) to cell growth, proliferation, and angiogenesis.

[0890] In addition to the above anti-cancer agents, the anti-EGFRADCs described herein may be administered in combination with the agents described in section II. Further, the aforementioned anti-cancer agents may also be used in the ADCs of the invention.

[0891] In particular embodiments, the ADCs of the invention can be administered alone or with another anti-cancer agent which acts in conjunction with or synergistically with the antibody to treat the disease associated with EGFR activity. Such anti-cancer agents include, for example, agents well known in the art (e.g., cytotoxins, chemotherapeutic agents, small molecules and radiation). Examples of anti-cancer agents include, but are not limited to, Panorex (Glaxo-Welcome), Rituxan (IDEC/Genentech/Hoffman la Roche), Mylotarg (Wyeth), Campath (Millennium), Zevalin (IDEC and Schering AG), Bexxar (Corixa/GSK), Erbitux (Imclone/BMS), Avastin (Genentech) and Herceptin (Genentech/Hoffman la Roche). Other anti-cancer agents include, but are not limited to, those disclosed in U.S. Pat. No. 7,598,028 and International Publication No. WO2008/100624, the contents of which are hereby incorporated by reference. One or more anti-cancer agents may be administered either simultaneously or before or after administration of an antibody or antigen binding portion thereof of the invention.

[0892] In particular embodiments of the invention, the ADCs described herein can be used in a combination therapy with an inhibitor of NAMPT (see examples of inhibitors in US 2013/0303509;

AbbVie, Inc., incorporated by reference herein) to treat a subject in need thereof. NAMPT (also known as pre-B-cell-colony-enhancing factor (PBEF) and visfatin) is an enzyme that catalyzes the phosphoribosylation of nicotinamide and is the rate-limiting enzyme in one of two pathways that salvage NAD. In one embodiment of the invention, anti-EGFR antibodies and ADCs described herein are administered in combination with a NAMPT inhibitor for the treatment of cancer in a subject.

[0893] In particular embodiments of the invention, the ADCs described herein can be used in a combination therapy with SN-38, which is the active metabolite of the topoisomerase inhibitor irinotecan.

[0894] In other embodiments of the invention, the ADCs described herein can be used in a combination therapy with a PARP (poly ADP ribose polymerase) inhibitor, e.g., veliparib, to treat cancer, including breast, ovarian and non-small cell lung cancers.

[0895] Further examples of additional therapeutic agents that can be co-administered and/or formulated with anti-EGFR ADCs described herein, include, but are not limited to, one or more of: inhaled steroids; beta-agonists, e.g., short-acting or long-acting beta-agonists; antagonists of leukotrienes or leukotriene receptors; combination drugs such as ADVAIR; IgE inhibitors, e.g., anti-IgE antibodies (e.g., XOLAIR, omalizumab); phosphodiesterase inhibitors (e.g., PDE4 inhibitors); xanthines; anticholinergic drugs; mast cell-stabilizing agents such as cromolyn; IL-4 inhibitors; IL-5 inhibitors; eotaxin/CCR3 inhibitors; antagonists of histamine or its receptors including H1, H2, H3, and H4, and antagonists of prostaglandin D or its receptors (DP1 and CRTH2). Such combinations can be used to treat, for example, asthma and other respiratory disorders. Other examples of additional therapeutic agents that can be co-administered and/or formulated with anti-EGFR ADCs described herein, include, but are not limited to, one or more of, temozolomide, ibrutinib, duvelisib, and idelalisib.

[0896] In certain embodiments, the ADC is administered in combination with an additional agent or an additional therapy, where the additional agent is selected from the group consisting of an anti-PD1 antibody, an anti-CTLA-4 antibody, temozolomide, ibrutinib, duvelisib, idelalisib, in combination with auristatin ADCs.

[0897] Additional examples of therapeutic agents that can be co-administered and/or formulated with one or more anti-EGFR antibodies or fragments thereof include one or more of: TNF antagonists (e.g., a soluble fragment of a TNF receptor, e.g., p55 or p75 human TNF receptor or derivatives thereof, e.g., 75 kD TNFR-IgG (75 kD TNF receptor-IgG fusion protein, ENBREL)); TNF enzyme antagonists, e.g., TNF converting enzyme (TACE) inhibitors; muscarinic receptor antagonists; TGF-beta antagonists; interferon gamma; perfenidone; chemotherapeutic agents, e.g., methotrexate, leflunomide, or a sirolimus (rapamycin) or an analog thereof, e.g., CCI-779; COX2 and cPLA2 inhibitors; NSAIDs; immunomodulators; p38 inhibitors, TPL-2, MK-2 and NFkB inhibitors, among others.

[0898] Other preferred combinations are cytokine suppressive anti-inflammatory drug(s) (CSAIDs); antibodies to or antagonists of other human cytokines or growth factors, for example, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-15, IL-16, IL-18, IL-21, IL-31, interferons, EMAP-II, GM-CSF, FGF, EGF, PDGF, and edothelin-1, as well as the receptors of these cytokines and growth factors.

[0899] Antibodies of the invention, or antigen binding portions thereof, can be combined with antibodies to cell surface molecules such as CD2, CD3, CD4, CD8, CD25, CD28, CD30, CD40, CD45, CD69, CD80 (B7.1), CD86 (B7.2), CD90, CTLA, CTLA-4, PD-1, or their ligands including CD154 (gp39 or CD40L).

[0900] Preferred combinations of therapeutic agents may interfere at different points in the inflammatory cascade; preferred examples include TNF antagonists like chimeric, humanized or human TNF antibodies, adalimumab, (HUMIRA; D2E7; PCT Publication No. WO 97/29131 and U.S. Pat. No. 6,090,382, incorporated by reference herein), CA2 (Remicade®), CDP 571, and

soluble p55 or p75 TNF receptors, derivatives, thereof, (p75TNFR1gG (Enbrel®) or p55TNFR1gG (Lenercept), and also TNF converting enzyme (TACE) inhibitors; similarly IL-1 inhibitors (Interleukin-1-converting enzyme inhibitors, IL-1RA etc.) may be effective for the same reason. Other preferred combinations include Interleukin 4.

[0901] In certain embodiments, the ADC is administered in combination with a taxane to treat non small cell lung cancer. In other embodiments, the ADC is administered in combination with venetoclax in SCLC.

[0902] The pharmaceutical compositions of the invention may include a “therapeutically effective amount” or a “prophylactically effective amount” of an antibody or antibody portion of the invention.

[0903] A “therapeutically effective amount” refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the antibody or antibody portion may be determined by a person skilled in the art and may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody or antibody portion to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody, or antibody portion, are outweighed by the therapeutically beneficial effects. A “prophylactically effective amount” refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

[0904] The amount of ADC administered will depend upon a variety of factors, including but not limited to, the particular disease being treated, the mode of administration, the desired therapeutic benefit, the stage or severity of the disease, the age, weight and other characteristics of the patient, etc. Determination of effective dosages is within the capabilities of those skilled in the art.

[0905] Dosage regimens may be adjusted to provide the optimum desired response (e.g., a therapeutic or prophylactic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic or prophylactic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

[0906] An exemplary, non-limiting range for a therapeutically or prophylactically effective amount of an ADC, is 0.1-20 mg/kg, more preferably 1-10 mg/kg. In one embodiment, the dose of the ADCs described herein is 1 to 6 mg/kg, including the individual doses recited therein, e.g., 1 mg/kg, 2 mg/kg, 3 mg/kg, 4 mg/kg, 5 mg/kg, and 6 mg/kg. In another embodiment, the dose of the ADCs described herein is 1 to 200 µg/kg, including the individual doses recited therein, e.g., 1 µg/kg, 2 µg/kg, 3 µg/kg, 4 µg/kg, 5 µg/kg, 10 µg/kg, 20 µg/kg, 30 µg/kg, 40 µg/kg, 50 µg/kg, 60 µg/kg, 80 µg/kg, 100 µg/kg, 120 µg/kg, 140 µg/kg, 160 µg/kg, 180 µg/kg and 200 µg/kg. It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

thereof, e.g., a subject having cancer, at a dose of 5 to 80 $\mu\text{g/kg}$. In another embodiment, an anti-EGFR ADC described herein is administered to a subject in need thereof, e.g., a subject having cancer, at a dose of 5 to 70 $\mu\text{g/kg}$. In another embodiment, an anti-EGFR ADC described herein is administered to a subject in need thereof, e.g., a subject having cancer, at a dose of 5 to 60 $\mu\text{g/kg}$. [0912] In another aspect, this application provides a method for detecting the presence of EGFR in a sample in vitro (e.g., a biological sample, such as serum, plasma, tissue, and biopsy). The subject method can be used to diagnose a disorder, e.g., a cancer. The method includes: (i) contacting the sample or a control sample with the anti-EGFR ADC as described herein; and (ii) detecting formation of a complex between the anti-EGFR ADC and the sample or the control sample, wherein a statistically significant change in the formation of the complex in the sample relative to the control sample is indicative of the presence of EGFR in the sample.

[0913] Given their ability to bind to human EGFR, the ADCs of the invention can be used to detect human EGFR (e.g., in a biological sample, such as serum or plasma), using a conventional immunoassay, such as an enzyme linked immunosorbent assays (ELISA), an radioimmunoassay (RIA) or tissue immunohistochemistry. In one aspect, the invention provides a method for detecting human EGFR in a biological sample comprising contacting a biological sample with an antibody, or antibody portion, of the invention and detecting either the antibody (or antibody portion) bound to human EGFR or unbound antibody (or antibody portion), to thereby detect human EGFR in the biological sample. The antibody is directly or indirectly labeled with a detectable substance to facilitate detection of the bound or unbound antibody. Suitable detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials and radioactive materials.

[0914] Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, P-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; and examples of suitable radioactive material include ^3H , ^{14}C , ^{35}S , ^{90}Y , ^{99}Tc , ^{111}In , ^{125}I , ^{131}I , ^{177}Lu , ^{166}Ho , or ^{153}Sm .

[0915] Alternative to labeling the antibody, human EGFR can be assayed in biological fluids by a competition immunoassay utilizing rhEGFR standards labeled with a detectable substance and an unlabeled anti-human EGFR ADC. In this assay, the biological sample, the labeled rhEGFR standards and the anti-human EGFR antibody are combined and the amount of labeled rhEGFR standard bound to the unlabeled antibody is determined. The amount of human EGFR in the biological sample is inversely proportional to the amount of labeled rhEGFR standard bound to the anti-EGFR antibody. Similarly, human EGFR can also be assayed in biological fluids by a competition immunoassay utilizing rhEGFR standards labeled with a detectable substance and an unlabeled anti-human EGFR ADC.

8. Pharmaceutical Compositions

[0916] The invention also provides pharmaceutical compositions comprising an ADC of the invention and a pharmaceutically acceptable carrier. The pharmaceutical compositions comprising ADCs of the invention are for use in, but not limited to, diagnosing, detecting, or monitoring a disorder, in preventing, treating, managing, or ameliorating of a disorder or one or more symptoms thereof, and/or in research. In a specific embodiment, a composition comprises one or more antibodies of the invention. In another embodiment, the pharmaceutical composition comprises one or more ADCs of the invention and one or more prophylactic or therapeutic agents other than ADCs of the invention for treating a disorder in which EGFR activity is detrimental. Preferably, the prophylactic or therapeutic agents known to be useful for or having been or currently being used in the prevention, treatment, management, or amelioration of a disorder or one or more symptoms thereof. In accordance with these embodiments, the composition may further comprise of a carrier,

diluent or excipient.

[0917] The ADCs described herein may be in the form of compositions comprising the ADC and one or more carriers, excipients and/or diluents. The compositions may be formulated for specific uses, such as for veterinary uses or pharmaceutical uses in humans. The form of the composition (e.g., dry powder, liquid formulation, etc.) and the excipients, diluents and/or carriers used will depend upon the intended uses of the ADCs and, for therapeutic uses, the mode of administration.

[0918] For therapeutic uses, the ADC compositions may be supplied as part of a sterile, pharmaceutical composition that includes a pharmaceutically acceptable carrier. This composition can be in any suitable form (depending upon the desired method of administering it to a patient). The pharmaceutical composition can be administered to a patient by a variety of routes such as orally, transdermally, subcutaneously, intranasally, intravenously, intramuscularly, intrathecally, topically or locally. The most suitable route for administration in any given case will depend on the particular Bcl-xL inhibitor or ADC, the subject, and the nature and severity of the disease and the physical condition of the subject. Typically, the ADCs will be administered orally or parenterally, and ADC pharmaceutical composition will be administered intravenously or subcutaneously.

[0919] Pharmaceutical compositions can be conveniently presented in unit dosage forms containing a predetermined amount of ADC described herein per dose. The quantity of inhibitor or ADC included in a unit dose will depend on the disease being treated, as well as other factors as are well known in the art. For ADCs, such unit dosages may be in the form of a lyophilized dry powder containing an amount of ADC suitable for a single administration, or in the form of a liquid. Dry powder unit dosage forms may be packaged in a kit with a syringe, a suitable quantity of diluent and/or other components useful for administration. Unit dosages in liquid form may be conveniently supplied in the form of a syringe pre-filled with a quantity of ADC suitable for a single administration.

[0920] The pharmaceutical compositions may also be supplied in bulk from containing quantities of ADC suitable for multiple administrations.

[0921] Pharmaceutical compositions of ADCs may be prepared for storage as lyophilized formulations or aqueous solutions by mixing an ADC having the desired degree of purity with optional pharmaceutically-acceptable carriers, excipients or stabilizers typically employed in the art (all of which are referred to herein as “carriers”), i.e., buffering agents, stabilizing agents, preservatives, isotonicifiers, non-ionic detergents, antioxidants, and other miscellaneous additives. See, Remington's Pharmaceutical Sciences, 16th edition (Osol, ed. 1980). Such additives should be nontoxic to the recipients at the dosages and concentrations employed.

[0922] Buffering agents help to maintain the pH in the range which approximates physiological conditions. They may be present at concentration ranging from about 2 mM to about 50 mM. Suitable buffering agents for use with the present disclosure include both organic and inorganic acids and salts thereof such as citrate buffers (e.g., monosodium citrate-disodium citrate mixture, citric acid-trisodium citrate mixture, citric acid-monosodium citrate mixture, etc.), succinate buffers (e.g., succinic acid-monosodium succinate mixture, succinic acid-sodium hydroxide mixture, succinic acid-disodium succinate mixture, etc.), tartrate buffers (e.g., tartaric acid-sodium tartrate mixture, tartaric acid-potassium tartrate mixture, tartaric acid-sodium hydroxide mixture, etc.), fumarate buffers (e.g., fumaric acid-monosodium fumarate mixture, fumaric acid-disodium fumarate mixture, monosodium fumarate-disodium fumarate mixture, etc.), gluconate buffers (e.g., gluconic acid-sodium gluconate mixture, gluconic acid-sodium hydroxide mixture, gluconic acid-potassium gluconate mixture, etc.), oxalate buffer (e.g., oxalic acid-sodium oxalate mixture, oxalic acid-sodium hydroxide mixture, oxalic acid-potassium oxalate mixture, etc.), lactate buffers (e.g., lactic acid-sodium lactate mixture, lactic acid-sodium hydroxide mixture, lactic acid-potassium lactate mixture, etc.) and acetate buffers (e.g., acetic acid-sodium acetate mixture, acetic acid-sodium hydroxide mixture, etc.). Additionally, phosphate buffers, histidine buffers and trimethylamine salts such as Tris can be used.

[0923] Preservatives may be added to retard microbial growth, and can be added in amounts ranging from about 0.2%-1% (w/v). Suitable preservatives for use with the present disclosure include phenol, benzyl alcohol, meta-cresol, methyl paraben, propyl paraben, octadecyldimethylbenzyl ammonium chloride, benzalconium halides (e.g., chloride, bromide, and iodide), hexamethonium chloride, and alkyl parabens such as methyl or propyl paraben, catechol, resorcinol, cyclohexanol, and 3-pentanol. Isotonicifiers sometimes known as “stabilizers” can be added to ensure isotonicity of liquid compositions of the present disclosure and include polyhydric sugar alcohols, for example trihydric or higher sugar alcohols, such as glycerin, erythritol, arabitol, xylitol, sorbitol and mannitol. Stabilizers refer to a broad category of excipients which can range in function from a bulking agent to an additive which solubilizes the therapeutic agent or helps to prevent denaturation or adherence to the container wall. Typical stabilizers can be polyhydric sugar alcohols (enumerated above); amino acids such as arginine, lysine, glycine, glutamine, asparagine, histidine, alanine, ornithine, L-leucine, 2-phenylalanine, glutamic acid, threonine, etc., organic sugars or sugar alcohols, such as lactose, trehalose, stachyose, mannitol, sorbitol, xylitol, ribitol, myoinositol, galactitol, glycerol and the like, including cyclitols such as inositol; polyethylene glycol; amino acid polymers; sulfur containing reducing agents, such as urea, glutathione, thiocetic acid, sodium thioglycolate, thioglycerol, α -monothioglycerol and sodium thio sulfate; low molecular weight polypeptides (e.g., peptides of 10 residues or fewer); proteins such as human serum albumin, bovine serum albumin, gelatin or immunoglobulins; hydrophylic polymers, such as polyvinylpyrrolidone monosaccharides, such as xylose, mannose, fructose, glucose; disaccharides such as lactose, maltose, sucrose and trisaccharides such as raffinose; and polysaccharides such as dextran.

[0924] Non-ionic surfactants or detergents (also known as “wetting agents”) may be added to help solubilize the glycoprotein as well as to protect the glycoprotein against agitation-induced aggregation, which also permits the formulation to be exposed to shear surface stressed without causing denaturation of the protein. Suitable non-ionic surfactants include polysorbates (20, 80, etc.), polyoxamers (184, 188, etc.), Pluronic polyols, polyoxyethylene sorbitan monoethers (TWEEN®-20, TWEEN®-80, etc.). Non-ionic surfactants may be present in a range of about 0.05 mg/mL to about 1.0 mg/mL, for example about 0.07 mg/mL to about 0.2 mg/mL.

[0925] Additional miscellaneous excipients include bulking agents (e.g., starch), chelating agents (e.g., EDTA), antioxidants (e.g., ascorbic acid, methionine, vitamin E), and cosolvents.

[0926] It will be readily apparent to those skilled in the art that other suitable modifications and adaptations of the methods of the invention described herein are obvious and may be made using suitable equivalents without departing from the scope of the invention or the embodiments disclosed herein. Having now described the invention in detail, the same will be more clearly understood by reference to the following examples, which are included for purposes of illustration only and are not intended to be limiting.

EXAMPLES

Example 1. Synthesis of Exemplary Bcl-xL Inhibitors

[0927] This Example provides synthetic methods for exemplary Bcl-xL inhibitory compounds W3.01-W3.43. Bcl-xL inhibitors (W3.01-W3.43) and synthons (Examples 2.1-2.72) were named using ACD/Name 2012 release (Build 56084, 5 Apr. 2012, Advanced Chemistry Development Inc., Toronto, Ontario), ACD/Name 2014 release (Build 66687, 25 Oct. 2013, Advanced Chemistry Development Inc., Toronto, Ontario), ChemDraw® Ver. 9.0.7 (CambridgeSoft, Cambridge, MA), ChemDraw® Ultra Ver. 12.0 (CambridgeSoft, Cambridge, MA), or ChemDraw® Professional Ver. 15.0.0.106. Bcl-xL inhibitor and synthon intermediates were named with ACD/Name 2012 release (Build 56084, 5 Apr. 2012, Advanced Chemistry Development Inc., Toronto, Ontario), ACD/Name 2014 release (Build 66687, 25 Oct. 2013, Advanced Chemistry Development Inc., Toronto, Ontario), ChemDraw® Ver. 9.0.7 (CambridgeSoft, Cambridge, MA), ChemDraw® Ultra Ver. 12.0 (CambridgeSoft, Cambridge, MA), or ChemDraw® Professional Ver. 15.0.0.106.

[0928] 1.1. Synthesis of 6-[1-(1,3-benzothiazol-2-ylcarbonyl)-1,2,3,4-tetrahydroquinolin-7-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl}-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid (Compound W3.01)

[0929] 1.1.1. 3-bromo-5,7-dimethyladamantanecarboxylic acid

[0930] To a 50 mL round-bottomed flask at 0° C. was added bromine (16 mL). Iron powder (7 g) was added, and the reaction was stirred at 0° C. for 30 minutes. 3,5-Dimethyladamantane-1-carboxylic acid (12 g) was then added. The mixture was then warmed to room temperature and stirred for 3 days. An ice/concentrated HCl mixture was poured into the reaction mixture. The resulting suspension was treated twice with Na.sub.2SO.sub.3 (50 g in 200 mL water) and extracted three times with dichloromethane. The combined organic layers were washed with 1N aqueous HCl, dried over Na.sub.2SO.sub.4, filtered, and concentrated to give the crude title compound.

[0931] 1.1.2. 3-bromo-5,7-dimethyladamantanemethanol

[0932] To a solution of Example 1.1.1 (15.4 g) in tetrahydrofuran (200 mL) was added BH.sub.3 (1M in tetrahydrofuran, 150 mL). The mixture was stirred at room temperature overnight. The reaction mixture was then carefully quenched via dropwise addition of methanol. The mixture was then concentrated under vacuum and the residue was partitioned between ethyl acetate (500 mL) and 2N aqueous HCl (100 mL). The aqueous layer was further extracted twice with ethyl acetate and the combined organic extracts were combined and washed with water and brine, and dried over Na.sub.2SO.sub.4. Filtration and evaporation of the solvent gave the title compound.

[0933] 1.1.3. 1-((3-bromo-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl)-1H-pyrazole

[0934] To a solution of Example 1.1.2 (8.0 g) in toluene (60 mL) was added 1H-pyrazole (1.55 g) and cyanomethylenetriethylphosphorane (2.0 g). The mixture was stirred at 90° C. overnight. The reaction mixture was then concentrated and the residue was purified by silica gel column chromatography (10:1 hexane:ethyl acetate) to provide the title compound. MS (ESI) m/e 324.2 (M+H)+.

[0935] 1.1.4. 2-([3,5-dimethyl-7-(1H-pyrazol-1-yl)methyl]tricyclo[3.3.1.1.sup.3,7]dec-1-yl)oxy}ethanol

[0936] To a solution of Example 1.1.3 (4.0 g) in ethane-1,2-diol (12 mL) was added triethylamine (3 mL). The mixture was stirred at 150° C. under microwave conditions (Biotage) for 45 minutes. The mixture was poured into water (100 mL) and extracted three times with ethyl acetate. The combined organic extracts were washed with water and brine, and dried over Na.sub.2SO.sub.4. Filtration and evaporation of the solvent gave the crude title compound which was purified via column chromatography, eluting with 20% ethyl acetate in hexane followed by 5% methanol in dichloromethane, to provide the title compound. MS (ESI) m/e 305.2 (M+H)+.

[0937] 1.1.5. 2-([3,5-dimethyl-7-[(5-methyl-1H-pyrazol-1-yl)methyl]tricyclo[3.3.1.1.sup.3,7]dec-1-yl)oxy}ethanol

[0938] To a cooled (-78° C.) solution of Example 1.1.4 (6.05 g) in tetrahydrofuran (100 mL) was added n-BuLi (40 mL, 2.5M in hexane). The mixture was stirred at -78° C. for 1.5 hours. Then, iodomethane (10 mL) was added through a syringe and the mixture was stirred at -78° C. for 3 hours. The reaction mixture was then quenched with aqueous NH.sub.4Cl and extracted twice with ethyl acetate, and the combined organic extracts were washed with water and brine. After drying over Na.sub.2SO.sub.4, the solution was filtered and concentrated and the residue was purified by silica gel column chromatography (5% methanol in dichloromethane) to provide the title compound. MS (ESI) m/e 319.5 (M+H)+.

[0939] 1.1.6. 1-([3,5-dimethyl-7-[2-(hydroxy)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl)-4-iodo-5-methyl-1H-pyrazole

[0940] To a solution of Example 1.1.5 (3.5 g) in N,N-dimethylformamide (30 mL) was added N-iodosuccinimide (3.2 g). The mixture was stirred at room temperature for 1.5 hours. The reaction mixture was then diluted with ethyl acetate (600 mL) and washed with aqueous NaHSO.sub.3,

water, and brine. After drying over Na.sub.2SO.sub.4, the solution was filtered and concentrated and the residue was purified by silica gel chromatography (20% ethyl acetate in dichloromethane) to give the title compound. MS (ESI) m/e 445.3 (M+H)+.

[0941] 1.1.7. 2-((3-((4-iodo-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl methanesulfonate

[0942] To a cooled solution (0° C.) of Example 1.1.6 (5.45 g) in dichloromethane (100 mL) was added triethylamine (5.13 mL) and methanesulfonyl chloride (0.956 mL). The mixture was stirred at room temperature for 1.5 hours, diluted with ethyl acetate (600 mL) and washed with water (120 mL) and brine (120 mL). The organic layer was dried over Na.sub.2SO.sub.4, filtered, and concentrated to provide the title compound. MS (ESI) m/e 523.4 (M+H)+.

[0943] 1.1.8. 2-((3-((4-iodo-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)-N-methylethanamine

[0944] A solution of Example 1.1.7 (6.41 g) in 2M methylamine in ethanol (15 mL) was stirred at overnight and concentrated. The residue was diluted with ethyl acetate and washed with aqueous NaHCO.sub.3, water and brine. The organic layer was dried over Na.sub.2SO.sub.4, filtered, and concentrated to provide the title compound. MS (ESI) m/e 458.4 (M+H)+.

[0945] 1.1.9. tert-butyl[2-({3-[(4-iodo-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethyl]methylcarbamate

[0946] To a solution of Example 1.1.8 (2.2 g) in tetrahydrofuran (30 mL) was added di-tert-butyl dicarbonate (1.26 g) and a catalytic amount of 4-dimethylaminopyridine. The mixture was stirred at room temperature for 1.5 hours and then diluted with ethyl acetate (300 mL). The solution was washed with saturated aqueous NaHCO.sub.3, water (60 mL) and brine (60 mL). The organic layer was dried with Na.sub.2SO.sub.4, filtered and concentrated. The residue was purified by silica gel chromatography, eluting with 20% ethyl acetate in dichloromethane, to provide the title compound. MS (ESI) m/e 558.5 (M+H).sup.+.

[0947] 1.1.10. tert-butyl (2-((3,5-dimethyl-7-((5-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazol-1-yl)methyl)adamantan-1-yl)oxy)ethyl)(methyl)carbamate

[0948] To a solution of Example 1.1.9 (1.2 g) in dioxane was added bis(benzonitrile)palladium(II) chloride (0.04 g), 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (0.937 mL) and triethylamine (0.9 mL). The mixture was heated at reflux overnight, diluted with ethyl acetate and washed with water (60 mL) and brine (60 mL). The organic layer was dried over Na.sub.2SO.sub.4, filtered and concentrated to provide the title compound. MS (ESI) m/e 558.5 (M+H)+.

[0949] 1.1.11. tert-butyl 3-(1-((3-(2-((tert-butoxycarbonyl)(methyl)amino) ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-chloropicolinate

[0950] To Example 1.1.10 (100 mg) and tert-butyl 3-bromo-6-chloropicolinate (52.5 mg) in dioxane (2 mL) was added tris(dibenzylideneacetone)dipalladium(0) (8.2 mg), K.sub.3PO.sub.4 (114 mg), 1,3,5,7-tetramethyl-8-phenyl-2,4,6-trioxa-8-phosphaadamantane (5.24 mg) and water (0.8 mL). The mixture was stirred at 95° C. for 4 hours, diluted with ethyl acetate and washed with water and brine. The organic layer was dried over Na.sub.2SO.sub.4, filtered, concentrated and purified by flash chromatography, eluting with 20% ethyl acetate in heptanes and then with 5% methanol in dichloromethane, to provide the title compound. MS (ESI) m/e 643.3 (M+H).sup.+.

[0951] 1.1.12. tert-butyl 3-(1-((3-(2-((tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(1,2,3,4-tetrahydroquinolin-7-yl)picolinate

[0952] A mixture of Example 1.1.11 (480 mg), 7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2,3,4-tetrahydroquinoline (387 mg), dichlorobis(triphenylphosphine)-palladium(II) (78 mg) and CsF (340 mg) in dioxane (12 mL) and water (5 mL) was heated at 100° C. for 5 hours. After this time the reaction mixture was allowed to cool to room temperature and then diluted with ethyl acetate. The resulting mixture was washed with water and brine, and the organic layer was dried over Na.sub.2SO.sub.4, filtered, and concentrated. The residue was purified by flash

chromatography, eluting with 50% ethyl acetate in heptanes to provide the title compound. MS (APCI) m/e 740.4 (M+H)+.

[0953] 1.1.13. tert-butyl 6-(1-(benzo[d]thiazol-2-ylcarbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl)-3-(1-((3-(2-((tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinate

[0954] To a solution of benzo[d]thiazol-2-amine (114 mg) in acetonitrile (5 mL) was added bis(2,5-dioxopyrrolidin-1-yl) carbonate (194 mg). The mixture was stirred for 1 hour, and Example 1.1.12 (432 mg) in acetonitrile (5 mL) was added. The mixture was stirred overnight, diluted with ethyl acetate, washed with water and brine, and the organic layer was dried over Na.sub.2SO.sub.4, filtered, and concentrated. The residue was purified by flash chromatography, eluting with 50% ethyl acetate in heptanes to provide the title compound.

[0955] 1.1.14. 6-(1-(benzo[d]thiazol-2-ylcarbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl)-3-(1-((3,5-dimethyl-7-(2-(methylamino)ethoxy)adamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid

[0956] Example 1.1.13 (200 mg) in dichloromethane (5 mL) was treated with trifluoroacetic acid (2.5 mL) overnight. The mixture was concentrated to provide the title compound. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 8.40 (s, 1H), 8.30 (s, 2H), 8.02 (d, 1H), 7.85 (d, 1H), 7.74-7.83 (m, 2H), 7.42-7.53 (m, 2H), 7.38 (t, 1H), 7.30 (d, 1H), 7.23 (t, 1H), 3.93-4.05 (m, 2H), 3.52-3.62 (m, 2H), 2.97-3.10 (m, 2H), 2.84 (t, 2H), 2.56 (t, 2H), 2.23 (s, 3H), 1.88-2.00 (m, 2H), 1.45 (s, 2H), 1.25-1.39 (m, 4H), 1.12-1.22 (m, 4H), 1.00-1.09 (m, 2H), 0.89 (s, 6H). MS (ESI) m/e 760.1 (M+H).sup.+.

[0957] 1.2. Synthesis of 6-[4-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydro-2H-1,4-benzoxazin-6-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid (Compound W3.02)

[0958] 1.2.1. tert-butyl 3-(1-(((3-(2-((tert-butoxycarbonyl)(methyl) amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)picolinate

[0959] To a solution of 6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,4-dihydro-2H-benzo[b][1,4]oxazine (122 mg) in dioxane (4 mL) and water (1 mL) was added Example 1.1.11 (300 mg), bis(triphenylphosphine)palladium(II) dichloride (32.7 mg), and CsF (212 mg). The mixture was stirred at reflux overnight. The mixture was diluted with ethyl acetate (500 mL) and washed with water, brine and dried over Na.sub.2SO.sub.4. Filtration and evaporation of the solvents gave crude material which was purified via column chromatography (20% ethyl acetate in heptane followed by 5% methanol in dichloromethane) to provide the title compound. MS (ESI) m/e 742.4 (M+H)+.

[0960] 1.2.2. 6-[4-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydro-2H-1,4-benzoxazin-6-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid

[0961] To an ambient suspension of bis(2,5-dioxopyrrolidin-1-yl) carbonate (70.4 mg) in acetonitrile (4 mL) was added benzo[d]thiazol-2-amine (41.3 mg) and the mixture was stirred for one hour. A solution of Example 1.2.1 (170 mg) in acetonitrile (1 mL) and water (10 mL) was added, and the suspension was stirred vigorously overnight. The mixture was diluted with ethyl acetate (500 mL) and washed with water, brine and dried over Na.sub.2SO.sub.4. Filtration and evaporation of the solvents afforded a residue which was loaded on a column and eluted with 20% ethyl acetate in heptane followed by 5% methanol in dichloromethane. The resultant material was treated with 20% TFA in dichloromethane overnight. After evaporation of the solvent, the residue was purified via HPLC (Gilson system, eluting with 10-85% acetonitrile in 0.1% TFA in water) to provide the title compound. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 8.76 (s, 1H), 8.24-8.46 (m, 2H), 7.97 (d, 1H), 7.70-7.89 (m, 3H), 7.47 (s, 1H), 7.35-7.47 (m, 2H), 7.24 (t, 1H), 7.02 (d, 1H), 4.32-4.42 (m, 3H), 4.14-4.23 (m, 3H), 3.90 (s, 3H), 3.57 (t, 3H), 2.93-3.11 (m, 2H), 2.57 (t, 3H), 2.23 (s, 3H), 1.46 (s, 2H), 1.24-1.39 (m, 4H), 0.98-1.25 (m, 5H), 0.89 (s, 6H).

MS (ESI) m/e 760.4 (M+H)+.

[0962] 1.3. Synthesis of 6-[4-(1,3-benzothiazol-2-ylcarbamoyl)-1-methyl-1,2,3,4-tetrahydroquinoxalin-6-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid (Compound W3.03)

[0963] 1.3.1. tert-butyl 3-(1-((3-(2-((tert-butoxycarbonyl)(methyl)amino) ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(1-methyl-1,2,3,4-tetrahydroquinoxalin-6-yl)picolinate

[0964] To a solution of 1-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2,3,4-tetrahydroquinoxaline (140 mg) in dioxane (4 mL) and water (1 mL) was added Example 1.1.11 (328 mg), bis(triphenylphosphine)palladium(II) dichloride (35.8 mg), and CsF (232 mg). The mixture was stirred at reflux overnight. The mixture was diluted with ethyl acetate (500 mL) and washed with water, brine and dried over Na.sub.2SO.sub.4. Filtration and evaporation of the solvent gave crude material which was purified via column chromatography, eluting with 20% ethyl acetate in heptane followed by 5% methanol in dichloromethane, to provide the title compound. MS (ESI) m/e 755.5 (M+H)+.

[0965] 1.3.2. 6-[4-(1,3-benzothiazol-2-ylcarbamoyl)-1-methyl-1,2,3,4-tetrahydroquinoxalin-6-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid

[0966] To an ambient suspension of bis(2,5-dioxopyrrolidin-1-yl) carbonate (307 mg) in acetonitrile (10 mL) was added benzo[d]thiazol-2-amine (180 mg) and the mixture was stirred for one hour. A solution of Example 1.3.1 (600 mg) in acetonitrile (3 mL) was added, and the suspension was vigorously stirred overnight. The mixture was diluted with ethyl acetate (500 mL) and washed with water and brine and dried over Na.sub.2SO.sub.4. Filtration and evaporation of the solvents afforded a residue which was loaded on a column and eluted with 20% ethyl acetate in heptane (1 L) followed by 5% methanol in dichloromethane. The resultant material was treated with 20% TFA in dichloromethane overnight. After evaporation of solvent, the residue was purified on an HPLC (Gilson system, eluting with 10-85% acetonitrile in 0.1% TFA in water) to give the title compound. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 8.17-8.44 (m, 3H), 7.90 (d, 1H), 7.68-7.84 (m, 3H), 7.45 (s, 2H), 7.37 (t, 1H), 7.22 (t, 1H), 6.83 (d, 1H), 3.96-4.12 (m, 2H), 3.89 (s, 3H), 3.57 (t, 2H), 3.44 (t, 2H), 2.93-3.09 (m, 4H), 2.56 (t, 3H), 2.21 (s, 3H), 1.45 (s, 2H), 1.25-1.39 (m, 4H), 0.99-1.22 (m, 7H), 0.89 (s, 6H). MS (ESI) m/e 760.4 (M+H).sup.+.

[0967] 1.4. Synthesis of 3-(1-{{3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl}-5-methyl-1H-pyrazol-4-yl)-6-[1-(1,3-benzothiazol-2-ylcarbamoyl)-5,6-dihydroimidazo[1,5-a]pyrazin-7(8H)-yl]pyridine-2-carboxylic acid (Compound W3.04)

[0968] 1.4.1. 2-((3-((4-iodo-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethanamine

[0969] A solution of Example 1.1.7 (4.5 g) in 7N ammonium in methanol (15 mL) was stirred at 100° C. for 20 minutes under microwave conditions (Biotage Initiator). The reaction mixture was concentrated under vacuum. The residue was diluted with ethyl acetate (400 mL) and washed with aqueous NaHCO.sub.3, water (60 mL) and brine (60 mL). The organic layer was dried (anhydrous Na.sub.2SO.sub.4), the solution was filtered and concentrated, and the residue was used in the next reaction without further purification. MS (ESI) m/e 444.2 (M+H).sup.+.

[0970] 1.4.2. tert-butyl (2-((3-((4-iodo-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)carbamate

[0971] To a solution of Example 1.4.1 (4.4 g) in tetrahydrofuran (100 mL) was added di-tert-butyl dicarbonate (2.6 g) and N,N-dimethyl-4-aminopyridine (100 mg). The mixture was stirred for 1.5 hours. The reaction mixture was diluted with ethyl acetate (300 mL) and washed with aqueous NaHCO.sub.3, water (60 mL) and brine (60 mL). After drying (anhydrous Na.sub.2SO.sub.4), the solution was filtered and concentrated, and the residue was purified by silica gel column

chromatography (20% ethyl acetate in dichloromethane) to give the title compound. MS (ESI) m/e 544.2 (M+H)+.

[0972] 1.4.3. 6-fluoro-3-bromopicolinic acid

[0973] A slurry of 6-amino-3-bromopicolinic acid (25 g) in 400 mL 1:1 dichloromethane/chloroform was added to nitrosonium tetrafluoroborate (18.2 g) in dichloromethane (100 mL) at 5° C. over 1 hour. The resulting mixture was stirred for another 30 minutes, warmed to 35° C., and stirred overnight. The reaction mixture was cooled to room temperature and adjusted to pH 4 with a NaH.sub.2PO.sub.4 solution. The resulting solution was extracted three times with dichloromethane, and the combined extracts were washed with brine, dried over sodium sulfate, filtered and concentrated to provide the title compound.

[0974] 1.4.4. Tert-butyl 3-bromo-6-fluoropicolinate

[0975] Para-toluenesulfonyl chloride (27.6 g) was added to a solution of Example 1.4.3 (14.5 g), pyridine (26.7 mL) and tert-butanol (80 mL) in dichloromethane (100 mL) at 0° C. The reaction was stirred for 15 minutes, warmed to room temperature, and stirred overnight. The solution was concentrated and partitioned between ethyl acetate and Na.sub.2CO.sub.3 solution. The layers were separated, and the aqueous layer was extracted with ethyl acetate. The organic layers were combined, rinsed with Na.sub.2CO.sub.3 solution and brine, dried over sodium sulfate, filtered, and concentrated to provide the title compound.

[0976] 1.4.5. Ethyl 7-(5-bromo-6-(tert-butoxycarbonyl)pyridin-2-yl)-5,6,7,8-tetrahydroimidazo[1,5-a]pyrazine-1-carboxylate

[0977] Ethyl 5,6,7,8-tetrahydroimidazo[1,5-a]pyrazine-1-carboxylate hydrochloride (692 mg) and Example 1.4.4 (750 mg) were dissolved in dimethyl sulfoxide (6 mL). N,N-Diisopropylethylamine (1.2 mL) was added, and the solution was heated at 50° C. for 16 hours. The solution was cooled, diluted with water (20 mL), and extracted with ethyl acetate (50 mL). The organic portion was washed with brine and dried on anhydrous sodium sulfate. The solution was concentrated and, upon standing for 16 hours, solid crystals formed. The crystals were washed with diethyl ether to yield the title compound. MS (ESI) m/e 451, 453 (M+H).sup.+, 395, 397 (M-tert-butyl).sup.+

[0978] 1.4.6. Ethyl 7-(6-(tert-butoxycarbonyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-yl)-5,6,7,8-tetrahydroimidazo[1,5-a]pyrazine-1-carboxylate

[0979] The title compound was prepared by substituting Example 1.4.5 for Example 1.1.9 in Example 1.1.10. MS (ESI) m/e 499 (M+H).sup.+, 443 (M-tert-butyl).sup.+, 529 (M+MeOH-H).sup.-.

[0980] 1.4.7. Ethyl 7-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-((tert-butoxycarbonyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-5,6,7,8-tetrahydroimidazo[1,5-a]pyrazine-1-carboxylate

[0981] Example 1.4.6 (136 mg) and Example 1.4.2 (148 mg) were dissolved in 1,4-dioxane (3 mL) and water (0.85 mL). Tripotassium phosphate (290 mg) was added, and the solution was degassed and flushed with nitrogen three times. Tris(dibenzylideneacetone)dipalladium(0) (13 mg) and 1,3,5,7-tetramethyl-8-tetradecyl-2,4,6-trioxa-8-phosphaadamantane (12 mg) were added. The solution was degassed, flushed with nitrogen once, and heated to 70° C. for 16 hours. The reaction was cooled and diluted with ethyl acetate (10 mL) and water (3 mL). The layers were separated, and the organic layer was washed with brine and dried on anhydrous sodium sulfate. After filtration, the filtrate was concentrated and purified by flash column chromatography on silica gel, eluting with 5% methanol in ethyl acetate. The solvent was removed under reduced pressure to give the title compound. MS (ESI) m/e 760 (M+H).sup.+, 758 (M-H).sup.-.

[0982] 1.4.8. 7-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-((tert-butoxycarbonyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-5,6,7,8-tetrahydroimidazo[1,5-a]pyrazine-1-carboxylic acid

[0983] Example 1.4.7 (200 mg) was dissolved in tetrahydrofuran (0.7 mL), methanol (0.35 mL), and water (0.35 mL). Lithium hydroxide monohydrate (21 mg) was added, and the solution was

stirred at room temperature for 16 hours. HCl (1M, 0.48 mL) was added and the water was removed by azeotroping twice with ethyl acetate (20 mL). The solvent was removed under reduced pressure, and the material was dried under vacuum. The material was dissolved in dichloromethane (5 mL) and ethyl acetate (1 mL) and dried over anhydrous sodium sulfate. After filtration, the solvent was removed under reduced pressure to give the title compound. MS (ESI) m/e 760 (M+H).sup.+, 758 (M-H).sup.-.

[0984] 1.4.9. Tert-butyl 6-(1-(benzo[d]thiazol-2-ylcarbamoyl)-5,6-dihydroimidazo[1,5-a]pyrazin-7(8H)-yl)-3-(1-((3-(2-((tert-butoxycarbonyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinate

[0985] Example 1.4.8 (160 mg) and benzo[d]thiazol-2-amine (35 mg) were dissolved in dichloromethane (1.5 mL). 1-Ethyl-3-[3-(dimethylamino)propyl]-carbodiimide hydrochloride (85 mg) and 4-(dimethylamino)pyridine (54 mg) were added, and the solution was stirred at room temperature for 16 hours. The material was purified by flash column chromatography on silica gel, eluting with 2.5-5% methanol in ethyl acetate. The solvent was removed under reduced pressure to give the title compound. MS (ESI) m/e 892 (M+H).sup.+, 890 (M-H).sup.-.

[0986] 1.4.10. 3-(1-{{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[1-(1,3-benzothiazol-2-ylcarbamoyl)-5,6-dihydroimidazo[1,5-a]pyrazin-7(8H)-yl]pyridine-2-carboxylic acid

[0987] The title compound was prepared by substituting Example 1.4.9 for Example 1.1.13 in Example 1.1.14. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 11.50 (bs, 1H), 8.21 (d, 1H), 7.98 (d, 1H), 7.93 (s, 1H), 7.76 (d, 1H), 7.66 (bs, 3H), 7.58 (d, 1H), 7.44 (t, 1H), 7.33 (s, 1H), 7.31 (t, 1H), 7.15 (d, 1H), 6.97 (d, 1H), 5.10 (s, 2H), 4.26 (m, 2H), 4.08 (t, 2H), 3.84 (s, 2H), 2.90 (m, 4H), 2.13 (s, 3H), 1.42 (s, 2H), 1.30 (q, 4H), 1.15 (m, 2H), 1.04 (q, 4H), 0.87 (s, 6H). MS (ESI) m/e 736 (M+H).sup.+, 734 (M-H).sup.-.

[0988] 1.5. Synthesis of 3-(1-{{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-hydroxy-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid (Compound W3.05)

[0989] 1.5.1. tert-butyldiphenyl(vinyl)silane

[0990] The title compound was prepared as described in J Org Chem, 70(4), 1467 (2005).

[0991] 1.5.2. 2-(tert-butyldiphenylsilyl)ethanol

[0992] Example 1.5.1 (8.2 g) was dissolved in tetrahydrofuran (30 mL), then a 0.5M solution of 9-borabicyclo[3.3.1]nonane in tetrahydrofuran (63 mL) was added and the reaction was stirred at room temperature for 2.5 hours. The reaction was warmed to 37° C., then 3.0N aqueous NaOH (11 mL) was added, followed by the very careful dropwise addition of 30% aqueous H.sub.2O.sub.2(11 mL). Once the peroxide addition was completed, the reaction was stirred for one hour, and water (200 mL) and diethyl ether (200 mL) were added. The organic layer was washed with brine and dried over sodium sulfate. After filtration and concentration, purification by silica gel chromatography, eluting with heptanes/ethyl acetate (3/1), gave the title compound.

[0993] 1.5.3. 5-(2-(tert-butyldiphenylsilyl)ethoxy)isoquinoline

[0994] Triphenylphosphine (262 mg) was dissolved in tetrahydrofuran (2 mL). Example 1.5.2 (285 mg), isoquinolin-5-ol (121 mg), and diisopropyl azodicarboxylate (203 mg) were added. The reaction was stirred at room temperature for 30 minutes, then more isoquinolin-5-ol (41 mg) was added and the reaction was stirred overnight. The reaction was then concentrated and purification by flash chromatography, eluting with heptanes/ethyl acetate (83/17), gave the title compound. MS (DCI) m/e 412.2 (M+H).sup.+,

[0995] 1.5.4. 8-bromo-5-(2-(tert-butyldiphenylsilyl)ethoxy)isoquinoline

[0996] Example 1.5.3 (6.2 g) was dissolved in acetic acid (40 mL), and sodium acetate (2.2 g) was added. A solution of bromine (0.70 mL) in acetic acid (13 mL) was added slowly. The reaction was stirred at room temperature overnight. The reaction was carefully added to 2M aqueous Na.sub.2CO.sub.3 and extracted with ethyl acetate. The organic layer was washed with brine and

dried over sodium sulfate. After filtration and concentration, purification by silica gel chromatography, eluting with heptanes/ethyl acetate (9/1), gave the title compound. MS (DCI) m/e 490.1, 492.1 (M+H).sup.+.

[0997] 1.5.5. 8-bromo-5-(2-(tert-butyldiphenylsilyl)ethoxy)-1,2,3,4-tetrahydroisoquinoline

[0998] Example 1.5.4 (4.46 g) was dissolved in methanol (45 mL). Sodium cyanoborohydride (2.0 g) was added followed by trifluoroborane etherate (4.0 mL, 31.6 mmol). The mixture was heated under reflux for two hours and then cooled to room temperature. Additional sodium cyanoborohydride (2.0 g) and trifluoroborane etherate (4.0 mL) were added, and the mixture was heated under reflux for two more hours. The reaction was cooled, then added to 1/1 water/2M aqueous Na.sub.2CO.sub.3 (150 mL). The mixture was extracted with dichloromethane (twice with 100 mL). The organic layer was dried over sodium sulfate. Filtration and concentration provided the title compound that was used in the next step with no further purification. MS (DCI) m/e 494.1, 496.1 (M+H).sup.+.

[0999] 1.5.6. tert-butyl 8-bromo-5-(2-(tert-butyldiphenylsilyl)ethoxy)-3,4-dihydroisoquinoline-2(1H)-carboxylate

[1000] Example 1.5.5 (3.9 g) was dissolved in dichloromethane (25 mL), and triethylamine (3.3 mL) and di-tert-butyl dicarbonate (1.9 g) were added. The reaction mixture was stirred at room temperature for three hours. The reaction was then concentrated and purified by flash chromatography, eluting with heptanes/ethyl acetate (96/4), to provide the title compound.

[1001] 1.5.7. 2-tert-butyl 8-methyl 5-(2-(tert-butyldiphenylsilyl)ethoxy)-3,4-dihydroisoquinoline-2,8(1H)-dicarboxylate

[1002] Example 1.5.6 (3.6 g) and [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) dichloromethane (0.025 g) were placed in a 250 mL SS pressure bottle, and methanol (10 mL) and triethylamine (0.469 mL) were added. After degassing the reactor with argon several times, the flask was charged with carbon monoxide and heated to 100° C. for 16 hours at 40 psi. The reaction mixture was cooled, concentrated, and purified by flash silica gel chromatography, eluting heptanes/ethyl acetate (88/12), to provide the title compound.

[1003] 1.5.8. methyl 5-(2-(tert-butyldiphenylsilyl)ethoxy)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1004] Example 1.5.7 (1.8 g) was dissolved in 4N HCl in dioxane (25 mL) and stirred at room temperature for 45 minutes. The reaction was then concentrated to provide the title compound as a hydrochloride salt. MS (DCI) m/e 474.2 (M+H).sup.+.

[1005] 1.5.9. methyl 2-(5-bromo-6-(tert-butoxycarbonyl)pyridin-2-yl)-5-(2-(tert-butyldiphenylsilyl)ethoxy)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1006] To a solution of Example 1.5.8 (1.6 g) and Example 1.4.4 (1.0 g) in dimethyl sulfoxide (6 mL) was added N,N-diisopropylethylamine (1.4 mL). The mixture was stirred at 50° C. for 24 hours. The mixture was then diluted with diethyl ether and washed with water and brine, and dried over Na.sub.2SO.sub.4. Filtration and evaporation of the solvent and silica gel column purification (eluting with 5% ethyl acetate in hexane) gave the title compound.

[1007] 1.5.10. 1-((3-(2-azidoethoxy)-5,7-dimethyladamantan-1-yl)methyl)-4-iodo-5-methyl-1H-pyrazole

[1008] Example 1.1.6 (2 g) was dissolved in dichloromethane (20 mL), and triethylamine (0.84 mL) was added. After cooling the reaction solution to 5° C., mesyl chloride (0.46 mL) was added dropwise. The cooling bath was removed and the reaction was stirred at room temperature for two hours. Saturated NaHCO.sub.3 was added, the layers were separated, and the organic layer was washed with brine, and dried over Na.sub.2SO.sub.4. After filtration and concentration, the residue was dissolved in N,N dimethylformamide (15 mL) and sodium azide (0.88 g) was added, and the reaction was heated to 80° C. for two hours. The reaction was then cooled to room temperature and poured into diethyl ether and water. The organic layer was separated and washed with brine and dried over Na.sub.2SO.sub.4. After filtration and concentration, purification by silica gel

chromatography, eluting with heptanes/ethyl acetate (4/1), gave the title compound. MS (DCI) m/e 470.0 (M+H).sup.+.

[1009] 1.5.11. methyl 2-(6-(tert-butoxycarbonyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-yl)-5-(2-(tert-butyldiphenylsilyl)ethoxy)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate
[1010] Example 1.5.9 (1.5 g), 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (0.46 mL), [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) dichloromethane (86 mg), and triethylamine (0.59 mL) were dissolved in acetonitrile (6.5 mL) under a nitrogen atmosphere, then the reaction was heated under reflux overnight. The reaction was then cooled to room temperature and ethyl acetate and water were added. The organic layer was washed with brine and dried over Na.sub.2SO.sub.4. After filtration and concentration, purification by silica gel chromatography, using a gradient of 10-20% ethyl acetate in heptanes, gave the title compound. MS (ESI) m/e 777.1 (M+H).sup.+.

[1011] 1.5.12. methyl 2-(5-(1-((3-(2-azidoethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(tert-butoxycarbonyl)pyridin-2-yl)-5-(2-(tert-butyldiphenylsilyl)ethoxy)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1012] Example 1.5.11 (1.22 g) and Example 1.5.10 (0.74 g) were dissolved in tetrahydrofuran (16 mL) under a nitrogen atmosphere, and tripotassium phosphate (4.5 g) and water (5 mL) were added. Tris(dibenzylideneacetone)dipalladium(0) (70 mg) and 1,3,5,7-tetramethyl-8-tetradecyl-2,4,6-trioxa-8-phosphaadamantane (66 mg) were then added, the reaction was heated at reflux overnight, and then allowed to cool to room temperature. Ethyl acetate and water were then added, and the organic layer washed with brine and dried over Na.sub.2SO.sub.4. After filtration and concentration, the crude material was purified by silica gel chromatography, eluting with heptanes/ethyl acetate (7/3), gave the title compound. MS (DCI) m/e 992.3 (M+H).sup.+.

[1013] 1.5.13. 2-(5-(1-((3-(2-azidoethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(tert-butoxycarbonyl)pyridin-2-yl)-5-(2-(tert-butyldiphenylsilyl)ethoxy)-1,2,3,4-tetrahydroisoquinoline-8-carboxylic acid

[1014] Example 1.5.12 (1.15 g) was dissolved in tetrahydrofuran (4.5 mL), and methanol (2.2 mL), water (2.2 mL), and lithium hydroxide monohydrate (96 mg) were added. The reaction mixture was stirred at room temperature for five days. Water (20 mL) and 2N aqueous HCl (1.1 mL) were added. The mixture was extracted with ethyl acetate, and the organic layer was washed with brine and dried over Na.sub.2SO.sub.4. After filtration and concentration, purification by silica gel chromatography, eluting with dichloromethane/ethyl acetate (70/30) followed by dichloromethane/ethyl acetate/acetic acid (70/30/1), gave the title compound.

[1015] 1.5.14. tert-butyl 3-(1-((3-(2-azidoethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbonyl)-5-(2-(tert-butyldiphenylsilyl)ethoxy)-3,4-dihydroisoquinolin-2(1H)-yl)picolinate

[1016] Example 1.5.13 (80 mg) and benzo[d]thiazol-2-amine (14 mg) were dissolved in dichloromethane (1.2 mL). N,N-Dimethylpyridin-4-amine (17 mg) and N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide hydrochloride (27 mg) were added and the reaction was stirred at room temperature overnight. The reaction was concentrated and the crude residue was purified by silica gel chromatography, eluting with dichloromethane/ethyl acetate (90/10), to provide the title compound. MS (ESI) m/e 1110.3 (M+H).sup.+.

[1017] 1.5.15. tert-butyl 3-(1-((3-(2-azidoethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbonyl)-5-hydroxy-3,4-dihydroisoquinolin-2(1H)-yl)picolinate

[1018] Example 1.5.14 (160 mg) was dissolved in a 1.0M solution of tetrabutylammonium fluoride in 95/5 tetrahydrofuran/water (1.15 mL) and the reaction was heated at 60° C. for two days. Powdered 4A molecular sieves were added, and the mixture was heated at 60° C. for another day. The reaction was cooled, then concentrated and the crude residue was purified by silica gel chromatography, eluting with 70/30/1 dichloromethane/ethyl acetate/acetic acid, to provide the title

compound. MS (ESI) m/e 844.2 (M+H).sup.+.

[1019] 1.5.16. tert-butyl 3-(1-((3-(2-aminoethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-5-hydroxy-3,4-dihydroisoquinolin-2(1H)-yl)picolinate

[1020] Example 1.5.15 (70 mg) was dissolved in tetrahydrofuran (2 mL), 10% palladium on carbon (20 mg) was added, and the mixture was stirred under a hydrogen balloon overnight. After filtration through diatomaceous earth and evaporation of the solvent, the crude title compound was purified by reverse phase chromatography (C18 column), eluting with 10-90% acetonitrile in 0.1% TFA water, to provide the title compound as a trifluoroacetic acid salt.

[1021] 1.5.17. 3-(1-((3-(2-aminoethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-5-hydroxy-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

[1022] Example 1.5.16 (11 mg) was dissolved in 4N HCl in dioxane (0.5 mL) and stirred at room temperature overnight. The solids were filtered off and washed with dioxane to provide the title compound as a hydrochloride salt. .sup.1H NMR (500 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 12.60 (v br s, 1H), 10.40 (br s, 1H), 8.00 (d, 1H) 7.76 (d, 1H), 7.75 (br s, 3H), 7.60 (d, 1H), 7.51 (d, 1H), 7.46 (t, 1H), 7.33 (t, 1H), 7.30 (s, 1H), 6.98 (d, 1H), 6.82 (d, 1H), 4.99 (s, 2H), 3.89 (m, 2H), 3.83 (s, 2H), 3.50 (m, 2H), 2.88 (m, 2H), 2.79 (m, 2H), 2.11 (s, 3H), 1.41 (s, 2H), 1.29 (m, 4H), 1.14 (m, 4H), 1.04 (m, 2H), 0.87 (s, 6H). MS (ESI) m/e 762.2 (M+H).sup.+.

[1023] 1.6. Synthesis of 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)naphthalen-2-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid (Compound W3.06)

[1024] 1.6.1. tert-butyl 3-(1-((3-(2-((tert-butoxycarbonyl)(methyl)amino) ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(methoxycarbonyl)naphthalen-2-yl)picolinate

[1025] To a solution of methyl 7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-naphthoate (2.47 g) in dioxane (40 mL) and water (20 mL) was added Example 1.1.11 (4.2 g), bis(triphenylphosphine)palladium(II) dichloride (556 mg), and CsF (3.61 g). The mixture was stirred at reflux overnight. The mixture was diluted with ethyl acetate (400 mL) and washed with water and brine, and dried over Na.sub.2SO.sub.4. After filtration and evaporation of the solvent, the crude material was purified via column chromatography, eluting with 20% ethyl acetate in heptane followed by 5% methanol in dichloromethane, to provide the title compound. MS (ESI) m/e 793.4 (M+H).sup.+.

[1026] 1.6.2. 7-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-((tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-1-naphthoic acid

[1027] To a solution of Example 1.6.1 (500 mg) in tetrahydrofuran (4 mL), methanol (2 mL) and water (2 mL) was added lithium hydroxide monohydrate (500 mg). The mixture was stirred for 3 hours. The mixture was then acidified with 1N aqueous HCl and diluted with ethyl acetate (200 mL). The organic layer was washed with water and brine, and dried over Na.sub.2SO.sub.4. Filtration and evaporation of the solvent gave the crude title compound which was used in the next reaction without further purification. MS (ESI) m/e 779.4 (M+H).sup.+.

[1028] 1.6.3. 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)naphthalen-2-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid

[1029] To a solution of Example 1.6.2 (79 mg) in N,N-dimethylformamide (2 mL) was added benzo[d]thiazol-2-amine (23 mg), fluoro-N,N,N',N'-tetramethylformamidinium hexafluorophosphate (41 mg) and N,N-diisopropylethylamine (150 mg). The mixture was stirred at 60° C. for 3 hours. The reaction mixture was diluted with ethyl acetate (200 mL) and washed with water and brine, and dried over Na.sub.2SO.sub.4. Filtration and evaporation of the solvent gave a

crude intermediate which was dissolved in dichloromethane/TFA (1:1, 6 mL) and left to sit overnight. Evaporation of the solvent gave a residue which was dissolved in dimethyl sulfoxide/methanol (1:1, 9 mL) and purified by HPLC (Gilson system, eluting with 10-85% acetonitrile in 0.1% TFA in water) to give the pure title compound. ¹H NMR (501 MHz, dimethyl sulfoxide-d₆) δ ppm 13.11 (s, 1H), 9.02 (s, 1H), 8.38 (dd, 1H), 8.26-8.34 (m, 2H), 8.13-8.27 (m, 3H), 8.07 (d, 1H), 8.02 (d, 1H), 7.93 (d, 1H), 7.82 (d, 1H), 7.67-7.75 (m, 1H), 7.44-7.53 (m, 2H), 7.30-7.41 (m, 1H), 3.90 (s, 3H), 2.94-3.12 (m, 3H), 2.53-2.60 (m, 4H), 2.20-2.31 (m, 3H), 1.45 (s, 2H), 1.25-1.39 (m, 4H), 0.99-1.23 (m, 4H), 0.89 (s, 6H). MS (ESI) m/e 755.4 (M+H).⁺

[1030] 1.7. Synthesis of 3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.^{sup}.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]-6-[8-([1,3]thiazolo[5,4-b]pyridin-2-ylcarbamoyl)naphthalen-2-yl]pyridine-2-carboxylic acid (Compound W3.07)

[1031] The title compound was prepared by substituting thiazolo[5,4-b]pyridin-2-amine for benzo[d]thiazol-2-amine in Example 1.6.3. ¹H NMR (400 MHz, dimethyl sulfoxide-d₆) δ ppm 13.25 (s, 1H), 9.02 (s, 1H), 8.54 (dd, 1H), 8.39 (dd, 1H), 8.14-8.35 (m, 6H), 8.04 (d, 1H), 7.93 (d, 1H), 7.66-7.75 (m, 1H), 7.55 (dd, 1H), 7.49 (s, 1H), 3.57 (t, 3H), 2.95-3.10 (m, 2H), 2.51-2.62 (m, 3H), 2.19-2.28 (m, 3H), 1.45 (s, 2H), 1.24-1.38 (m, 4H), 0.98-1.24 (m, 6H), 0.89 (s, 6H). MS (ESI) m/e 756.3 (M+H).⁺

[1032] 1.8. Synthesis of 3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.^{sup}.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]-6-[8-([1,3]thiazolo[4,5-b]pyridin-2-ylcarbamoyl)naphthalen-2-yl]pyridine-2-carboxylic acid (Compound W3.08)

[1033] The title compound was prepared by substituting thiazolo[4,5-c]pyridin-2-amine for benzo[d]thiazol-2-amine in Example 1.6.3. ¹H NMR (501 MHz, dimethyl sulfoxide-d₆) δ ppm 13.40 (s, 1H), 9.04 (s, 1H), 8.62 (dd, 1H), 8.56 (dd, 1H), 8.39 (dd, 1H), 8.13-8.34 (m, 5H), 8.06 (d, 1H), 7.94 (d, 1H), 7.68-7.79 (m, 1H), 7.45-7.54 (m, 1H), 7.39 (dd, 1H), 3.90 (s, 3H), 3.54-3.60 (m, 3H), 2.94-3.08 (m, 2H), 2.51-2.60 (m, 4H), 2.18-2.31 (m, 3H), 1.46 (s, 2H), 1.24-1.40 (m, 4H), 1.01-1.21 (m, 6H), 0.83-0.89 (m, 5H). MS (ESI) m/e 756.3 (M+H).⁺

[1034] 1.9. Synthesis of 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.^{sup}.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid (Compound W3.09)

[1035] 1.9.1. tert-butyl 8-bromo-5-hydroxy-3,4-dihydroisoquinoline-2(1H)-carboxylate

[1036] To a solution of tert-butyl 5-hydroxy-3,4-dihydroisoquinoline-2(1H)-carboxylate (9 g) in N,N-dimethylformamide (150 mL) was added N-bromosuccinimide (6.43 g). The mixture was stirred overnight and quenched with water (200 mL). The mixture was diluted with ethyl acetate (500 mL) and washed with water and brine, and dried over sodium sulfate. Filtration and evaporation of the solvent gave crude title compound which was used in the next reaction without further purification. MS(ESI) m/e 329.2 (M+H).⁺

[1037] 1.9.2. tert-butyl 5-(benzyloxy)-8-bromo-3,4-dihydroisoquinoline-2(1H)-carboxylate

[1038] To a solution of Example 1.9.1 (11.8 g) in acetone (200 mL) was added benzyl bromide (7.42 g) and K₂CO₃ (5 g). The mixture was stirred at reflux overnight. The mixture was concentrated and the residue was partitioned between ethyl acetate (600 mL) and water (200 mL). The organic layer was washed with water and brine, and dried over sodium sulfate. Filtration and evaporation of the solvent gave crude title compound which was purified on a silica gel column and eluted with 10% ethyl acetate in heptane to provide the title compound. MS (ESI) m/e 418.1 (M+H).⁺

[1039] 1.9.3. 2-tert-butyl 8-methyl 5-(benzyloxy)-3,4-dihydroisoquinoline-2,8(1H)-dicarboxylate

[1040] Methanol (100 mL) and triethylamine (9.15 mL) were added to Example 1.9.2 (10.8 g) and [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (0.48 g) in a 500 mL stainless steel

pressure reactor. The vessel was sparged with argon several times. The reactor was pressurized with carbon monoxide and stirred for 2 hours at 100° C. under 60 psi of carbon monoxide. After cooling, the crude reaction mixture was concentrated under vacuum. The residue was partitioned between ethyl acetate (500 mL) and water (200 mL). The organic layer was further washed with water and brine, and dried over sodium sulfate. After filtration and evaporation of the solvent, the residue was purified on a 330 g silica gel column, eluting with 10-20% ethyl acetate in heptane, to provide the title compound. MS (ESI) m/e 398.1 (M+H).sup.+.

[1041] 1.9.4. methyl 5-(benzyloxy)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate hydrochloride

[1042] To a solution of Example 1.9.3 (3.78 g) in tetrahydrofuran (20 mL) was added 4N HCl in dioxane (20 mL). The mixture was stirred overnight and the mixture was concentrated under vacuum and the crude title compound was used in the next reaction without further purification. MS (ESI) m/e 298.1 (M+H).sup.+.

[1043] 1.9.5. methyl 5-(benzyloxy)-2-(5-bromo-6-(tert-butoxycarbonyl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1044] To a solution of Example 1.9.4 (3.03 g) in dimethyl sulfoxide (50 mL) was added Example 1.4.4 (2.52 g) and triethylamine (3.8 mL). The mixture was stirred at 60° C. overnight under nitrogen. The reaction mixture was diluted with ethyl acetate (500 mL) and washed with water and brine, and dried over sodium sulfate. After filtration and evaporation of the solvent, the crude material was purified on a silica gel column, eluting with 20% ethyl acetate in heptane, to give the title compound. MS (ESI) m/e 553.1 (M+H).sup.+.

[1045] 1.9.6. methyl 5-(benzyloxy)-2-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-((tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1046] To a solution of Example 1.9.5 (2.58 g) in tetrahydrofuran (40 mL) and water (20 mL) was added Example 1.1.10 (2.66 g), 1,3,5,7-tetramethyl-6-phenyl-2,4,8-trioxo-6-phosphaadamante (341 mg), tris(dibenzylideneacetone)dipalladium(0) (214 mg), and K.sub.3PO.sub.4 (4.95 g). The mixture was stirred at reflux for 4 hours. The mixture was diluted with ethyl acetate (500 mL) and washed with water and brine, and dried over sodium sulfate. After filtration and evaporation of the solvent, the crude material was purified on a silica gel column, eluting with 20% ethyl acetate in dichloromethane, to give the title compound. MS (ESI) m/e 904.5 (M+H).sup.+.

[1047] 1.9.7. methyl 2-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-((tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-5-hydroxy-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1048] Example 1.9.6 (3.0 g) in tetrahydrofuran (60 mL) was added to Pd(OH).sub.2 (0.6 g, Degussa #E101NE/W, 20% on carbon, 49% water content) in a 250 mL SS pressure bottle. The mixture was agitated for 16 hours under 30 psi of hydrogen gas at 50° C. The mixture was then filtered through a nylon membrane, and the solvent concentrated under vacuum to provide the title compound. MS (ESI) m/e 815.1(M+H).sup.+.

[1049] 1.9.8. methyl 2-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-((tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-5-methoxy-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1050] Example 1.9.7 (170 mg) was dissolved in dichloromethane (0.8 mL) and methanol (0.2 mL). To the mixture was added a 2.0 M solution of (trimethylsilyl)diazomethane in diethyl ether (0.17 mL) and the reaction was stirred at room temperature overnight. Additional 2.0 M (trimethylsilyl)diazomethane in diethyl ether (0.10 mL) was added, and the reaction was allowed to stir for 24 hours. The reaction mixture was then concentrated and the title compound was used without further purification. MS (ESI) m/e 828.2 (M+H).sup.+.

[1051] 1.9.9. 2-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-((tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-5-methoxy-1,2,3,4-tetrahydroisoquinoline-8-carboxylic acid

[1052] The title compound was prepared by substituting Example 1.9.8 for Example 1.5.12 in Example 1.5.13. MS (ESI) m/e 814.1 (M+H).sup.+.

[1053] 1.9.10. tert-butyl 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-((3-(2-((tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinate

[1054] The title compound was prepared by substituting Example 1.9.9 for Example 1.5.13 in Example 1.5.14. MS (ESI) m/e 946.1 (M+H).sup.+.

[1055] 1.9.11. 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-((3,5-dimethyl-7-(2-(methylamino)ethoxy)adamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid

[1056] The title compound was prepared by substituting Example 1.9.10 for Example 1.5.16 in Example 1.5.17. .sup.1H NMR (500 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 8.74 (br s, 2H), 8.02 (d, 1H) 7.77 (m, 2H), 7.54 (d, 1H), 7.47 (t, 1H), 7.34 (m, 2H), 7.01 (d, 2H), 5.01 (s, 2H), 3.90 (m, 2H), 3.89 (s, 3H), 3.85 (s, 2H), 3.58 (m, 2H), 3.57 (s, 3H), 2.98 (m, 2H), 2.82 (m, 2H), 2.12 (s, 3H), 1.41 (s, 2H), 1.30 (m, 4H), 1.14 (m, 4H), 1.04 (m, 2H), 0.87 (s, 6H). MS (ESI) m/e 790.2 (M+H).sup.+.

[1057] 1.10. Synthesis of 6-[5-(1,3-benzothiazol-2-ylcarbamoyl)quinolin-3-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid (Compound W3.10)

[1058] 1.10.1. 3-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-((tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)quinoline-5-carboxylic acid

[1059] A mixture of 3-bromoquinoline-5-carboxylic acid (300 mg), 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (363 mg), and potassium acetate (350 mg) in dioxane (5 mL) was purged with nitrogen gas for 5 minutes, and PdCl.sub.2(dppf)-CH.sub.2Cl.sub.2 adduct (58.3 mg) was added. The mixture was heated at 100° C. overnight and cooled. To this mixture was added Example 1.1.11 (510 mg), dichlorobis(triphenylphosphine)-palladium(II) (83 mg), CsF (362 mg), and water (3 mL). The resulting mixture was heated at 100° C. overnight and filtered through diatomaceous earth. The filtrate was concentrated, and the residue was dissolved in dimethyl sulfoxide, loaded onto a C18 column (300 g), and eluted with a gradient of 50-100% acetonitrile in a 0.1% TFA/water solution to provide the title compound. MS (ESI) m/e 780.5 (M+H).sup.+.

[1060] 1.10.2. tert-butyl 6-(5-(benzo[d]thiazol-2-ylcarbamoyl)quinolin-3-yl)-3-(1-((3-(2-((tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinate

[1061] To a mixture of Example 1.10.1 (120 mg), benzo[d]thiazol-2-amine (46.2 mg), and O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU, 117 mg) in N,N-dimethylformamide (0.5 mL) was added N,N-diisopropylethylamine (134 μ L). The mixture was stirred overnight and loaded onto a C18 column (300 g), eluting with a gradient of 50-100% acetonitrile in 0.1% TFA/water solution to provide the title compound. MS (ESI) m/e 913.4 (M+H).sup.+.

[1062] 1.10.3. 6-[5-(1,3-benzothiazol-2-ylcarbamoyl)quinolin-3-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid

[1063] Example 1.10.2 (50 mg) in dichloromethane (3 mL) was treated with trifluoroacetic acid (2 mL) overnight and concentrated. The residue was dissolved in a mixture of dimethyl sulfoxide (5 mL), loaded onto a C18 column (300 g), and eluted with a gradient of 10-70% acetonitrile in 0.1% TFA water solution to provide the title compound. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 13.22 (s, 1H), 9.73 (d, 1H), 9.41 (s, 1H), 8.34 (dd, 2H), 8.27 (s, 3H), 8.18 (d, 1H), 8.08 (d, 1H), 8.02-7.93 (m, 2H), 7.82 (d, 1H), 7.55-7.46 (m, 2H), 7.38 (t, 1H), 3.91 (s, 2H), 3.03 (p, 2H), 2.59-2.53 (m, 4H), 2.25 (s, 3H), 1.46 (s, 2H), 1.38-1.25 (m, 4H), 1.18 (s, 4H), 1.11-1.01 (m,

2H), 0.89 (s, 6H). MS (ESI) m/e 756.2 (M+H).sup.+.

[1064] 1.11. Synthesis of 6-[4-(1,3-benzothiazol-2-ylcarbamoyl)quinolin-6-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid (Compound W3.11)

[1065] 1.11.1. ethyl 6-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-((tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)quinoline-4-carboxylate

[1066] The title compound was prepared as described in Example 1.10.1, replacing 3-bromoquinoline-5-carboxylic acid with ethyl 6-bromoquinoline-4-carboxylate. MS (ESI) m/e 808.4 (M+H).sup.+.

[1067] 1.11.2. 6-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-((tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)quinoline-4-carboxylic acid

[1068] To a solution of Example 1.11.1 (100 mg) in dimethyl sulfoxide (2 mL) was added methanol (2 mL) and 1M lithium hydroxide (248 µL). The mixture was stirred for 30 minutes, acidified to pH 4 with 10% HCl, diluted with ethyl acetate and washed with water and brine to provide the title compound. MS (ESI) m/e 780.4 (M+H).sup.+.

[1069] 1.11.3. tert-butyl 6-(4-(benzo[d]thiazol-2-ylcarbamoyl)quinolin-6-yl)-3-(1-((3-(2-((tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinate

[1070] The title compound was prepared as described in Example 1.10.2, replacing Example 1.10.1 with Example 1.11.2. MS (ESI) m/e 912.3 (M+H).sup.+.

[1071] 1.11.4. 6-[4-(1,3-benzothiazol-2-ylcarbamoyl)quinolin-6-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid

[1072] The title compound was prepared as described in Example 1.10.3, replacing Example 1.10.2 with Example 1.11.3. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 13.34 (s, 2H), 9.14 (d, 1H), 8.94 (s, 1H), 8.63 (dd, 1H), 8.27 (dd, 4H), 8.09 (d, 1H), 8.00-7.90 (m, 2H), 7.83 (d, 1H), 7.50 (d, 2H), 7.40 (t, 1H), 3.90 (s, 2H), 3.03 (p, 2H), 2.56 (t, 4H), 2.23 (s, 3H), 1.45 (s, 2H), 1.32 (d, 3H), 1.18 (s, 4H), 1.11-0.98 (m, 2H), 0.89 (s, 6H). MS (ESI) m/e 756.2 (M+H).sup.+.

[1073] 1.12. Synthesis of 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{2-[(2-methoxyethyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid (Compound W3.12)

[1074] 1.12.1. methyl 5-(benzyloxy)-2-(6-(tert-butoxycarbonyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1075] The title compound was prepared by substituting Example 1.9.5 for Example 1.5.9 in Example 1.5.11. MS (DCI) m/e 601.0 (M+H).sup.+.

[1076] 1.12.2. 2-((3-((4-iodo-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)acetaldehyde

[1077] Dimethyl sulfoxide (4.8 mL) was dissolved in dichloromethane (150 mL). The mixture was cooled to -75° C., and oxalyl chloride (2.6 mL) was added dropwise. The reaction mixture was stirred at -75° C. for 45 minutes, and a solution of Example 1.1.6 (7.1 g) in dichloromethane (45 mL) was added dropwise. The reaction mixture was stirred at -75° C. for 30 minutes, and triethylamine (5.0 mL) was added. The reaction was warmed to room temperature, poured into water, and extracted with diethyl ether. The organic layer was washed with brine and dried over Na.sub.2SO.sub.4. After filtration and concentration, purification by silica gel chromatography, eluting with dichloromethane/ethyl acetate 85/15, gave the title compound. MS (DCI) m/e 443.0 (M+H).sup.+.

[1078] 1.12.3. 2-((3-((4-iodo-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-

yl)oxy)-N-(2-methoxyethyl)ethanamine

[1079] Example 1.12.2 (4.0 g) and 2-methoxyethanamine (0.90 mL) were dissolved in dichloromethane (40 mL) and the mixture was stirred at room temperature for two hours. A suspension of sodium borohydride (500 mg) in methanol (7 mL) was added and the resulting mixture was stirred for 45 minutes. The reaction was then added to saturated aqueous NaHCO₃ and resultant mixture extracted with ethyl acetate. The organic layer was washed with brine and dried over Na₂SO₄. The title compound was obtained after filtration and concentration and was used without purification. MS (DCI) m/e 502.1 (M+H)⁺.

[1080] 1.12.4. tert-butyl (2-((3-((4-iodo-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)oxy)ethyl)(2-methoxyethyl)carbamate

[1081] Example 1.12.3 (4.4 g) was dissolved in tetrahydrofuran (60 mL), and di-tert-butyl dicarbonate (3.0 g) and N,N-dimethylpyridin-4-amine (0.15 g) were added. The reaction was stirred at room temperature overnight. The reaction was then concentrated and purified by flash chromatography, eluting with dichloromethane/ethyl acetate (3/1), to provide the title compound.

[1082] 1.12.5. methyl 5-(benzyloxy)-2-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-((tert-butoxycarbonyl)(2-methoxyethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1083] The title compound was prepared by substituting Example 1.12.1 for Example 1.5.11 and Example 1.12.4 for Example 1.5.10 in Example 1.5.12. MS (ESI) m/e 948.2 (M+H)⁺.

[1084] 1.12.6. methyl 2-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-((tert-butoxycarbonyl)(2-methoxyethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-5-hydroxy-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1085] Example 1.12.5 (5.2 g) was dissolved in tetrahydrofuran (100 mL). 20% Palladium hydroxide on activated charcoal (1.0 g) was then added, and the reaction mixture agitated on a Parr reactor under a hydrogen atmosphere at 30 psi and 50° C. for 3 hours. After filtration and concentration, purification by silica gel chromatography, eluting with heptanes/ethyl acetate (2/3), gave the title compound. MS (ESI) m/e 858.1 (M+H)⁺.

[1086] 1.12.7. methyl 2-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-((tert-butoxycarbonyl)(2-methoxyethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-5-methoxy-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1087] The title compound was prepared by substituting Example 1.12.6 for Example 1.9.7 in Example 1.9.8. MS (ESI) m/e 872.2 (M+H)⁺.

[1088] 1.12.8. 2-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-((tert-butoxycarbonyl)(2-methoxyethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-5-methoxy-1,2,3,4-tetrahydroisoquinoline-8-carboxylic acid

[1089] The title compound was prepared by substituting Example 1.12.7 for Example 1.5.12 in Example 1.5.13. MS (ESI) m/e 858.1 (M+H)⁺.

[1090] 1.12.9. tert-butyl 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-((3-(2-((tert-butoxycarbonyl)(2-methoxyethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinate

[1091] The title compound was prepared by substituting Example 1.12.8 for Example 1.5.13 in Example 1.5.14. MS (ESI) m/e 990.1 (M+H)⁺.

[1092] 1.12.10. 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-(((1R,3S,5R,7S)-3-(2-((2-methoxyethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid

[1093] Example 1.12.9 (2.6 g) was dissolved in dioxane (20 mL), then 4N HCl in dioxane (100 mL) was added, and the reaction was stirred at room temperature overnight. The precipitants were allowed to settle and the supernatant was drawn off. The remaining solids were purified by reverse phase chromatography (C18 column), eluting with 10-90% acetonitrile in 0.1% TFA/water, to provide the title compound as a trifluoroacetic acid salt. ¹H NMR (500 MHz, dimethyl

sulfoxide-d.sub.6) δ ppm 8.41 (v br s, 2H), 8.01 (d, 1H) 7.77 (m, 2H), 7.50 (d, 1H), 7.47 (m, 1H), 7.34 (t, 1H), 7.29 (s, 1H), 7.01 (dd, 2H), 5.00 (s, 2H), 3.90 (m, 2H), 3.89 (s, 3H), 3.83 (s, 2H), 3.56 (m, 4H), 3.29 (s, 3H), 3.12 (m, 2H), 3.05 (m, 2H), 2.81 (m, 2H), 2.11 (s, 3H), 1.41 (s, 2H), 1.30 (m, 4H), 1.14 (m, 4H), 1.04 (m, 2H), 0.87 (s, 6H). MS (ESI) m/e 834.3 (M+H).sup.+.

[1094] 1.13. Synthesis of 3-(1-([3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-cyano-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid (Compound W3.13)

[1095] 1.13.1. 4-Bromo-3-cyanomethyl-benzoic acid methyl ester

[1096] Trimethylsilanecarbonitrile (3.59 mL) was added to tetrahydrofuran (6 mL). 1M Tetrabutylammonium fluoride (26.8 mL) was added dropwise over 30 minutes. The solution was then stirred at room temperature for 30 minutes. Methyl 4-bromo-3-(bromomethyl)benzoate (7.50 g) was dissolved in acetonitrile (30 mL) and the resultant solution added to the first solution dropwise over 30 minutes. The solution was then heated to 80° C. for 30 minutes and then allowed to cool to room temperature. The solution was concentrated under reduced pressure and purified by flash column chromatography on silica gel, eluting with 20-30% ethyl acetate in heptanes. The solvent was evaporated under reduced pressure to provide the title compound.

[1097] 1.13.2. 3-(2-Aminoethyl)-4-bromobenzoic acid methyl ester

[1098] Example 1.13.1 (5.69 g) was dissolved in tetrahydrofuran (135 mL), and 1 M borane (in tetrahydrofuran, 24.6 mL) was added. The solution was stirred at room temperature for 16 hours and then slowly quenched with methanol and 1M HCL. 4M HCL (150 mL) was added, and the solution was stirred at room temperature for 16 hours. The mixture was concentrated under reduced pressure, and the pH adjusted to between 11 and 12 using solid potassium carbonate. The solution was then extracted with dichloromethane (3×100 mL). The organic extracts were combined and dried over anhydrous sodium sulfate. The solution was filtered and concentrated under reduced pressure, and the material was purified by flash column chromatography on silica gel, eluting with 10-20% methanol in dichloromethane. The solvent was evaporated under reduced pressure to provide the title compound. MS (ESI) m/e 258, 260 (M+H).sup.+.

[1099] 1.13.3. 4-Bromo-3-[2-(2,2,2-trifluoroacetyl-amino)-ethyl]-benzoic acid methyl ester

[1100] Example 1.13.2 (3.21 g) was dissolved in dichloromethane (60 mL). The solution was cooled to 0° C., and triethylamine (2.1 mL) was added. Trifluoroacetic anhydride (2.6 mL) was then added dropwise. The solution was stirred at 0° C. for ten minutes and then allowed to warm to room temperature while stirring for one hour. Water (50 mL) was added and the solution was diluted with ethyl acetate (100 mL). 1M HCL was added (50 mL) and the organic layer was separated, washed with 1M HCL, and then washed with brine. The organic layer was then dried on anhydrous sodium sulfate. After filtration, the solvent was evaporated under reduced pressure to provide the title compound. MS (ESI) m/e 371, 373 (M+H).sup.+.

[1101] 1.13.4. 5-Bromo-2-(2,2,2-trifluoroacetyl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylic acid methyl ester

[1102] Example 1.13.3 (4.40 g) and paraformaldehyde (1.865 g) were placed in a flask and concentrated sulfuric acid (32 mL) was added. The solution was stirred at room temperature for one hour. Cold water (120 mL) was added. The solution was extracted with ethyl acetate (3×100 mL). The extracts were combined, washed with saturated aqueous sodium bicarbonate (100 mL), washed with water (100 mL), and dried over anhydrous sodium sulfate. The solution was concentrated under reduced pressure, and the material was purified by flash column chromatography on silica gel, eluting with 20-30% ethyl acetate in heptanes. The solvent was evaporated under reduced pressure to provide the title compound. MS (ESI) m/e 366, 368 (M+H).sup.+.

[1103] 1.13.5. 5-Cyano-2-(2,2,2-trifluoroacetyl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylic acid methyl ester

[1104] Example 1.13.4 (500 mg) and dicyanozinc (88 mg) were added to N,N-dimethylformamide (4 mL). The solution was degassed and flushed with nitrogen three times.

Tetrakis(triphenylphosphine)palladium(0) (79 mg) was added, and the solution was degassed and flushed with nitrogen once. The solution was then stirred at 80° C. for 16 hours. The solution was cooled, diluted with 50% ethyl acetate in heptanes (20 mL), and washed with 1 M hydrochloric acid (15 mL) twice. The organic layer was washed with brine and dried over anhydrous sodium sulfate. The solution was filtered and concentrated under reduced pressure, and the material was purified by flash column chromatography on silica gel, eluting with 20-30% ethyl acetate in heptanes. The solvent was evaporated under reduced pressure to provide the title compound.

[1105] 1.13.6. 5-Cyano-1,2,3,4-tetrahydroisoquinoline-8-carboxylic acid methyl ester

[1106] Example 1.13.5 (2.00 g) was dissolved in methanol (18 mL) and tetrahydrofuran (18 mL). Water (9 mL) was added followed by potassium carbonate (1.064 g). The reaction was stirred at room temperature for 135 minutes and then diluted with ethyl acetate (100 mL). The solution was washed with saturated aqueous sodium bicarbonate and dried on anhydrous sodium sulfate. The solvent was filtered and evaporated under reduced pressure to provide the title compound. MS (ESI) m/e 217 (M+H).sup.+.

[1107] 1.13.7. 2-(5-Bromo-6-tert-butoxycarbonylpyridin-2-yl)-5-cyano-1,2,3,4-tetrahydroisoquinoline-8-carboxylic acid methyl ester

[1108] Example 1.13.6 (1.424 g) and Example 1.4.4 (1.827 g) were dissolved in dimethyl sulfoxide (13 mL). N,N-Diisopropylethylamine (1.73 mL) was added, and the solution was heated to 50° C. for 16 hours. Additional Example 1.4.4 (0.600 g) was added, and the solution was heated at 50° C. for another 16 hours. The solution was allowed to cool to room temperature, diluted with ethyl acetate (50 mL), washed with water (25 mL) twice, washed with brine, and then dried on anhydrous sodium sulfate. The solution was filtered and concentrated under reduced pressure, and the material was purified by flash column chromatography on silica gel, eluting with 20-50% ethyl acetate in heptanes. The solvent was evaporated under reduced pressure to provide the title compound. MS (ESI) m/e 472, 474 (M+H).sup.+.

[1109] 1.13.8. 2-[6-tert-Butoxycarbonyl-5-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-pyridin-2-yl]-5-cyano-1,2,3,4-tetrahydroisoquinoline-8-carboxylic acid methyl ester

[1110] Example 1.13.7 (2.267 g) and triethylamine (1.34 mL) were added to acetonitrile (15 mL). The solution was degassed and flushed with nitrogen three times. 4,4,5,5-Tetramethyl-1,3,2-dioxaborolane (1.05 mL) was added followed by dichloro[1,1'-bis(diphenylphosphino)ferrocene]palladium(II) (196 mg). The solution was degassed and flushed with nitrogen once and heated to reflux for 16 hours. The solution was cooled, diluted with ethyl acetate (50 mL), washed with water (10 mL), washed with brine, and dried on anhydrous sodium sulfate. The solution was concentrated under reduced pressure, and the material was purified by flash column chromatography on silica gel, eluting with 20-30% ethyl acetate in heptanes. The solvent was evaporated under reduced pressure to provide the title compound. MS (ESI) m/e 520 (M+H).sup.+.

[1111] 1.13.9. 2-(6-tert-Butoxycarbonyl-5-{1-[5-(2-tert-butoxycarbonylamino-ethoxy)-3,7-dimethyl-adamantan-1-ylmethyl]-5-methyl-1H-pyrazol-4-yl}-pyridin-2-yl)-5-cyano-1,2,3,4-tetrahydro-isoquinoline-8-carboxylic acid methyl ester

[1112] Example 1.13.8 (140 mg) and Example 1.4.2 (146 mg) were dissolved in tetrahydrofuran (3 mL). Potassium phosphate (286 mg) and water (0.85 mL) were added. The solution was degassed and flushed with nitrogen three times. (1S,3R,5R,7S)-1,3,5,7-Tetramethyl-8-tetradecyl-2,4,6-trioxa-8-phosphaadamantane (11 mg) and tris(dibenzylideneacetone)dipalladium(0) (12 mg) were added, and the solution was degassed and flushed with nitrogen once. The solution was heated to 62° C. for 16 hours. The solution was cooled, then diluted with water (5 mL) and ethyl acetate (25 mL). The organic layer was separated and washed with brine and dried on anhydrous sodium sulfate. The solution was filtered and concentrated under reduced pressure, and the material was purified by flash column chromatography on silica gel, eluting with 30-50% ethyl acetate in heptanes. The solvent was evaporated under reduced pressure to provide the title compound. MS

(ESI) m/e 809 (M+H).sup.+.

[1113] 1.13.10.2-(6-tert-Butoxycarbonyl-5-{1-[5-(2-tert-butoxycarbonylamino-ethoxy)-3,7-dimethyl-adamantan-1-ylmethyl]-5-methyl-1H-pyrazol-4-yl}-pyridin-2-yl)-5-cyano-1,2,3,4-tetrahydro-isoquinoline-8-carboxylic acid

[1114] Example 1.13.9 (114 mg) was dissolved in tetrahydrofuran (0.7 mL) and methanol (0.35 mL). Water (0.35 mL) was added followed by lithium hydroxide monohydrate (11 mg). The solution was stirred at room temperature for 16 hours, and 1 M hydrochloric acid (0.27 mL) was added. Water (1 mL) was added and the solution was extracted with ethyl acetate (5 mL) three times. The extracts were combined and dried on anhydrous sodium sulfate and filtered. The solvent was evaporated under reduced pressure to provide the title compound. MS (ESI) m/e 795 (M+H).sup.+.

[1115] 1.13.11.6-[8-(Benzothiazol-2-ylcarbamoyl)-5-cyano-3,4-dihydro-1H-isoquinolin-2-yl]-3-{1-[5-(2-tert-butoxycarbonylamino-ethoxy)-3,7-dimethyl-adamantan-1-ylmethyl]-5-methyl-1H-pyrazol-4-yl}-pyridine-2-carboxylic acid tert-butyl ester

[1116] Example 1.13.10 (89 mg) and benzo[d]thiazol-2-amine (18 mg) were dissolved in dichloromethane (1.2 mL). N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (39 mg) and N,N-dimethylpyridin-4-amine (25 mg) were added, and the solution was stirred at room temperature for 16 hours. The material was purified by flash column chromatography on silica gel, eluting with 50% ethyl acetate in heptanes. The solvent was evaporated under reduced pressure to provide the title compound. MS (ESI) m/e 927 (M+H).sup.+.

[1117] 1.13.12.3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-cyano-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid

[1118] Example 1.13.11 (44 mg) was dissolved in dichloromethane (1 mL). Trifluoroacetic acid (0.144 mL) was added and the solution stirred at room temperature for 16 hours. The solvents were then evaporated under reduced pressure, the residue was dissolved in dichloromethane (1 mL), and the solvent removed under reduced pressure. Diethyl ether was added (2 mL) and was removed under reduced pressure. Diethyl ether (2 mL) was added again and removed under reduced pressure to provide the title compound as the trifluoroacetic acid salt. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 8.52 (bs, 1H), 8.05 (d, 1H), 7.92 (d, 1H), 7.82-7.75 (m, 2H), 7.63 (m, 2H), 7.50 (dd, 2H), 7.42-7.28 (m, 3H), 7.16 (t, 1H), 7.04 (d, 1H), 4.98 (s, 2H), 3.96 (t, 2H), 3.83 (s, 2H), 3.49 (t, 2H), 3.15 (t, 2H), 2.90 (q, 2H), 2.10 (s, 3H), 1.41 (s, 2H), 1.35-1.22 (m, 4H), 1.18-0.99 (m, 6H), 0.87 (bs, 6H). MS (ESI) m/e 771 (M+H).sup.+.

[1119] 1.14. Synthesis of 6-[1-(1,3-benzothiazol-2-ylcarbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl]-3-{1-[3-(2-[(2-methoxyethyl)amino]ethoxy)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid (Compound W3.14)

[1120] 1.14.1. 2-((3,5-dimethyl-7-((5-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazol-1-yl)methyl)adamantan-1-yl)oxy)ethanol

[1121] To a solution of Example 1.1.6 (4.45 g) and PdCl.sub.2(dppf)-CH.sub.2Cl.sub.2 adduct (409 mg) in acetonitrile (60 mL) was added triethylamine (5 mL) and pinacolborane (6.4 mL). The mixture was refluxed overnight. The mixture was used directly in the next step without work up. MS (ESI) m/e 444.80 (M+H).sup.+.

[1122] 1.14.2. tert-butyl 6-chloro-3-(1-((3-(2-hydroxyethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinate

[1123] To a solution of tert-butyl 3-bromo-6-chloropicolinate (3.06 g) in tetrahydrofuran (50 mL) and water (20 mL) was added Example 1.14.1 (4.45 g), 1,3,5,7-tetramethyl-8-tetradecyl-2,4,6-trioxa-8-phosphaadamantane (0.732 g), Pd.sub.2(dba).sub.3 (0.479 g), and K.sub.3PO.sub.4 (11 g). The mixture was stirred at reflux overnight and concentrated. The residue was dissolved in ethyl acetate (500 mL) and washed with water and brine. The organic layer was dried over Na.sub.2SO.sub.4, filtered, and concentrated. The residue was purified by flash chromatography,

eluting with a gradient of 20-40% ethyl acetate in dichloromethane, to provide the title compound. MS (ESI) m/e 530.23 (M+H).sup.+.

[1124] 1.14.3. tert-butyl 6-chloro-3-(1-((3,5-dimethyl-7-(2-((methylsulfonyl)oxy)ethoxy)adamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinate

[1125] To a cooled (0° C.) stirring solution of Example 1.14.2 (3.88 g) in dichloromethane (30 mL) and triethylamine (6 mL) was added methanesulfonyl chloride (2.52 g). The mixture was stirred at room temperature for 4 hours, diluted with ethyl acetate (400 mL), and washed with water and brine. The organic layer was dried over Na.sub.2SO.sub.4. Filtration and evaporation of the solvents afforded the title compound. MS (ESI) m/e 608.20 (M+H).sup.+.

[1126] 1.14.4. tert-butyl 3-(1-((3-(2-aminoethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-chloropicolinate

[1127] A solution of Example 1.14.3 (2.2 g) in 7N ammonium in CH.sub.3OH (20 mL) was heated at 100° C. under microwave conditions (Biotage Initiator) for 45 minutes and concentrated to dryness. The residue was dissolved in ethyl acetate and washed with water and brine. The organic layer was dried over Na.sub.2SO.sub.4, filtered, and concentrated to provide the title compound. MS (ESI) m/e 529.33 (M+H).sup.+.

[1128] 1.14.5. tert-butyl 6-chloro-3-(1-((3,5-dimethyl-7-(2-(2-(trimethylsilyl)ethylsulfonamido)ethoxy)adamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinate

[1129] To a cooled (0° C.) solution of Example 1.14.4 (3.0 g) in dichloromethane (30 mL) was added triethylamine (3 mL), followed by 2-(trimethylsilyl)ethanesulfonyl chloride (2.3 g). The mixture was stirred at room temperature for 3 hours and concentrated to dryness. The residue was dissolved in ethyl acetate (400 mL) and washed with aqueous NaHCO.sub.3, water, and brine. The residue was dried over Na.sub.2SO.sub.4, filtered, concentrated, and purified by flash chromatography, eluting with 20% ethyl acetate in heptane, to provide the title compound. MS (ESI) m/e 693.04 (M+H).sup.+.

[1130] 1.14.6. tert-butyl 6-chloro-3-(1-((3-(2-(N-(2-methoxyethyl)-2-(trimethylsilyl)ethylsulfonamido)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinate

[1131] To a solution of Example 1.14.5 (415 mg) in toluene (15 mL) was added 2-methoxyethanol (91 mg), followed by cyanomethylenetriethylphosphorane (289 mg). The mixture was stirred at 70° C. for 3 hours and concentrated to dryness. The residue was purified by flash chromatography, eluting with 20% ethyl acetate in heptane, to provide the title compound. MS (ESI) m/e 751.04 (M+H).sup.+.

[1132] 1.14.7. tert-butyl 3-(1-((3-(2-(N-(2-methoxyethyl)-2-(trimethylsilyl)ethylsulfonamido)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(1,2,3,4-tetrahydroquinolin-7-yl)picolinate

[1133] To a solution of 7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2,3,4-tetrahydroquinoline (172 mg) in dioxane (10 mL) and water (5 mL) was added Example 1.14.6 (500 mg), (Ph.sub.3P).sub.2PdCl.sub.2 (45.6 mg) and CsF (296 mg). The mixture was stirred at 120° C. for 30 minutes under microwave conditions (Biotage Initiator), diluted with ethyl acetate (200 mL) and washed with water and brine. The organic layer was dried over Na.sub.2SO.sub.4, filtered, and concentrated. The residue was purified by flash chromatography, eluting with 20% ethyl acetate in dichloromethane, to provide the title compound. MS (ESI) m/e 848.09 (M+H).sup.+.

[1134] 1.14.8. tert-butyl 6-(1-(benzo[d]thiazol-2-ylcarbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl)-3-(1-((3-(2-(N-(2-methoxyethyl)-2-(trimethylsilyl)ethylsulfonamido)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinate

[1135] To a suspension of bis(2,5-dioxopyrrolidin-1-yl) carbonate (63 mg) in acetonitrile (10 mL) was added benzo[d]thiazol-2-amine (37.2 mg). The mixture was stirred for 1 hour. A solution of Example 1.14.7 (210 mg) in acetonitrile (2 mL) was added, and the suspension was vigorously

stirred overnight, diluted with ethyl acetate, and washed with water and brine. The organic layer was dried over Na.sub.2SO.sub.4, filtered, and concentrated to provide the title compound. MS (ESI) m/e 1024.50 (M+H).sup.+.

[1136] 1.14.9. 6-[1-(1,3-benzothiazol-2-ylcarbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl]-3-{1-[(3-{2-[(2-methoxyethyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid

[1137] To a solution of Example 1.14.8 (230 mg) in tetrahydrofuran (10 mL) was added tetrabutyl ammonium fluoride (TBAF 10 mL, 1M in tetrahydrofuran). The mixture was stirred at room temperature overnight, diluted with ethyl acetate, and washed with water and brine. The organic layer was dried over Na.sub.2SO.sub.4, filtered, and concentrated. The residue was dissolved in dichloromethane (5 mL) and treated with trifluoroacetic acid (5 mL) overnight. The mixture was concentrated, and the residue was purified by reverse HPLC (Gilson), eluting with 10-85% acetonitrile in 0.1% TFA/water to provide the title compound. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 8.40 (d, 3H), 8.00 (d, 1H), 7.90-7.72 (m, 3H), 7.46 (s, 1H), 7.40-7.32 (m, 1H), 7.28 (d, 1H), 7.24-7.17 (m, 1H), 3.95 (d, 3H), 3.88 (s, 16H), 3.56 (dt, 5H), 3.28 (s, 3H), 3.18-2.96 (m, 5H), 2.82 (t, 2H), 2.21 (s, 3H), 1.93 (p, 2H), 1.43 (s, 2H), 1.30 (q, 5H), 1.21-0.97 (m, 7H), 0.86 (s, 6H) MS (ESI) m/e 804.3 (M+H).sup.+.

[1138] 1.15. Synthesis of 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)naphthalen-2-yl]-3-{1-[(3-{2-[(2-methoxyethyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid (Compound W3.15)

[1139] 1.15.1. 7-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-(N-(2-methoxyethyl)-2-(trimethylsilyl)ethylsulfonamido)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-1-naphthoic acid

[1140] To a solution of methyl 7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-naphthoate (208 mg) in dioxane (10 mL) and water (5 mL) was added Example 1.14.6 (500 mg), (Ph.sub.3P).sub.2PdCl.sub.2 (45.6 mg) and CsF (296 mg). The mixture was stirred at 120° C. for 30 minutes under microwave conditions (Biotage Initiator), diluted with ethyl acetate and washed with water and brine. The organic layer was dried over Na.sub.2SO.sub.4, filtered, and concentrated. The residue was purified by flash chromatography, eluting with 20% ethyl acetate in dichloromethane, to give the ester intermediate. The ester was dissolved in a mixture of tetrahydrofuran (10 mL), methanol (5 mL) and H.sub.2O (5 mL) and treated with lithium hydroxide monohydrate (200 mg). The mixture was stirred at room temperature for 4 hours, acidified with 1N aqueous HCl solution and diluted with ethyl acetate (300 mL). After washing with water and brine, the organic layer was dried over Na.sub.2SO.sub.4. After filtration, evaporation of the solvent afforded the title compound. MS (ESI) m/e 888.20 (M+H).sup.+.

[1141] 1.15.2. 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)naphthalen-2-yl]-3-{1-[(3-{2-[(2-methoxyethyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid

[1142] To a solution of Example 1.15.1 (500 mg) in dichloromethane (10 mL) was added benzo[d]thiazol-2-amine (85 mg), 1-ethyl-3-[3-(dimethylamino)propyl]-carbodiimide hydrochloride (216 mg) and 4-(dimethylamino)pyridine (138 mg). The mixture was stirred at room temperature overnight, diluted with ethyl acetate, and washed with water and brine. The organic layer was then dried over Na.sub.2SO.sub.4, filtered, and concentrated to dryness. The residue was dissolved in tetrahydrofuran (10 mL) and treated with tetrabutyl ammonium fluoride (10 mL, 1M in tetrahydrofuran) overnight. The reaction mixture was diluted with ethyl acetate and washed with water and brine. The organic layer was dried over Na.sub.2SO.sub.4, filtered, and concentrated to dryness. The residue was dissolved in dichloromethane (5 mL) and treated with trifluoroacetic acid (5 mL) overnight. The mixture was then concentrated and the residue was purified by reverse HPLC (Gilson), eluting with 10-85% acetonitrile in 0.1% TFA in water, to give the title compound. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 13.11 (s, 1H), 9.00 (s, 1H), 8.60-8.29

(m, 3H), 8.26-8.13 (m, 3H), 8.03 (ddd, 2H), 7.92 (d, 1H), 7.80 (d, 1H), 7.74-7.62 (m, 1H), 7.51-7.42 (m, 2H), 7.36 (td, 1H), 3.88 (s, 2H), 3.61-3.52 (m, 2H), 3.27 (s, 3H), 3.17-2.95 (m, 4H), 2.22 (s, 3H), 1.43 (s, 2H), 1.30 (q, 4H), 1.23-0.96 (m, 6H), 0.86 (s, 6H). MS (ESI) m/e 799.2 (M+H).sup.+.

[1143] 1.16. Synthesis of 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-[1-({3,5-dimethyl-7-[2-(oxetan-3-ylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid (Compound W3.16)

[1144] 1.16.1. methyl 2-(5-bromo-6-(tert-butoxycarbonyl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1145] To a solution of methyl 1,2,3,4-tetrahydroisoquinoline-8-carboxylate hydrochloride (12.37 g) and Example 1.4.4 (15 g) in dimethyl sulfoxide (100 mL) was added N,N-diisopropylethylamine (12 mL). The mixture was stirred at 50° C. for 24 hours. The mixture was diluted with ethyl acetate (500 mL), washed with water and brine, and dried over Na.sub.2SO.sub.4. After filtration and evaporation of the solvent, the crude material was purified via silica gel column chromatography, eluting with 20% ethyl acetate in hexane, to give the title compound. MS (ESI) m/e 448.4 (M+H).sup.+.

[1146] 1.16.2. methyl 2-(6-(tert-butoxycarbonyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1147] To a solution of Example 1.16.1 (2.25 g) and [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (205 mg) in acetonitrile (30 mL) was added triethylamine (3 mL) and pinacolborane (2 mL). The mixture was stirred at reflux for 3 hours. The mixture was diluted with ethyl acetate (200 mL), washed with water and brine, and dried over Na.sub.2SO.sub.4. Filtration, evaporation of the solvent, and silica gel chromatography (eluting with 20% ethyl acetate in hexane) gave the title compound. MS (ESI) m/e 495.4 (M+H).sup.+.

[1148] 1.16.3. methyl 2-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-((tert-butoxycarbonyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1149] To a solution of Example 1.16.2 (4.94 g) in tetrahydrofuran (60 mL) and water (20 mL) was added Example 1.4.2 (5.57 g), 1,3,5,7-tetramethyl-8-tetradecyl-2,4,6-trioxa-8-phosphaadamantane (412 mg), tris(dibenzylideneacetone)dipalladium(0) (457 mg), and K.sub.3PO.sub.4 (11 g). The mixture was stirred at reflux overnight. The reaction mixture was diluted with ethyl acetate (500 mL), washed with water and brine, and dried over Na.sub.2SO.sub.4. After filtration and evaporation of the solvent, the crude material was purified via column chromatography, eluting with 20% ethyl acetate in heptane, to give the title compound. MS (ESI) m/e 784.4 (M+H).sup.+.

[1150] 1.16.4. 2-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-((tert-butoxycarbonyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylic acid

[1151] To a solution of Example 1.16.3 (10 g) in tetrahydrofuran (60 mL), methanol (30 mL) and water (30 mL), was added lithium hydroxide monohydrate (1.2 g). The mixture was stirred at room temperature for 24 hours. The reaction mixture was neutralized with 2% aqueous HCl and concentrated under vacuum. The residue was diluted with ethyl acetate (800 mL), washed with water and brine, and dried over Na.sub.2SO.sub.4. Filtration and evaporation of the solvent gave the title compound. MS (ESI) m/e 770.4 (M+H).sup.+.

[1152] 1.16.5. tert-butyl 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-((3-(2-((tert-butoxycarbonyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinate

[1153] To a solution of Example 1.16.4 (3.69 g) in N,N-dimethylformamide (20 mL) was added benzo[d]thiazol-2-amine (1.1 g), fluoro-N,N,N',N'-tetramethylformamidinium hexafluorophosphate (1.9 g) and N,N diisopropylethylamine (1.86 g). The mixture was stirred at 60° C. for 3 hours. The

reaction mixture was diluted with ethyl acetate (500 mL), washed with water and brine, and dried over Na.sub.2SO.sub.4. Filtration, evaporation of the solvent, and column purification (20% ethyl acetate in heptane) gave the title compound. MS (ESI) m/e 902.2(M+H).sup.+.

[1154] 1.16.6. 3-(1-((3-(2-aminoethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

[1155] Example 1.16.5 (2 g) was dissolved in 50% TFA in dichloromethane (20 mL) and stirred overnight. The solvents were removed under vacuum and the residue was loaded on a reverse-phase column and eluted with 20-80% acetonitrile in water (0.1% TFA) to give the title compound. MS (ESI) m/e 746.3 (M+H).sup.+.

[1156] 1.16.7. 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-[1-({3,5-dimethyl-7-[2-(oxetan-3-ylamino)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid

[1157] A solution of Example 1.16.6 (0.050 g), oxetan-3-one (5 mg) and sodium triacetoxymethylborohydride (0.018 g) was stirred together in dichloromethane (1 mL) at room temperature. After stirring for 1 hour, additional oxetan-3-one (5 mg) and sodium triacetoxymethylborohydride (0.018 g) were added and the reaction was stirred overnight. The reaction was concentrated, dissolved in a 1:1 mixture of dimethyl sulfoxide/methanol (2 mL) and purified by HPLC using a Gilson system (20-60% acetonitrile in water containing 0.1% v/v trifluoroacetic acid). The desired fractions were combined and freeze-dried to provide the title compound. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 12.95 (s, 1H), 9.26 (s, 2H), 8.12 (d, 1H), 7.88 (d, 1H), 7.71 (d, 1H), 7.63-7.50 (m, 3H), 7.50-7.41 (m, 2H), 7.38 (s, 1H), 7.05 (d, 1H), 5.05 (s, 2H), 4.79 (t, 2H), 4.68 (dd, 2H), 4.54-4.41 (m, 1H), 3.98 (t, 2H), 3.92 (s, 2H), 3.63 (t, 2H), 3.16-3.04 (m, 4H), 2.20 (s, 3H), 1.52 (s, 2H), 1.47-1.06 (m, 10H), 0.96 (s, 6H). MS (ESI) m/e 802.2 (M+H).sup.+.

[1158] 1.17. Synthesis of 6-[6-(3-aminopyrrolidin-1-yl)-8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{{3-(2-methoxyethoxy)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl}methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid (Compound W3.17)

[1159] 1.17.1. 4-iodo-1-((3-(2-methoxyethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazole

[1160] Example 1.1.6 (3.00 g) was dissolved in 1,4-dioxane (40 mL), and sodium hydride (60% in mineral oil, 568 mg) was added. The solution was mixed at room temperature for 15 minutes, and methyl iodide (1.64 mL) was added. The solution was stirred at room temperature for three days, and then 0.01 M aqueous HCl solution (50 mL) was added. The solution was extracted with diethyl ether three times. The combined organic extracts were washed with brine and dried on anhydrous sodium sulfate. After filtration, the solvent was removed under reduced pressure and then under high vacuum to yield the title compound. MS (ESI) m/e 459 (M+H).sup.+.

[1161] 1.17.2. benzyl 4-oxopent-2-ynoate

[1162] Benzyl 4-hydroxypent-2-ynoate (40.5 g) and Dess-Martin Periodinane (93.0 g) in dichloromethane (500 mL) were stirred for 1 hour at 0° C. The solution was poured into diethyl ether (1 L), and the combined organics were washed three times with 1M aqueous NaOH and brine, dried over Na.sub.2SO.sub.4, filtered, and concentrated. The residue was chromatographed on silica gel using 5% ethyl acetate in heptanes to give the title compound.

[1163] 1.17.3. (S)-benzyl 6-(3-((tert-butoxycarbonyl)amino)pyrrolidin-1-yl)-2-(2,2,2-trifluoroacetyl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1164] A solution of 1-(2,2,2-trifluoroacetyl)piperidin-4-one (6.29 g), (S)-tert-butyl pyrrolidin-3-ylcarbamate (6.0 g), and p-toluenesulfonic acid monohydrate (0.613 g) in ethanol (80 mL) was stirred for 1 hour at room temperature. Example 1.17.2 (6.51 g) was then added and the reaction was stirred for 24 hours at room temperature, and heated to 45° C. for 3 days. The reaction was then cooled and poured into diethyl ether (600 mL). The resulting solution was washed twice with

water and brine, dried over Na.sub.2SO.sub.4, filtered, and concentrated. The residue was chromatographed on silica gel using 5-50% ethyl acetate in heptanes to give the product.

[1165] 1.17.4. (S)-benzyl 6-(3-((tert-butoxycarbonyl)amino)pyrrolidin-1-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1166] A solution of Example 1.17.3 (3.1 g) and potassium carbonate (1.8 g) in a mixture of tetrahydrofuran (30 mL), methanol (10 mL), and water (25 mL) was stirred for 48 hours at 45° C. The reaction was then cooled and diluted with dichloromethane (300 mL). The layers were separated and the organic layer was dried over Na.sub.2SO.sub.4, filtered, and concentrated to give the title compound.

[1167] 1.17.5. (S)-benzyl 2-(5-bromo-6-(tert-butoxycarbonyl)pyridin-2-yl)-6-(3-((tert-butoxycarbonyl)amino)pyrrolidin-1-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1168] A solution of Example 1.17.4 (1.6 g), Example 1.4.4 (1.08 g), and triethylamine (0.59 mL) in N,N-dimethylformamide (10 mL) was heated to 50° C. for 24 hours. The reaction was cooled and poured into ethyl acetate (400 mL). The resulting solution was washed three times with water and brine, dried over Na.sub.2SO.sub.4, filtered, and concentrated. The residue was chromatographed on silica gel using 5-50% ethyl acetate in heptanes to give the product.

[1169] 1.17.6. (S)-benzyl 2-(6-(tert-butoxycarbonyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-yl)-6-(3-((tert-butoxycarbonyl)amino)pyrrolidin-1-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1170] A solution of Example 1.17.5 (500 mg), 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (136 mg), and triethylamine (0.200 mL) in acetonitrile (5 mL) was heated to 75° C. for 24 hours. The reaction was allowed to cool to room temperature and concentrated to dryness. The crude material was then purified via column chromatography, eluting with 5-50% ethyl acetate in heptanes, to give the title compound.

[1171] 1.17.7. benzyl 2-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-methoxyethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-6-((S)-3-((tert-butoxycarbonyl)amino)pyrrolidin-1-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1172] A solution of Example 1.17.6 (240 mg), Example 1.17.1 (146 mg), 1,3,5,7-tetramethyl-8-tetradecyl-2,4,6-trioxa-8-phosphaadamantane (13 mg), palladium (II)acetate (14.6 mg), and tripotassium phosphate (270 mg) in dioxane (7 mL) and water (3 mL) was heated to 70° C. for 24 hours. The reaction was allowed to cool to room temperature and was concentrated to dryness. The crude material was then purified via column chromatography, eluting with 5-25% ethyl acetate in heptanes, to give the title compound.

[1173] 1.17.8. 2-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-methoxyethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-6-((S)-3-((tert-butoxycarbonyl)amino)pyrrolidin-1-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylic acid

[1174] A solution of Example 1.17.7 (1.6 g) and lithium hydroxide monohydrate (5 mg) in a 3:1:1 mixture of tetrahydrofuran/methanol/water (10 mL) was stirred for 4 days. The reaction was acidified with 1M aqueous HCl solution and poured into ethyl acetate (150 mL). The resulting solution was washed with brine, dried over Na.sub.2SO.sub.4, filtered, and concentrated to give the title compound.

[1175] 1.17.9. tert-butyl 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-6-((S)-3-((tert-butoxycarbonyl)amino)pyrrolidin-1-yl)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-((3-(2-methoxyethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinate

[1176] A solution of Example 1.17.8 (78 mg), benzo[d]thiazol-2-amine (16 mg), O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (48 mg), and diisopropylethylamine (0.024 mL) in N,N-dimethylformamide (3 mL) was heated to 50° C. for 48 hours. The reaction was then cooled and poured into ethyl acetate (100 mL). The resulting solution was washed three times with water and brine, dried over Na.sub.2SO.sub.4, filtered, and concentrated. The residue was purified via column chromatography, eluting with 20-100% ethyl

acetate in heptanes, to give the title compound.

[1177] 1.17.10.6-[6-(3-aminopyrrolidin-1-yl)-8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[3-(2-methoxyethoxy)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid

[1178] Example 1.17.9 (40 mg) in dichloromethane (3 mL) was treated with trifluoroacetic acid (2 mL) overnight. The mixture was concentrated to provide the title compound as a TFA salt. MS (ESI) m/e 845.7 (M+H).sup.+.

[1179] 1.18. Synthesis of 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3,5-dimethyl-7-{2-[(2-sulfamoyl)ethyl]amino}ethoxy)tricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid (Compound W3.18) 1.18.1. 3-bromo-5,7-dimethyladamantanecarboxylic acid

[1180] Into a 50 mL round-bottomed flask at 0° C., was added bromine (16 mL). Iron powder (7 g) was added, and the reaction was stirred at 0° C. for 30 minutes. 3,5-Dimethyladamantane-1-carboxylic acid (12 g) was added. The mixture was warmed up to room temperature and stirred for 3 days. A mixture of ice and concentrated HCl was poured into the reaction mixture. The resulting suspension was treated twice with Na.sub.2SO.sub.3 (50 g in 200 mL water) and extracted three times with dichloromethane. The combined organics were washed with 1N aqueous HCl, dried over sodium sulfate, filtered, and concentrated to give the title compound.

[1181] 1.18.2. 3-bromo-5,7-dimethyladamantanemethanol

[1182] To a solution of Example 1.18.1 (15.4 g) in tetrahydrofuran (200 mL) was added BH.sub.3 (1M in tetrahydrofuran, 150 mL), and the mixture was stirred at room temperature overnight. The reaction mixture was then carefully quenched by adding methanol dropwise. The mixture was then concentrated under vacuum, and the residue was balanced between ethyl acetate (500 mL) and 2N aqueous HCl (100 mL). The aqueous layer was further extracted twice with ethyl acetate, and the combined organic extracts were washed with water and brine, dried over sodium sulfate, and filtered. Evaporation of the solvent gave the title compound.

[1183] 1.18.3. 1-((3-bromo-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl)-1H-pyrazole

[1184] To a solution of Example 1.18.2 (8.0 g) in toluene (60 mL) was added 1H-pyrazole (1.55 g) and cyanomethylenetriethylphosphorane (2.0 g), and the mixture was stirred at 90° C. overnight. The reaction mixture was concentrated, and the residue was purified by silica gel column chromatography (10:1 heptane:ethyl acetate) to give the title compound. MS (ESI) m/e 324.2 (M+H).sup.+.

[1185] 1.18.4. 2-{[3,5-dimethyl-7-(1H-pyrazol-1-yl)methyl]tricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy}ethanol

[1186] To a solution of Example 1.18.3 (4.0 g) in ethane-1,2-diol (12 mL) was added triethylamine (3 mL). The mixture was stirred at 150° C. under microwave conditions (Biotage Initiator) for 45 minutes. The mixture was poured into water (100 mL) and extracted three times with ethyl acetate. The combined organic extracts were washed with water and brine, dried over sodium sulfate, and filtered. Evaporation of the solvent gave a residue that was purified by silica gel chromatography, eluting with 20% ethyl acetate in heptane, followed by 5% methanol in dichloromethane, to give the title compound. MS (ESI) m/e 305.2 (M+H).sup.+.

[1187] 1.18.5. 2-({3,5-dimethyl-7-[(5-methyl-1H-pyrazol-1-yl)methyl]tricyclo[3.3.1.1.sup.3,7]dec-1-yl}oxy)ethanol

[1188] To a cooled (-78° C.) solution of Example 1.18.4 (6.05 g) in tetrahydrofuran (100 mL) was added n-BuLi (40 mL, 2.5M in hexane), and the mixture was stirred at -78° C. for 1.5 hours. Iodomethane (10 mL) was added through a syringe, and the mixture was stirred at -78° C. for 3 hours. The reaction mixture was then quenched with aqueous NH.sub.4Cl and extracted twice with ethyl acetate, and the combined organic extracts were washed with water and brine. After drying over sodium sulfate, the solution was filtered and concentrated, and the residue was purified by

silica gel column chromatography, eluting with 5% methanol in dichloromethane, to give the title compound. MS (ESI) m/e 319.5 (M+H).sup.+.

[1189] 1.18.6. 1-((3,5-dimethyl-7-[2-(hydroxy)ethoxy]tricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl)-4-iodo-5-methyl-1H-pyrazole

[1190] To a solution of Example 1.18.5 (3.5 g) in N,N-dimethylformamide (30 mL) was added N-iodosuccinimide (3.2 g), and the mixture was stirred at room temperature for 1.5 hours. The reaction mixture was diluted with ethyl acetate (600 mL) and washed with aqueous NaHSO₃, water and brine. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluting with 20% ethyl acetate in dichloromethane, to give the title compound. MS (ESI) m/e 445.3 (M+H).sup.+.

[1191] 1.18.7. 1-((3-(2-((tert-butyldimethylsilyl)oxy)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-4-iodo-5-methyl-1H-pyrazole

[1192] Tert-butyldimethylsilyl trifluoromethanesulfonate (5.34 mL) was added to a solution of Example 1.18.6 (8.6 g) and 2,6-lutidine (3.16 mL) in dichloromethane (125 mL) at -40° C., and the reaction was allowed to warm to room temperature overnight. The mixture was concentrated, and the residue was purified by silica gel chromatography, eluting with 5-20% ethyl acetate in heptanes, to give the title compound. MS (ESI) m/e 523.4 (M+H).sup.+.

[1193] 1.18.8. 1-((3-(2-((tert-butyldimethylsilyl)oxy)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole

[1194] n-Butyllithium (8.42 mL, 2.5M in hexanes) was added to Example 1.18.7 (9.8 g) in 120 mL tetrahydrofuran at -78° C., and the reaction was stirred for 1 minute. Trimethyl borate (3.92 mL) was added, and the reaction stirred for 5 minutes. Pinacol (6.22 g) was added, and the reaction was allowed to warm to room temperature and was stirred 2 hours. The reaction was quenched with pH 7 buffer, and the mixture was poured into ether. The layers were separated, and the organic layer was concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluting with 1-25% ethyl acetate in heptanes, to give the title compound.

[1195] 1.18.9. 6-fluoro-3-bromopicolinic acid

[1196] A slurry of 6-amino-3-bromopicolinic acid (25 g) in 400 mL 1:1 dichloromethane/chloroform was added to nitrosonium tetrafluoroborate (18.2 g) in dichloromethane (100 mL) at 5° C. over 1 hour. The resulting mixture was stirred for another 30 minutes, then warmed to 35° C. and stirred overnight. The reaction was cooled to room temperature, and then adjusted to pH 4 with aqueous NaH₂PO₄ solution. The resulting solution was extracted three times with dichloromethane, and the combined extracts were washed with brine, dried over sodium sulfate, filtered and concentrated to provide the title compound.

[1197] 1.18.10. Tert-butyl 3-bromo-6-fluoropicolinate

[1198] Para-toluenesulfonyl chloride (27.6 g) was added to a solution of Example 1.18.9 (14.5 g) and pyridine (26.7 mL) in dichloromethane (100 mL) and tert-butanol (80 mL) at 0° C. The reaction was stirred for 15 minutes, and then warmed to room temperature, and stirred overnight. The solution was concentrated and partitioned between ethyl acetate and aqueous Na₂CO₃ solution. The layers were separated, and the aqueous layer extracted with ethyl acetate. The organic layers were combined, rinsed with aqueous Na₂CO₃ solution and brine, dried over sodium sulfate, filtered, and concentrated to provide the title compound.

[1199] 1.18.11. methyl 2-(5-bromo-6-(tert-butoxycarbonyl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1200] To a solution of methyl 1,2,3,4-tetrahydroisoquinoline-8-carboxylate hydrochloride (12.37 g) and Example 1.18.10 (15 g) in dimethyl sulfoxide (100 mL) was added N,N-diisopropylethylamine (12 mL), and the mixture was stirred at 50° C. for 24 hours. The mixture was then diluted with ethyl acetate (500 mL) and washed with water and brine. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluting with 20% ethyl acetate in hexane, to give the title

compound. MS (ESI) m/e 448.4 (M+H).sup.+.

[1201] 1.18.12. methyl 2-(6-(tert-butoxycarbonyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1202] To a solution of Example 1.18.11 (2.25 g) and [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (205 mg) in acetonitrile (30 mL) was added triethylamine (3 mL) and pinacolborane (2 mL), and the mixture was stirred at reflux for 3 hours. The mixture was diluted with ethyl acetate (200 mL) and washed with water and brine. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification of the residue by silica gel chromatography, eluting with 20% ethyl acetate in hexane, provided the title compound.

[1203] 1.18.13. methyl 2-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-hydroxyethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1204] To a solution of Example 1.18.12 (2.25 g) in tetrahydrofuran (30 mL) and water (10 mL) was added Example 1.18.6 (2.0 g), 1,3,5,7-tetramethyl-6-phenyl-2,4,8-trioxa-6-phosphaadamantane (329 mg), tris(dibenzylideneacetone)dipalladium(0) (206 mg) and potassium phosphate tribasic (4.78 g). The mixture was refluxed overnight, cooled and diluted with ethyl acetate (500 mL). The resulting mixture was washed with water and brine, and the organic layer was dried over sodium sulfate, filtered and concentrated. The residue was purified by flash chromatography, eluting with 20% ethyl acetate in heptanes followed by 5% methanol in dichloromethane, to provide the title compound.

[1205] 1.18.14. methyl 2-(6-(tert-butoxycarbonyl)-5-(1-((3,5-dimethyl-7-(2-((methylsulfonyl)oxy)ethoxy)adamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1206] To a cold solution of Example 1.18.13 (3.32 g) in dichloromethane (100 mL) in an ice-bath was sequentially added triethylamine (3 mL) and methanesulfonyl chloride (1.1 g). The reaction mixture was stirred at room temperature for 1.5 hours and diluted with ethyl acetate, and washed with water and brine. The organic layer was dried over sodium sulfate, filtered, and concentrated to provide the title compound.

[1207] 1.18.15. methyl 2-(5-(1-((3-(2-azidoethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(tert-butoxycarbonyl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1208] To a solution of Example 1.18.14 (16.5 g) in N,N-dimethylformamide (120 mL) was added sodium azide (4.22 g). The mixture was heated at 80° C. for 3 hours, cooled, diluted with ethyl acetate and washed with water and brine. The organic layer was dried over sodium sulfate, filtered, and concentrated. The residue was purified by flash chromatography, eluting with 20% ethyl acetate in heptanes, to provide the title compound.

[1209] 1.18.16. 2-(5-(1-((3-(2-azidoethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(tert-butoxycarbonyl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylic acid

[1210] To a solution of Example 1.18.15 (10 g) in a mixture of tetrahydrofuran (60 mL), methanol (30 mL) and water (30 mL) was added lithium hydroxide monohydrate (1.2 g). The mixture was stirred at room temperature overnight and neutralized with 2% aqueous HCl. The resulting mixture was concentrated, and the residue was dissolved in ethyl acetate (800 mL), and washed with brine. The organic layer was dried over sodium sulfate, filtered, and concentrated to provide the title compound.

[1211] 1.18.17. tert-butyl 3-(1-((3-(2-azidoethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbonyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinate

[1212] A mixture of Example 1.18.16 (10 g), benzo[d]thiazol-2-amine (3.24 g), fluoro-N,N,N',N'-tetramethylformamidinium hexafluorophosphate (5.69 g) and N,N-diisopropylethylamine (5.57 g)

in N,N-dimethylformamide (20 mL) was heated at 60° C. for 3 hours, cooled and diluted with ethyl acetate. The resulting mixture was washed with water and brine. The organic layer was dried over sodium sulfate, filtered, and concentrated. The residue was purified by flash chromatography, eluting with 20% ethyl acetate in dichloromethane to give the title compound.

[1213] 1.18.18. tert-butyl 3-(1-(((3-(2-aminoethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinate

[1214] To a solution of Example 1.18.17 (2.0 g) in tetrahydrofuran (30 mL) was added Pd/C (10%, 200 mg). The mixture was stirred under a hydrogen atmosphere overnight. The insoluble material was filtered off and the filtrate was concentrated to provide the title compound.

[1215] 1.18.19. 3-(1-(((3-(2-aminoethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

[1216] Example 1.18.18 (200 mg) in dichloromethane (2.5 mL) was treated with trifluoroacetic acid (2.5 mL) overnight. The reaction mixture was concentrated, and the residue was purified by reverse phase chromatography (C18 column), eluting with 20-60% acetonitrile in water containing 0.1% v/v trifluoroacetic acid, to provide the title compound. MS (ESI) m/e 746.2 (M+H).sup.+.

[1217] 1.18.20. 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3,5-dimethyl-7-{2-[(2-sulfamoyl)ethyl]amino}ethoxy}tricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid

[1218] A mixture of Example 1.18.19 (18 mg) and ethenesulfonamide (5.2 mg) in N,N-dimethylformamide (1 mL) and water (0.3 mL) was stirred for one week. The mixture was purified by reverse phase chromatography (C18 column), eluting with 20-60% acetonitrile in water containing 0.1% v/v trifluoroacetic acid, to provide the title compound. .sup.1H NMR (500 MHz, dimethyl sulfoxide-d) δ ppm 8.03 (d, 1H), 7.79 (d, 1H), 7.61 (d, 1H), 7.45-7.50 (m, 1H), 7.41-7.44 (m, 1H), 7.33-7.39 (m, 3H), 7.23 (s, 1H), 6.73 (d, 1H), 4.87 (s, 2H), 3.89 (t, 2H), 3.79 (s, 2H), 3.12-3.20 (m, 2H), 2.99 (t, 2H), 2.85 (s, 2H), 2.09 (s, 3H), 1.32 (dd, 4H), 1.08-1.19 (m, 5H), 1.04 (d, 4H), 0.86 (s, 6H). MS (ESI) m/e 853.2(M+H).sup.+.

[1219] 1.19 Synthesis of 3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl)-6-[3-(1,3-benzothiazol-2-ylcarbamoyl)-6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl]pyridine-2-carboxylic acid (W3.19)

[1220] 1.19.1 6,7-dihydro-4H-thieno[3,2-c]pyridine-3,5-dicarboxylic acid 5-tert-butyl ester 3-methyl ester

[1221] Tert-butyl 3-bromo-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-carboxylate (1000 mg) and dichloro[1,1'-bis(diphenylphosphino)ferrocene]palladium(II) (69 mg) were placed in a 50 mL pressure bottle, and methanol (20 mL) was added, followed by trimethylamine (636 mg). The solution was degassed and flushed with argon three times. The solution was then degassed and flushed with carbon monoxide and heated to 100° C. for 18 hours under 60 psi of carbon monoxide. The solvent was removed under reduced pressure, and the residue was purified by flash column chromatography on silica gel, eluting with 50% ethyl acetate in heptanes. The solvent was removed under reduced pressure to yield the title compound.

[1222] 1.19.2 4,5,6,7-tetrahydro-thieno[3,2-c]pyridine-3-carboxylic acid methyl ester

[1223] Example 1.19.1 (940 mg) was dissolved in dichloromethane (12 mL). Trifluoroacetic acid (2220 mg) was added, and the solution was stirred for three hours. The solvent was removed under reduced pressure to yield the title compound as the trifluoroacetic acid salt, which was used without further purification.

[1224] 1.19.3 5-(5-bromo-6-tert-butoxycarbonyl-pyridin-2-yl)-4,5,6,7-tetrahydro-thieno[3,2-c]pyridine-3-carboxylic acid methyl ester

[1225] The title compound was prepared by substituting Example 1.19.2 for ethyl 5,6,7,8-tetrahydroimidazo[1,5-a]pyrazine-1-carboxylate hydrochloride in Example 1.4.5. MS (ESI) m/e 452, 450 (M+H).sup.+.

[1226] 1.19.4 5-[6-tert-butoxycarbonyl-5-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-pyridin-2-yl]-4,5,6,7-tetrahydro-thieno[3,2-c]pyridine-3-carboxylic acid methyl ester

[1227] The title compound was prepared by substituting Example 1.19.3 for Example 1.1.9 in Example 1.1.10. MS (ESI) m/e 500 (M+H).sup.+, 531 (M+CH.sub.3OH-H).sup.-.

[1228] 1.19.5 5-(6-tert-butoxycarbonyl-5-{1-[5-(2-tert-butoxycarbonylamino-ethoxy)-3,7-dimethyl-adamantan-1-ylmethyl]-5-methyl-1H-pyrazol-4-yl}-pyridin-2-yl)-4,5,6,7-tetrahydro-thieno[3,2-c]pyridine-3-carboxylic acid methyl ester

[1229] The title compound was prepared by substituting Example 1.19.4 for Example 1.4.6 in Example 1.4.7.

[1230] 1.19.6 5-(6-tert-butoxycarbonyl-5-{1-[5-(2-tert-butoxycarbonylamino-ethoxy)-3,7-dimethyl-adamantan-1-ylmethyl]-5-methyl-1H-pyrazol-4-yl}-pyridin-2-yl)-4,5,6,7-tetrahydro-thieno[3,2-c]pyridine-3-carboxylic acid

[1231] The title compound was prepared by substituting Example 1.19.5 for Example 1.4.7 in Example 1.4.8. MS (ESI) m/e 776 (M+H).sup.+, 774 (M-H).sup.-.

[1232] 1.19.7 6-[3-(benzothiazol-2-ylcarbamoyl)-6,7-dihydro-4H-thieno[3,2-c]pyridin-5-yl]-3-{1-[5-(2-tert-butoxycarbonylamino-ethoxy)-3,7-dimethyl-adamantan-1-ylmethyl]-5-methyl-1H-pyrazol-4-yl}-pyridine-2-carboxylic acid tert-butyl ester

[1233] The title compound was prepared by substituting Example 1.19.6 for Example 1.4.8 in Example 1.4.9. MS (ESI) m/e 892 (M+H).sup.+, 890 (M-H).sup.-.

[1234] 1.19.8 3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[3-(1,3-benzothiazol-2-ylcarbamoyl)-6,7-dihydrothieno[3,2-c]pyridin-5(4H)-yl]pyridine-2-carboxylic acid

[1235] The title compound was prepared by substituting Example 1.19.7 for Example 1.1.13 in Example 1.1.14. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 8.11 (bs, 1H), 8.00 (d, 1H), 7.77 (d, 1H), 7.68 (bs, 3H), 7.53 (d, 1H), 7.47 (t, 1H), 7.36-7.31 (m, 2H), 7.14 (d, 1H), 4.71 (s, 2H), 3.99 (t, 2H), 3.85 (s, 2H), 3.52 (m, 2H), 3.00 (t, 2H), 2.91 (q, 2H), 2.13 (s, 3H), 1.44 (s, 2H), 1.31 (q, 4H), 1.16 (m, 4H), 1.05 (q, 2H), 0.88 (s, 6H). MS (ESI) m/e 752 (M+H).sup.+, 750 (M-H).sup.-.

[1236] 1.20 Synthesis of 3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[1-(1,3-benzothiazol-2-ylcarbamoyl)-3-(trifluoromethyl)-5,6-dihydroimidazo[1,5-a]pyrazin-7(8H)-yl]pyridine-2-carboxylic acid (W3.20)

[1237] 1.20.1 7-(5-bromo-6-tert-butoxycarbonyl-pyridin-2-yl)-3-trifluoromethyl-5,6,7,8-tetrahydro-imidazo[1,5-a]pyrazine-1-carboxylic acid methyl ester

[1238] The title compound was prepared by substituting methyl 3-(trifluoromethyl)-5,6,7,8-tetrahydroimidazo[1,5-a]pyrazine-1-carboxylate for ethyl 5,6,7,8-tetrahydroimidazo[1,5-a]pyrazine-1-carboxylate hydrochloride in Example 1.4.5. MS (ESI) m/e 449 (M-tBu+H).sup.+, 503 (M-H).sup.-.

[1239] 1.20.2 7-[6-tert-butoxycarbonyl-5-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-pyridin-2-yl]-3-trifluoromethyl-5,6,7,8-tetrahydro-imidazo[1,5-a]pyrazine-1-carboxylic acid methyl ester

[1240] The title compound was prepared by substituting Example 1.20.1 for Example 1.1.9 in Example 1.1.10. MS (ESI) m/e 553 (M+H).sup.+,

[1241] 1.20.3 di-tert-butyl[2-({3-[(4-iodo-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]decan-1-yl}oxy)ethyl]-2-imidodicarbonate

[1242] Example 1.1.6 (5.000 g) was dissolved in dichloromethane (50 mL). Triethylamine (1.543 g) was added, and the solution was cooled on an ice bath. Methanesulfonyl chloride (1.691 g) was added dropwise. The solution was allowed to warm to room temperature and stir for 30 minutes. Saturated aqueous sodium bicarbonate solution (50 mL) was added. The layers were separated, and the organic layer was washed with brine (50 mL). The aqueous portions were then combined and back extracted with dichloromethane (50 mL). The organic portions were combined, dried over anhydrous sodium sulfate, filtered, and concentrated. The residue was dissolved in acetonitrile (50

mL). Di-tert-butyl iminodicarboxylate (2.689 g) and cesium carbonate (7.332 g) were added, and the solution was refluxed for 16 hours. The solution was cooled and added to diethyl ether (100 mL) and water (100 mL). The layers were separated. The organic portion was washed with brine (50 mL). The aqueous portions were then combined and back extracted with diethyl ether (100 mL). The organic portions were combined, dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure. The material was purified by flash column chromatography on silica gel, eluting with 20% ethyl acetate in heptanes. The solvent was evaporated under reduced pressure to provide the title compound. MS (ESI) m/e 666 (M+Na).sup.+.

[1243] 1.20.4 methyl 7-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-(di-(tert-butoxycarbonyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-3-(trifluoromethyl)-5,6,7,8-tetrahydroimidazo[1,5-a]pyrazine-1-carboxylate
[1244] The title compound was prepared by substituting Example 1.20.2 for Example 1.4.6 and Example 1.20.3 for Example 1.4.2 in Example 1.4.7. MS (ESI) m/e 964 (M+Na).sup.+, 940 (M-H).sup.-.

[1245] 1.20.5 7-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-(di-(tert-butoxycarbonyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-3-(trifluoromethyl)-5,6,7,8-tetrahydroimidazo[1,5-a]pyrazine-1-carboxylic acid

[1246] The title compound was prepared by substituting Example 1.20.4 for Example 1.4.7 in Example 1.4.8. MS (ESI) m/e 828 (M+H).sup.+, 826 (M-H).sup.-.

[1247] 1.20.6 tert-butyl 6-(1-(benzo[d]thiazol-2-ylcarbamoyl)-3-(trifluoromethyl)-5,6-dihydroimidazo[1,5-a]pyrazin-7(8H)-yl)-3-(1-((3-(2-(di-(tert-butoxycarbonyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinate

[1248] The title compound was prepared by substituting Example 1.20.5 for Example 1.4.8 in Example 1.4.9. MS (ESI) m/e 1058 (M-H).sup.-.

[1249] 1.20.7 3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[1-(1,3-benzothiazol-2-ylcarbamoyl)-3-(trifluoromethyl)-5,6-dihydroimidazo[1,5-a]pyrazin-7(8H)-yl]pyridine-2-carboxylic acid

[1250] The title compound was prepared by substituting Example 1.20.6 for Example 1.1.13 in Example 1.1.14. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 11.99 (bs, 1H), 8.00 (d, 1H), 7.79 (d, 1H), 7.66 (bs, 3H), 7.61 (d, 1H), 7.47 (t, 1H), 7.35 (t, 2H), 7.19 (d, 1H), 5.20 (s, 2H), 4.37 (t, 2H), 4.16 (t, 2H), 3.86 (s, 2H), 3.51 (t, 2H), 2.91 (q, 2H), 2.14 (s, 3H), 1.44 (s, 2H), 1.36-1.24 (m, 4H), 1.19-1.02 (m, 6H), 0.88 (s, 6H). MS (ESI) m/e 804 (M+H).sup.+, 802 (M-H).sup.-.

[1251] 1.21 Synthesis of 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-6-{methyl[2-(methylamino)ethyl]amino}-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[3-(2-methoxyethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid (W3.21)

[1252] 1.21.1 methyl 3-bromo-5-(bromomethyl)benzoate

[1253] AIBN (2,2'-azobis(2-methylpropionitrile)) (1.79 g) was added to methyl 3-bromo-5-methylbenzoate (50 g) and N-bromosuccinimide (44.7 g) in 350 mL acetonitrile, and the mixture was refluxed overnight. An additional 11 g of N-bromosuccinimide and 0.5 g of AIBN (2,2'-azobis(2-methylpropionitrile)) was added, and the refluxing was continued for 3 hours. The mixture was concentrated, and then taken up in 500 mL ether, and stirred for 30 minutes. The mixture was then filtered, and the resulting solution was concentrated. The crude product was chromatographed on silica gel using 10% ethyl acetate in heptane to give the title compound.

[1254] 1.21.2 methyl 3-bromo-5-(cyanomethyl)benzoate

[1255] Tetrabutylammonium cyanide (50 g) was added to Example 1.21.1 (67.1 g) in 300 mL acetonitrile, and the mixture was heated to 70° C. overnight. The mixture was cooled, poured into diethyl ether, and rinsed with water and brine. The mixture was concentrated and chromatographed on silica gel using 2-20% ethyl acetate in heptane to give the title compound.

[1256] 1.21.3 methyl 3-(2-aminoethyl)-5-bromobenzoate

[1257] Borane-tetrahydrofuran complex (126 mL, 1M solution) was added to a solution of Example 1.21.2 (16 g) in 200 mL tetrahydrofuran, and the mixture was stirred overnight. The reaction was carefully quenched with methanol (50 mL), and then concentrated to 50 mL volume. The mixture was then taken up in 120 mL methanol/120 mL 4M HCl/120 mL dioxane, and stirred overnight. The organics were removed by evaporation under reduced pressure, and the residue was extracted with diethyl ether (2×). The organic extracts were discarded. The aqueous layer was basified with solid K₂CO₃, and then extracted with ethyl acetate, and dichloromethane (2×). The extracts were combined, dried over Na₂SO₄, filtered and concentrated to give the title compound.

[1258] 1.21.4 methyl 3-bromo-5-(2-(2,2,2-trifluoroacetamido)ethyl)benzoate

[1259] Trifluoroacetic anhydride (9.52 mL) was added dropwise to a mixture of Example 1.21.3 (14.5 g) and triethylamine (11.74 mL) in 200 mL dichloromethane at 0° C. Upon addition, the mixture was allowed to warm to room temperature and was stirred for three days. The mixture was poured into diethyl ether, and washed with NaHCO₃ solution and brine. The mixture was concentrated and chromatographed on silica gel using 5-30% ethyl acetate in heptanes to give the title compound.

[1260] 1.21.5 methyl 6-bromo-2-(2,2,2-trifluoroacetyl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1261] Sulfuric acid was added to Example 1.21.4 (10 g) until it went into solution (40 mL), at which time paraformaldehyde (4.24 g) was added, and the mixture was stirred for 2 hours. The solution was then poured onto 400 mL ice, and stirred 10 minutes. It was then extracted with ethyl acetate (3×), and the combined extracts were washed with NaHCO₃ solution and brine, and then concentrated. The crude product was chromatographed on silica gel using 2-15% ethyl acetate in heptanes to give the title compound.

[1262] 1.21.6 methyl 6-((2-((tert-butoxycarbonyl)(methyl)amino)ethyl)(methyl)amino)-2-(2,2,2-trifluoroacetyl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1263] Example 1.21.5 (2.25 g), tert-butyl methyl(2-(methylamino)ethyl)carbamate (1.27 g), palladium (II) acetate (0.083 g), 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (0.213 g) and cesium carbonate (4.00 g) were stirred in 40 mL dioxane at 80° C. overnight. The mixture was concentrated and chromatographed on silica gel using 5-50% ethyl acetate in heptanes to give the title compound.

[1264] 1.21.7 methyl 2-(5-bromo-6-(tert-butoxycarbonyl)pyridin-2-yl)-6-((2-((tert-butoxycarbonyl)(methyl)amino)ethyl)(methyl)amino)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1265] Example 1.21.6 (3 g) and potassium carbonate (2.63 g) were stirred in 30 mL tetrahydrofuran, 20 mL methanol, and 25 mL water overnight. The mixture was concentrated and 60 mL N,N-dimethylformamide was added. To this was then added Example 1.4.4 (1.08 g) and triethylamine (0.6 mL), and the reaction was stirred at 50° C. overnight. The mixture was cooled to room temperature and poured into ethyl acetate (200 mL). The solution was washed with water (3×) and brine, then dried over Na₂SO₄, filtered, and concentrated. The residue was chromatographed on silica gel using 5-50% ethyl acetate in heptanes to give the title compound. MS (ESI) m/e 635 (M+H)⁺.

[1266] 1.21.8 methyl 6-((2-((tert-butoxycarbonyl)(methyl)amino)ethyl)(methyl)amino)-2-(6-(tert-butoxycarbonyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1267] The title compound was prepared by substituting Example 1.21.7 for Example 1.1.9 in Example 1.1.10.

[1268] 1.21.9 methyl 6-((2-((tert-butoxycarbonyl)(methyl)amino)ethyl)(methyl)amino)-2-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-methoxyethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1269] The title compound was prepared by substituting Example 1.21.8 for Example 1.5.11 and Example 1.17.1 for Example 1.5.10 in Example 1.5.12. MS (ESI) m/e 885.6 (M+H).sup.+.

[1270] 1.21.10 6-((2-((tert-butoxycarbonyl)(methyl)amino)ethyl)(methyl)amino)-2-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-methoxyethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylic acid

[1271] The title compound was prepared by substituting Example 1.21.9 for Example 1.4.7 in Example 1.4.8.

[1272] 1.21.11 tert-butyl 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-6-((2-((tert-butoxycarbonyl)(methyl)amino)ethyl)(methyl)amino)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-((3-(2-methoxyethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinate

[1273] The title compound was prepared by substituting Example 1.21.10 for Example 1.4.8 in Example 1.4.9. MS (ESI) m/e 1003.6 (M+H).sup.+.

[1274] 1.21.12 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-6-{methyl[2-(methylamino)ethyl]amino}-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[3-(2-methoxyethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid

[1275] Example 1.21.11 (40 mg) was stirred in 2 mL trifluoroacetic acid and 3 mL dichloromethane overnight. After evaporation of the solvent, the residue was purified on an HPLC (Gilson system, eluting with 10-85% acetonitrile in 0.1% trifluoroacetic acid in water) to give the title compound. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d₆) δ ppm 12.75 (bs, 1H), 12.50 (br s, 1H), 8.40 (m, 2H), 8.01 (d, 1H), 7.76 (d, 1H), 7.45 (m, 2H), 7.32 (t, 1H), 7.24 (s, 1H), 6.99 (d, 1H), 6.86 (d, 1H), 6.78 (d, 1H), 4.72 (m, 2H), 3.98 (m, 2H), 3.80 (m, 4H), 3.76 (s, 2H), 3.55 (m, 2H), 3.29 (d, 3H), 3.20 (s, 3H), 3.15 (m, 2H), 2.90 (s, 3H), 2.58 (t, 2H), 2.05 (s, 3H), 1.30 (s, 2H), 1.21 (m, 4H), 1.08 (m, 4H), 0.98 (m, 2H), 0.85 (s, 6H). MS (ESI) m/e 847.5 (M+H).sup.+.

[1276] 1.22 Synthesis of 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-6-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid (W3.22)

[1277] 1.22.1 methyl 6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-2-(2,2,2-trifluoroacetyl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1278] A mixture of Example 1.21.5 (4.5 g), 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (3.75 g), [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) dichloromethane (0.4 g), and potassium acetate (3.62 g) was stirred in 60 mL dioxane at 70° C. for 24 hours. The mixture was then diluted with ethyl acetate, and rinsed with water and brine. The mixture was concentrated and chromatographed on silica gel using 5-50% ethyl acetate in heptanes to give the title compound.

[1279] 1.22.2 methyl 6-hydroxy-2-(2,2,2-trifluoroacetyl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1280] Hydrogen peroxide (30%, 1.1 mL) was added to a mixture of Example 1.22.1 (4 g) and 1M aqueous NaOH solution (9.86 mL) in 40 mL tetrahydrofuran and 40 mL water, and the mixture was stirred for 90 minutes. The solution was acidified with concentrated HCl, and extracted twice with ethyl acetate. The combined extracts were washed with brine. The mixture was then concentrated and chromatographed on silica gel using 5-50% ethyl acetate in heptanes to give the title compound. MS (ESI) m/e 304.2 (M+H).sup.+.

[1281] 1.22.3 methyl 6-methoxy-2-(2,2,2-trifluoroacetyl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1282] Trimethylsilyldiazomethane (2.6 mL, 2M solution in diethyl ether) was added to Example 1.22.2 (800 mg) in 10 mL methanol, and the reaction was stirred for 24 hours. The mixture was then concentrated and chromatographed on silica gel using 5-25% ethyl acetate in heptanes to give the title compound. MS (ESI) m/e 318.2 (M+H).sup.+.

[1283] 1.22.4 methyl 2-(5-bromo-6-(tert-butoxycarbonyl)pyridin-2-yl)-6-methoxy-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1284] The title compound was prepared by substituting Example 1.22.3 for Example 1.21.6 in Example 1.21.7. MS (ESI) m/e 479.1 (M+H).sup.+.

[1285] 1.22.5 methyl 2-(6-(tert-butoxycarbonyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-yl)-6-methoxy-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1286] The title compound was prepared by substituting Example 1.22.4 for Example 1.1.9 in Example 1.1.10. MS (ESI) m/e 525.1 (M+H).sup.+.

[1287] 1.22.6 methyl 2-(6-(tert-butoxycarbonyl)-5-(1-((2-((tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-6-methoxy-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1288] The title compound was prepared by substituting Example 1.22.5 for Example 1.5.11 and Example 1.1.9 for Example 1.5.10 in Example 1.5.12. MS (ESI) m/e 829.6 (M+H).sup.+.

[1289] 1.22.7 2-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-((tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-6-methoxy-1,2,3,4-tetrahydroisoquinoline-8-carboxylic acid

[1290] The title compound was prepared by substituting Example 1.22.6 for Example 1.4.7 in Example 1.4.8. MS (ESI) m/e 814.6 (M+H).sup.+.

[1291] 1.22.8 tert-butyl 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-6-methoxy-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-((3-(2-((tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinate

[1292] The title compound was prepared by substituting Example 1.22.7 for Example 1.4.8 in Example 1.4.9. MS (ESI) m/e 946.5 (M+H).sup.+.

[1293] 1.22.9 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-6-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid

[1294] The title compound was prepared by substituting Example 1.22.8 for Example 1.21.11 in Example 1.21.12. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 12.75 (bs, 1H), 12.50 (br s, 1H), 8.21 (m, 2H), 8.01 (d, 1H), 7.76 (d, 1H), 7.44 (m, 2H), 7.32 (t, 1H), 7.25 (s, 1H), 7.20 (d, 1H), 6.99 (d, 1H), 6.90 (d, 1H), 4.72 (m, 2H), 3.80 (m, 4H), 3.55 (s, 3H), 3.50 (d, 3H), 2.98 (m, 4H), 2.51 (t, 2H), 2.05 (s, 3H), 1.35 (s, 2H), 1.26 (m, 4H), 1.10 (m, 4H), 1.00 (m, 2H), 0.85 (s, 6H). MS (ESI) m/e 790.4 (M+H).sup.+.

[1295] 1.23 Synthesis of 3-(1-({3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl)-6-[4-(1,3-benzothiazol-2-ylcarbamoyl)quinolin-6-yl]pyridine-2-carboxylic acid (W3.23)

[1296] 1.23.1 ethyl 6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline-4-carboxylate

[1297] To a solution of ethyl 6-bromoquinoline-4-carboxylate (140 mg) in N,N-dimethylformamide (2 mL) was added [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) dichloromethane (20 mg), potassium acetate (147 mg) and bis(pinacolato)diboron (190 mg). The mixture was stirred at 60° C. overnight. The mixture was cooled to room temperature and used in the next reaction directly. MS (ESI) m/e 328.1 (M+H).sup.+.

[1298] 1.23.2 di-tert-butyl {2-[(3,5-dimethyl-7-{{5-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazol-1-yl}methyl}tricyclo[3.3.1.1^{3,7}]decan-1-yl)oxy]ethyl}-2-imidodicarbonate

[1299] To a solution of Example 1.20.3 (13 g) in dioxane (100 mL) was added dicyclohexyl(2',6'-dimethoxy-[1,1'-biphenyl]-2-yl)phosphine (S-Phos) (1.0 g) and bis(benzonitrile)palladium(II) chloride (0.23 g) and the reaction was purged with several house vacuum/N.sub.2 refills. 4,4,5,5-Tetramethyl-1,3,2-dioxaborolane (8.8 mL) and triethylamine (8.4 mL) was added followed by a couple more house vacuum/nitrogen refills and then the reaction was heated to 85° C. under nitrogen for 90 minutes. The reaction was cooled, filtered through diatomaceous earth and rinsed

with methyl tert-butyl ether. The solution was then concentrated and chromatographed on silica gel using 25% ethyl acetate in heptanes to give the title compound.

[1300] 1.23.3 tert-butyl 3-{1-[(3-{2-[bis(tert-butoxycarbonyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]decan-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}-6-chloropyridine-2-carboxylate

[1301] To a solution of Example 1.23.2 (12.3 g) and tert-butyl 3-bromo-6-chloropicolinate (5.9 g) in dioxane (50 mL) was added (1S,3R,5R,7S)-1,3,5,7-tetramethyl-8-phenyl-2,4,6-trioxa-8-phosphaadamantane(CyTop) (0.52 g) and bis(dibenzylideneacetone)palladium(0) (0.66 g). After several house vacuum/nitrogen refills, potassium phosphate (4.06 g) and water (25 mL) were added and the reaction was heated at 80° C. under nitrogen for 30 minutes. The reaction was cooled and then water and ethyl acetate were added. The organic layer was separated and washed with brine. The combined aqueous layers were extracted with ethyl acetate, and dried over sodium sulfate. The solution was filtered, concentrated and chromatographed on silica gel using 33% ethyl acetate in heptanes to give the title compound.

[1302] 1.23.4 ethyl 6-[5-{1-[(3-{2-[bis(tert-butoxycarbonyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}-6-(tert-butoxycarbonyl)pyridin-2-yl]quinoline-4-carboxylate

[1303] To a solution of Example 1.23.1 (164 mg) in 1,4-dioxane (10 mL) and water (5 mL) was added Example 1.23.3 (365 mg), bis(triphenylphosphine)palladium(II) dichloride (35 mg), and CsF (228 mg). The mixture was stirred at 120° C. for 30 minutes under microwave conditions (Biotage Initiator). The mixture was diluted with ethyl acetate (200 mL) and washed with water and brine and dried over anhydrous sodium sulfate. Filtration and evaporation of the solvent gave a residue that purified by silica gel chromatography, eluting with 20% ethyl acetate in heptane, to give the title compound. MS (ESI) m/e 894.3(M+H).sup.+.

[1304] 1.23.5 6-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-((tert-butoxycarbonyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)quinoline-4-carboxylic acid

[1305] To a solution of Example 1.23.4 (3.1 g) in tetrahydrofuran (20 mL), methanol (10 mL) and water (10 mL) was added LiOH H.sub.2O (240 mg). The mixture was stirred at room temperature overnight. The mixture was acidified with aqueous 2N HCl, diluted with ethyl acetate (400 mL), washed with water and brine, and dried over anhydrous sodium sulfate. Filtration and evaporation of the solvent gave the title compound, which was used without further purification. MS (ESI) m/e 766.3(M+H).sup.+.

[1306] 1.23.6 3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-[4-(1,3-benzothiazol-2-ylcarbamoyl)quinolin-6-yl]pyridine-2-carboxylic acid

[1307] To a solution of Example 1.23.5 (4.2 g) in dichloromethane (30 mL) was added benzo[d]thiazol-2-amine (728 mg), 1-ethyl-3-[3-(dimethylamino)propyl]-carbodiimide hydrochloride (1.40 g) and 4-(dimethylamino)pyridine (890 mg). The mixture was stirred at room temperature overnight. The reaction mixture was diluted with ethyl acetate (500 mL), washed with water and brine, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was dissolved in dichloromethane and trifluoroacetic acid (10 mL, 1:1) and stirred overnight. The solvents were removed under reduced pressure. The residue was diluted with N,N-dimethylformamide (2 mL), filtered and purified by reverse-phase HPLC on a Gilson system (C18 column), eluting with 20-80% acetonitrile in water containing 0.1% trifluoroacetic acid, to give the title compound. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 9.12 (dd, 1H), 8.92 (s, 1H), 8.61 (dt, 1H), 8.35-8.16 (m, 2H), 8.07 (d, 1H), 7.97-7.87 (m, 2H), 7.81 (d, 1H), 7.66 (s, 3H), 7.53-7.44 (m, 2H), 7.38 (t, 1H), 3.88 (s, 2H), 3.49 (t, 2H), 2.89 (q, 2H), 2.22 (s, 4H), 1.43 (s, 2H), 1.29 (q, 4H), 1.15 (s, 4H), 1.09-0.96 (m, 2H), 0.86 (s, 7H). MS (ESI) m/e 742.2 (M+H).sup.+.

[1308] 1.24 Synthesis of 6-[5-amino-8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl}-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid (W3.24)

[1309] 1.24.1 5-tert-butoxycarbonylamino-2-(2,2,2-trifluoro-acetyl)-1,2,3,4-tetrahydro-isoquinoline-8-carboxylic acid methyl ester

[1310] Example 1.13.4 (5000 mg), tert-butyl carbamate (1920 mg), and cesium carbonate (6674 mg) were added to 1,4-dioxane (80 mL). The solution was degassed and flushed with nitrogen three times. Diacetoxypalladium (307 mg) and (9,9-dimethyl-9H-xanthene-4,5-diyl)bis(diphenylphosphine) (1580 mg) were added, and the solution was degassed and flushed with nitrogen once. The solution was heated to 80° C. for 16 hours. The solution was cooled, and 1 M aqueous HCl (150 mL) was added. The solution was extracted with 50% ethyl acetate in heptanes. The organic portion was washed with brine and dried on anhydrous sodium sulfate. The solution was filtered, concentrated and purified by flash column chromatography on silica gel, eluting with 30% ethyl acetate in heptanes. The solvent was removed under reduced pressure to yield the title compound. MS (ESI) m/e 420 (M+NH₄).sup.+, 401 (M-H).sup.-.

[1311] 1.24.2 5-tert-butoxycarbonylamino-1,2,3,4-tetrahydro-isoquinoline-8-carboxylic acid methyl ester

[1312] The title compound was prepared by substituting Example 1.24.1 for Example 1.13.5 in Example 1.13.6. MS (ESI) m/e 307 (M+H).sup.+, 305 (M-H).sup.-.

[1313] 1.24.3 2-(5-bromo-6-tert-butoxycarbonyl-pyridin-2-yl)-5-tert-butoxycarbonylamino-1,2,3,4-tetrahydro-isoquinoline-8-carboxylic acid methyl ester

[1314] The title compound was prepared by substituting Example 1.24.2 for Example 1.13.6 in Example 1.13.7. MS (ESI) m/e 562, 560 (M+H).sup.+, 560, 558 (M-H).sup.-.

[1315] 1.24.4 5-tert-butoxycarbonylamino-2-[6-tert-butoxycarbonyl-5-(4,4,5,5-tetramethyl-1,3,2)dioxaborolan-2-yl]-pyridin-2-yl]-1,2,3,4-tetrahydro-isoquinoline-8-carboxylic acid methyl ester

[1316] The title compound was prepared by substituting Example 1.24.3 for Example 1.13.7 in Example 1.13.8. MS (ESI) m/e 610 (M+H).sup.+, 608 (M-H).sup.-.

[1317] 1.24.5 methyl 2-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-((tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-5-((tert-butoxycarbonyl)amino)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1318] The title compound was prepared by substituting Example 1.24.4 for Example 1.13.8 and Example 1.1.9 for Example 1.4.2 in Example 1.13.9. MS (ESI) m/e 913 (M+H).sup.+, 911 (M-H).sup.-.

[1319] 1.24.6 2-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-((tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-5-((tert-butoxycarbonyl)amino)-1,2,3,4-tetrahydroisoquinoline-8-carboxylic acid

[1320] The title compound was prepared by substituting Example 1.24.5 for Example 1.13.9 in Example 1.13.10. MS (ESI) m/e 899 (M+H).sup.+, 897 (M-H).sup.-.

[1321] 1.24.7 tert-butyl 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-5-((tert-butoxycarbonyl)amino)-3,4-dihydroisoquinolin-2(1H)-yl)-3-[1-((3-(2-((tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl]picolinate

[1322] The title compound was prepared by substituting Example 1.24.6 for Example 1.13.10 in Example 1.13.11. MS (ESI) m/e 1031 (M+H).sup.+, 1029 (M-H).sup.-.

[1323] 1.24.8 6-[5-amino-8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl}-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid

[1324] The title compound was prepared by substituting Example 1.24.7 for Example 1.13.11 in Example 1.13.12. ¹H NMR (400 MHz, dimethyl sulfoxide-d₆) δ ppm 11.42 (s, 1H), 7.98 (d, 1H), 7.75 (d, 1H), 7.55 (d, 1H), 7.44 (t, 2H), 7.31 (t, 1H), 7.27 (s, 1H), 6.92 (d, 1H), 6.58 (d,

1H), 5.74 (s, 2H), 4.99 (s, 2H), 3.93 (t, 2H), 3.82 (s, 2H), 3.57 (s, 3H), 3.54 (m, 2H), 3.09 (q, 2H), 2.98 (bs, 2H), 2.11 (s, 3H), 1.35-1.04 (m, 12H), 0.87 (s, 6H). MS (ESI) m/e 775 (M+H).sup.+.

[1325] 1.25 Synthesis of 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-6-[3-(methylamino)prop-1-yn-1-yl]-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[3-(2-methoxyethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid (W3.25) 1.25.1 methyl 6-(3-((tert-butoxycarbonyl)(methyl)amino)prop-1-yn-1-yl)-2-(2,2,2-trifluoroacetyl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1326] A solution of Example 1.21.5 (1.97 g), tert-butyl methyl(prop-2-yn-1-yl)carbamate (1 g), bis(triphenylphosphine)palladium(II) dichloride (0.19 g), CuI (0.041 g), and triethylamine (2.25 mL) in 20 mL dioxane was stirred at 50° C. overnight. The mixture was then concentrated and chromatographed on silica gel using 10-50% ethyl acetate in heptanes to give the title compound.

[1327] 1.25.2 methyl 2-(5-bromo-6-(tert-butoxycarbonyl)pyridin-2-yl)-6-(3-((tert-butoxycarbonyl)(methyl)amino)prop-1-yn-1-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1328] The title compound was prepared by substituting Example 1.25.1 for Example 1.21.6 in Example 1.21.7. MS (ESI) m/e 616 (M+H).sup.+.

[1329] 1.25.3 methyl 6-(3-((tert-butoxycarbonyl)(methyl)amino)prop-1-yn-1-yl)-2-(6-(tert-butoxycarbonyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1330] The title compound was prepared by substituting Example 1.25.2 for Example 1.1.9 in Example 1.1.10. MS (ESI) m/e 662.3 (M+H).sup.+.

[1331] 1.25.4 methyl 6-(3-((tert-butoxycarbonyl)(methyl)amino)prop-1-yn-1-yl)-2-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-methoxyethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1332] The title compound was prepared by substituting Example 1.25.3 for Example 1.5.11 and Example 1.17.1 for Example 1.5.10 in Example 1.5.12.

[1333] 1.25.5 6-(3-((tert-butoxycarbonyl)(methyl)amino)prop-1-yn-1-yl)-2-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-methoxyethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylic acid

[1334] The title compound was prepared by substituting Example 1.25.4 for Example 1.4.7 in Example 1.4.8. 1.25.6 tert-butyl 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-6-(3-((tert-butoxycarbonyl)(methyl)amino)prop-1-yn-1-yl)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-((3-(2-methoxyethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinate The title compound was prepared by substituting Example 1.25.5 for Example 1.4.8 in Example 1.4.9.

[1335] 1.25.7 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-6-[3-(methylamino)prop-1-yn-1-yl]-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[3-(2-methoxyethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid

[1336] The title compound was prepared by substituting Example 1.25.6 for Example 1.21.11 in Example 1.21.12. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 12.95 (bs, 1H), 8.70 (m, 1H), 8.02 (d, 1H), 7.77 (d, 1H), 7.74 (m, 1H), 7.47 (m, 2H), 7.34 (m, 2H), 7.24 (s, 1H), 6.95 (m, 1H), 6.78 (m, 1H), 4.92 (s, 2H), 4.28 (t, 2H), 3.95 (t, 2H), 3.40 (s, 3H), 3.30 (m, 2H), 3.20 (s, 3H), 3.00 (m, 2H), 2.57 (t, 2H), 2.07 (s, 3H), 1.85 (m, 2H), 1.29 (d, 2H), 1.10-1.24 (m, 10H), 0.85 (s, 6H).

[1337] 1.26 Synthesis of 6-[4-(1,3-benzothiazol-2-ylcarbamoyl)isoquinolin-6-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid (W3.26)

[1338] 1.26.1 methyl 2-(3-bromophenyl)-2-cyanoacetate

[1339] To a solution of 2-(3-bromophenyl)acetonitrile (5 g) in tetrahydrofuran (50 mL) was added sodium hydride (3.00 g) portion wise at 23° C. The mixture was heated to 50° C. for 20 minutes. Dimethyl carbonate (8.60 mL) was added dropwise. The mixture was heated at reflux for 2 hours. The mixture was poured into cold and slightly acidic water. The aqueous layer was extracted with

ethyl acetate (2×200 mL). The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered through a Büchner funnel and concentrated to give a residue, which was purified by silica gel column chromatography, eluting with 0%-25% dichloromethane/petroleum ether to afford the title compound. MS (LC-MS) m/e 256.0 (M+H)+

[1340] 1.26.2 methyl 3-amino-2-(3-bromophenyl)propanoate

[1341] Sodium borohydride (14.89 g, 394 mmol) was added portionwise to a solution of Example 1.26.1 (10 g) and cobalt(II) chloride hexahydrate (18.73 g) in methanol (200 mL) at -20° C. The mixture was stirred for 1 hour and the pH was adjusted to 3 with 2N aqueous HCl. The mixture was concentrated. The residue was basified with 2 M aqueous sodium hydroxide and extracted with ethyl acetate. The combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated to provide the title compound. MS (LC-MS) m/e 260.0 (M+H).sup.+.

[1342] 1.26.3 methyl 2-(3-bromophenyl)-3-formamidopropanoate

[1343] A solution of Example 1.26.2 (3.6 g) in ethyl formate (54 mL) was heated at 80° C. for 5 hours. The solvent was removed, and the residue was purified by silica gel column chromatography eluting with petroleum/ethyl acetate (2:1-1:2) to give the title compound. MS (LC-MS) m/e 288.0 (M+H).sup.+.

[1344] 1.26.4 methyl 8-bromo-2,3-dioxo-3,5,6,10b-tetrahydro-2H-oxazolo[2,3-a]isoquinoline-6-carboxylate

[1345] Oxalyl chloride (1.901 mL) was slowly added to a solution of Example 1.26.3 (5.65 g) in dichloromethane (190 mL). The resulting mixture was stirred at 20° C. for 2 hours. The mixture was cooled to -20° C., and iron(III) chloride (3.84 g) was added. The resulting mixture was stirred at 20° C. for 3 hours. Aqueous hydrochloric acid (2M, 45 mL) was added in one portion, and the resulting biphasic mixture was vigorously stirred for 0.5 hours at room temperature. The biphasic mixture was poured into a separatory funnel, and the phases were separated. The organic layer was washed with brine, dried with sodium sulfate, and filtered. The solvent was evaporated under reduced pressure to provide the title compound. The crude product was directly used in subsequent step without purification. MS (LC-MS) m/e 342.0 (M+H).sup.+.

[1346] 1.26.5 methyl 6-bromo-3,4-dihydroisoquinoline-4-carboxylate

[1347] Example 1.26.4 (13.0 g) in methanol (345 mL) and sulfuric acid (23 mL) was heated at 80° C. for 16 hours. The mixture was concentrated, and the residue was diluted with water, basified with saturated aqueous sodium bicarbonate solution and extracted with ethyl acetate. The combined organic layers were washed with brine, dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by silica gel column chromatography, eluting with petroleum ether/ethyl acetate (2:1-1:2) to give the title compound. MS (LC-MS) m/e 268.0 (M+H).sup.+.

[1348] 1.26.6 methyl 6-bromoisoquinoline-4-carboxylate

[1349] To a solution of Example 1.26.5 (5.25 g) in 1,4-dioxane (200 mL) at 60° C. was added manganese(IV) dioxide (8.5 g). The mixture was heated to 110° C. for 3 hours. The reaction mixture was filtered through a pad of diatomaceous earth and washed with dichloromethane and ethyl acetate. The filtrate was concentrated to dryness. The crude material was adsorbed onto silica gel and purified by silica gel chromatography, eluting with 5-30% ethyl acetate in dichloromethane to give the title compound. MS (LC-MS) m/e 267.9 (M+H).sup.+.

[1350] 1.26.7 methyl 6-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-((tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)isoquinoline-4-carboxylate

[1351] Example 1.26.6 (229 mg), 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (328 mg) and potassium acetate (253 mg) in N,N-dimethylformamide (5 mL) was purged with N.sub.2 for 5 minutes and [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) dichloromethane (42.2 mg) was added. The mixture was heated at 100° C. overnight and cooled. To the mixture was added Example 1.1.11 (0.369 g), dichlorobis(triphenylphosphine)palladium(II) (0.060 g), cesium

fluoride (0.261 g) and water (2 mL). The resulting mixture was heated at 100° C. for 10 hours and filtered. The filtrate was concentrated. The residue was dissolved in dimethyl sulfoxide and purified by reverse-phase HPLC on a Gilson system (C18 column), eluting with 20-80% acetonitrile in water containing 0.1% trifluoroacetic acid, to give the title compound. MS (ESI) m/e 794.5 (M+H).sup.+.

[1352] 1.26.8 6-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-((tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)isoquinoline-4-carboxylic acid

[1353] Example 1.26.7 (220 mg) in tetrahydrofuran-methanol was treated with 1 M aqueous sodium hydroxide (1.66 mL) for 2 days. The mixture was neutralized with acetic acid and concentrated. The residue was dissolved in dimethyl sulfoxide and purified by reverse-phase HPLC on a Gilson system (C18 column), eluting with 20-80% acetonitrile in water containing 0.1% trifluoroacetic acid, to give the title compound. MS (ESI) m/e 780.5 (M+H).sup.+.

[1354] 1.26.9 tert-butyl 6-(4-(benzo[d]thiazol-2-ylcarbamoyl)isoquinolin-6-yl)-3-(1-((3-(2-((tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinate

[1355] To a mixture of Example 1.26.8 (122 mg), benzo[d]thiazol-2-amine (47.0 mg), O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (119 mg) in N,N-dimethylformamide (0.5 mL) was added N,N-diisopropylethylamine (273 µL). The mixture was stirred overnight and loaded onto an 80 g silica gel column, eluting with 5-100% heptanes in ethyl acetate to provide the title compound. MS (ESI) m/e 912.5 (M+H).sup.+.

[1356] 1.26.10 6-[4-(1,3-benzothiazol-2-ylcarbamoyl)isoquinolin-6-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid

[1357] Example 1.26.9 (100 mg) in dichloromethane (4 mL) was treated with trifluoroacetic acid (2 mL) for 3 hours and the mixture was concentrated. The residue was dissolved in dimethyl sulfoxide (5 mL) and purified by reverse-phase HPLC on a Gilson system (C18 column), eluting with 20-80% acetonitrile in water containing 0.1% trifluoroacetic acid, to give the title compound. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d₆) 6 13.27 (s, 1H), 9.58 (s, 1H), 9.03 (d, 2H), 8.53 (dd, 1H), 8.42 (d, 1H), 8.25 (t, 3H), 8.06 (d, 1H), 7.97 (d, 1H), 7.81 (d, 1H), 7.56-7.45 (m, 2H), 7.37 (t, 1H), 3.89 (s, 2H), 3.55 (t, 2H), 3.01 (t, 2H), 2.54 (t, 4H), 2.23 (s, 3H), 1.44 (s, 2H), 1.36-1.23 (m, 4H), 1.16 (s, 4H), 0.87 (s, 6H). MS (ESI) m/e 756.1 (M+H).sup.+.

[1358] 1.27 Synthesis of 6-[7-(1,3-benzothiazol-2-ylcarbamoyl)-1H-indol-2-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid (W3.27)

[1359] 1.27.1 methyl 2-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-((tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-1H-indole-7-carboxylate

[1360] To a stirred solution of methyl 2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-indole-7-carboxylate (370 mg), tris(dibenzylideneacetone)dipalladium(0) (30 mg), 1,2,3,4,5-pentaphenyl-1'- (di-tert-butylphosphino)ferrocene (30 mg) and potassium phosphate (550 mg) in tetrahydrofuran (2 mL) was added Example 1.1.11 (735 mg). The mixture was purged with nitrogen and stirred at 70° C. for 3 hours. The reaction was diluted with ethyl acetate and washed with water and brine. The aqueous layer was back extracted by ethyl acetate. The combined organic layers were dried over sodium sulfate, filtered and concentrated. The residue was purified via silica gel chromatography, eluting with 0-20% ethyl acetate in heptanes, to give the title compound. MS (ESI) m/e 780.4 (M-H).sup.-.

[1361] 1.27.2 2-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-((tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-1H-indole-7-carboxylic acid

[1362] The title compound was prepared as described in Example 1.4.8, replacing Example 1.4.7 with Example 1.27.1. MS (ESI) m/e 766.4 (M-H).sup.-.

[1363] 1.27.3 tert-butyl 6-(7-(benzo[d]thiazol-2-ylcarbamoyl)-1H-indol-2-yl)-3-(1-((3-(2-((tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinate

[1364] The title compound was prepared as described in Example 1.4.9, replacing Example 1.4.8 with Example 1.27.2. MS (ESI) m/e 898.4 (M-H).sup.-.

[1365] 1.27.4 6-[7-(1,3-benzothiazol-2-ylcarbamoyl)-1H-indol-2-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl}-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid

[1366] The title compound was prepared by substituting Example 1.27.3 for Example 1.1.13 in Example 1.1.14. .sup.1H NMR (501 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 13.01 (s, 1H), 11.19 (s, 1H), 8.27 (dd, 4H), 8.04 (d, 1H), 7.99 (d, 1H), 7.91 (d, 1H), 7.53-7.45 (m, 3H), 7.36 (t, 1H), 7.27 (t, 1H), 3.91 (s, 2H), 3.57 (t, 3H), 3.03 (t, 3H), 2.58-2.54 (m, 4H), 2.24 (s, 3H), 1.46 (s, 2H), 1.38-1.27 (m, 4H), 1.24-1.01 (m, 6H), 0.89 (s, 6H). MS (ESI) m/e 744.2 (M+H).sup.+.

[1367] 1.28 Synthesis of 3-(1-{{3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl}-5-methyl-1H-pyrazol-4-yl)-6-[7-(1,3-benzothiazol-2-ylcarbamoyl)-1H-indol-2-yl]pyridine-2-carboxylic acid (W3.28)

[1368] 1.28.1 methyl 2-[5-{1-[(3-{2-[bis(tert-butoxycarbonyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}-6-(tert-butoxycarbonyl)pyridin-2-yl]-1H-indole-7-carboxylate

[1369] The title compound was prepared by substituting Example 1.23.3 for Example 1.1.11 in Example 1.27.1. MS (ESI) m/e 866.3 (M-H).sup.-.

[1370] 1.28.2 2-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-((tert-butoxycarbonyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-1H-indole-7-carboxylic acid

[1371] The title compound was prepared as described in Example 1.4.8, replacing Example 1.4.7 with Example 1.28.1. MS (ESI) m/e 754.4 (M+H).sup.+.

[1372] 1.28.3 tert-butyl 6-(7-(benzo[d]thiazol-2-ylcarbamoyl)-1H-indol-2-yl)-3-(1-((3-(2-((tert-butoxycarbonyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinate

[1373] The title compound was prepared as described in Example 1.4.9, replacing Example 1.4.8 with Example 1.28.2. MS (ESI) m/e 886.5 (M+H).sup.+.

[1374] 1.28.4 3-(1-{{3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl}-5-methyl-1H-pyrazol-4-yl)-6-[7-(1,3-benzothiazol-2-ylcarbamoyl)-1H-indol-2-yl]pyridine-2-carboxylic acid

[1375] The title compound was prepared by substituting Example 1.28.3 for Example 1.1.13 in Example 1.1.14. .sup.1H NMR (501 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 13.00 (s, 1H), 11.19 (s, 1H), 8.29 (d, 1H), 8.23 (d, 1H), 8.03 (d, 1H), 7.98 (d, 1H), 7.90 (d, 1H), 7.80 (s, 1H), 7.63 (s, 3H), 7.50 (s, 1H), 7.49-7.44 (m, 2H), 7.39-7.32 (m, 1H), 7.25 (t, 1H), 3.90 (s, 2H), 2.90 (q, 2H), 2.23 (s, 3H), 1.45 (s, 2H), 1.31 (q, 4H), 1.23-1.00 (m, 7H), 0.88 (s, 6H). MS (ESI) m/e 730.2 (M+H).sup.+.

[1376] 1.29 Synthesis of 6-[7-(1,3-benzothiazol-2-ylcarbamoyl)-3-methyl-1H-indol-2-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl}-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid (W3.29) 1.29.1 methyl 3-methyl-1H-indole-7-carboxylate

[1377] To 7-bromo-3-methyl-1H-indole (1 g), dichloro[1,1'-bis(diphenylphosphino)ferrocene]palladium(II) dichloromethane adduct (0.070 g) in a 50 mL pressure bottle was added methanol (20 mL) and trimethylamine (1.327 mL). The reactor was purged with inert gas, followed by carbon monoxide. The reaction was heated to 100° C. for 20 hours at 60 psi. The solution was filtered and concentrated. The residue was purified by silica gel

chromatography, eluting with a gradient of 5-30% ethyl acetate in heptanes, to give the title compound. MS (ESI) m/e 189.9 (M+H).sup.+.

[1378] 1.29.2 methyl 2-bromo-3-methyl-1H-indole-7-carboxylate

[1379] To a stirred suspension of Example 1.29.1 (70 mg) and 70 mg silica gel in dichloromethane (2 mL) was added 1-bromopyrrolidine-2,5-dione (70 mg). The mixture was protected from light by with aluminum foil and was stirred at room temperature under nitrogen for 30 minutes. The reaction mixture was filtered, washed with dichloromethane and purified via silica gel chromatography, eluting with 10-50% ethyl acetate in heptane, to provide the title compound. MS (ESI) m/e 267.6 (M+H).sup.+.

[1380] 1.29.3 methyl 3-methyl-2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-indole-7-carboxylate

[1381] To a stirred suspension of Example 1.29.2 (398 mg), 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (1.2 g,) and potassium acetate (450 mg) in 1,4-dioxane (2 mL) was added bis(triphenylphosphine)palladium(II) dichloride (55 mg). The mixture was purged with nitrogen and heated at 115° C. under microwave conditions (Biotage Initiator) for 3 hours. The reaction was diluted with ethyl acetate and washed with water and brine. The aqueous layer was back extracted with ethyl acetate. The combined organic layer was dried over sodium sulfate, filtered and concentrated. The residue was purified via silica gel chromatography, eluting with 5-50% ethyl acetate in heptane, to give the title compound. MS (ESI) m/e 315.9 (M+H).sup.+.

[1382] 1.29.4 methyl 2-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-((tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-3-methyl-1H-indole-7-carboxylate

[1383] Example 1.29.4 was prepared by substituting Example 1.29.3 for methyl 2-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-indole-7-carboxylate in Example 1.27.1. MS (ESI) m/e 794.4 (M-H).sup.-.

[1384] 1.29.5 2-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-((tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-3-methyl-1H-indole-7-carboxylic acid

[1385] Example 1.29.5 was prepared by substituting Example 1.29.4 for Example 1.4.7 in Example 1.4.8. MS (ESI) m/e 780.4 (M-H).sup.-.

[1386] 1.29.6 tert-butyl 6-(7-(benzo[d]thiazol-2-ylcarbamoyl)-3-methyl-1H-indol-2-yl)-3-(1-((3-(2-((tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinate

[1387] Example 1.29.6 was prepared by substituting Example 1.29.5 for Example 1.4.8 in Example 1.4.9. MS (ESI) m/e 912.4 (M-H).sup.-.

[1388] 1.29.7 6-[7-(1,3-benzothiazol-2-ylcarbamoyl)-3-methyl-1H-indol-2-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid

[1389] The title compound was prepared by substituting Example 1.29.6 for Example 1.1.13 in Example 1.1.14. .sup.1H NMR (501 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 12.97 (s, 1H), 11.04 (s, 1H), 8.34-8.23 (m, 3H), 8.06 (d, 1H), 8.02 (dd, 2H), 7.93 (d, 1H), 7.79 (d, 1H), 7.51 (s, 1H), 7.48 (ddd, 1H), 7.38-7.32 (m, 1H), 7.25 (t, 1H), 3.91 (s, 2H), 3.56 (t, 2H), 3.03 (p, 2H), 2.67 (s, 3H), 2.56 (t, 3H), 2.25 (s, 3H), 1.46 (s, 2H), 1.38-1.26 (m, 4H), 1.24-1.13 (m, 4H), 1.06 (q, 2H), 0.89 (s, 6H). MS (ESI) m/e 758.2 (M+H).sup.+.

[1390] 1.30 Synthesis of 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-({[3,5-dimethyl-7-(2-{[1-(methylsulfonyl)piperidin-4-yl]amino}ethoxy)tricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid (W3.30)

[1391] 1.30.1 tert-butyl 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-(((1r,7r)-3,5-dimethyl-7-(2-((1-(methylsulfonyl)piperidin-4-yl)amino)ethoxy)adamantan-1-

yl)methyl]-5-methyl-1H-pyrazol-4-yl)picolinate

[1392] A solution of Example 1.18.18 (0.060 g), 1-(methylsulfonyl)piperidin-4-one (0.015 g) and sodium triacetoxyborohydride (0.024 g) was stirred in dichloromethane (0.5 mL) at room temperature. After 30 minutes, the reaction mixture was concentrated. The crude material was dissolved in N,N-dimethylformamide (1.5 mL) and water (0.5 mL) and purified by preparatory reverse-phase HPLC on a Gilson 2020 system using a gradient of 5% to 85% acetonitrile/water. The product-containing fractions were lyophilized to give the title compound as a trifluoroacetic acid salt. MS (ESI) m/e 963.9 (M+H).sup.+.

[1393] 1.30.2 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[3,5-dimethyl-7-(2-{[1-(methylsulfonyl)piperidin-4-yl]amino}ethoxy)tricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid

[1394] A solution of Example 1.30.1 (0.060 g) was dissolved in dichloromethane (0.5 mL) and treated with trifluoroacetic acid (0.5 mL) overnight. The reaction mixture was concentrated. The residue was dissolved in N,N-dimethylformamide (1.5 mL) and water (0.5 mL) and was purified by preparatory reverse-phase HPLC on a Gilson 2020 system using a gradient of 5% to 85% acetonitrile/water. The product-containing fractions were lyophilized to give the title compound.

.sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ 12.90 (s, 1H), 8.53 (d, 2H), 8.08 (d, 1H), 7.84 (d, 1H), 7.66 (d, 1H), 7.58-7.45 (m, 4H), 7.41 (td, 2H), 7.33 (s, 1H), 7.00 (d, 1H), 5.00 (s, 2H), 3.93 (s, 2H), 3.88 (s, 2H), 3.62 (d, 4H), 3.22 (h, 2H), 3.12, 3.06 (s, 2H), 2.93 (s, 3H), 2.79 (d, 2H), 2.15 (s, 3H), 2.11 (s, 1H), 1.61 (qd, 2H), 1.48 (s, 2H), 1.37 (s, 2H), 1.19 (s, 4H), 1.10 (s, 2H), 0.91 (s, 8H). MS (ESI) m/e 907.2 (M+H).sup.+.

[1395] 1.31 Synthesis of 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[3,5-dimethyl-7-(2-{[1-(methylsulfonyl)azetidin-3-yl]amino}ethoxy)tricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid (W3.31)

[1396] A solution of Example 1.18.18 (0.050 g), 1-(methylsulfonyl)azetidin-3-one (0.014 g) and sodium triacetoxyborohydride (0.020 g) was stirred in dichloromethane (0.50 mL) at room temperature. After 30 minutes, acetic acid (5.35 μ L) was added and stirring was continued at room temperature overnight. Trifluoroacetic acid (0.5 mL) was added to the reaction and was stirring continued overnight. The reaction mixture was concentrated. The residue was dissolved in a mixture of N,N-dimethylformamide (2 mL) and water (0.5 mL) and was purified by preparatory reverse-phase HPLC on a Gilson 2020 system using a gradient of 5% to 70% acetonitrile/water. The product-containing fractions were lyophilized to give the title compound. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ 12.86 (s, 1H), 9.13 (s, 2H), 8.03 (d, 1H), 7.79 (d, 1H), 7.62 (d, 1H), 7.54-7.41 (m, 3H), 7.36 (td, 2H), 7.29 (s, 1H), 6.96 (d, 1H), 4.96 (s, 2H), 4.09 (s, 2H), 4.08 (s, 1H), 3.98 (s, 2H), 3.89 (s, 2H), 3.84 (s, 2H), 3.56 (s, 2H), 3.05 (s, 3H), 3.03 (s, 2H), 3.02 (s, 1H), 2.11 (s, 2H), 1.44 (s, 2H), 1.31 (q, 4H), 1.14 (s, 4H), 1.06 (s, 2H), 0.87 (s, 6H). MS (ESI) m/e 879.7 (M+H).sup.+.

[1397] 1.32 Synthesis of 3-{1-[(3-{2-[(3-amino-3-oxopropyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid (W3.32)

[1398] 1.32.1 tert-butyl 3-(1-((3-(2-((3-amino-3-oxopropyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinate

[1399] A mixture of Example 1.18.18 (245 mg) and acrylamide (217 mg) in N,N-dimethylformamide (5 mL) was heated at 50° C. for 3 days and was purified by reverse phase HPLC, eluted with 30%-80% acetonitrile in 0.1% trifluoroacetic acid in water solution, to provide the title compound. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ 12.83 (s, 1H), 8.30 (s, 2H), 8.00 (dd, 1H), 7.76 (d, 1H), 7.57 (d, 2H), 7.44 (ddd, 3H), 7.39-7.29 (m, 2H), 7.21 (s, 1H), 7.13 (s, 1H), 6.91 (d, 1H), 4.95 (s, 2H), 3.81 (d, 4H), 3.53 (t, 2H), 3.05 (dq, 6H), 2.06 (s, 3H), 1.43 (s,

2H), 1.27 (q, 4H), 1.13 (d, 15H), 0.82 (s, 6H). MS (ESI) m/e 873.8 (M+H).sup.+.

[1400] 1.32.2 3-{1-[(3-{2-[(3-amino-3-oxopropyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid

[1401] The title compound was prepared using the procedure in Example 1.26.10, replacing Example 1.26.9 with Example 1.32.1. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ 8.29 (s, 2H), 8.00 (dd, 1H), 7.76 (d, 1H), 7.63-7.52 (m, 2H), 7.49-7.38 (m, 3H), 7.37-7.29 (m, 2H), 7.25 (s, 1H), 7.11 (s, 1H), 6.92 (d, 1H), 4.92 (s, 2H), 3.53 (t, 2H), 3.04 (ddt, 6H), 2.07 (s, 3H), 1.39 (s, 2H), 1.26 (q, 4H), 1.16-0.93 (m, 6H), 0.83 (s, 6H). MS (ESI) m/e 817.2 (M+H).sup.+.

[1402] 1.33 Synthesis of 6-[3-(1,3-benzothiazol-2-ylcarbamoyl)-1H-indazol-5-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid (W3.33)

[1403] 1.33.1 5-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-1-(2-trimethylsilyl-ethoxymethyl)-1H-indazole-3-carboxylic acid ethyl ester

[1404] Ethyl 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-indazole-3-carboxylate (1000 mg) was dissolved in N,N-dimethylformamide (30 mL). Sodium hydride (60% in mineral oil, 83 mg) was added, and the solution was stirred at room temperature for 20 minutes. (2-(Chloromethoxy)ethyl)trimethylsilane (580 mg) was added, and the solution was stirred at room temperature for 90 minutes. The reaction was quenched with saturated aqueous ammonium chloride (10 mL) and diluted with water (90 mL). The solution was extracted with 70% ethyl acetate in heptanes (50 mL) twice. The combined organic portions were washed with water (25 mL) and then brine (25 mL). The solution was dried on anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography on silica gel, eluting with 10-30% ethyl acetate in heptanes. The solvent was removed under reduced pressure to yield the title compound. MS (ESI) m/e 447 (M+H).sup.+.

[1405] 1.33.2 ethyl 5-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-((tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-indazole-3-carboxylate

[1406] Example 1.33.1 (335 mg) and Example 1.1.11 (483 mg) were dissolved in 1,4-dioxane (3 mL). 2 M aqueous sodium carbonate (1.13 mL) was added, and the solution was degassed and flushed with nitrogen three times. Dichloro[1,1'-bis(diphenylphosphino)ferrocene]palladium(II) (61 mg) was added, and the solution was degassed and flushed with nitrogen once. The solution was heated at 75° C. for 16 hours. The solution was cooled, and 0.1 M aqueous HCl (25 mL) was added. The solution was extracted with ethyl acetate (50 mL) twice. The combined organic portions were washed with brine (25 mL) and dried on anhydrous sodium sulfate. The solution was filtered, concentrated under reduced pressure and purified by flash column chromatography on silica gel, eluting with 50% ethyl acetate in heptanes. The solvent was removed under reduced pressure to yield the title compound. MS (ESI) m/e 927 (M+NH.sub.4—H.sub.2O).sup.+.

[1407] 1.33.3 5-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-((tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-indazole-3-carboxylic acid

[1408] The title compound was prepared by substituting Example 1.33.2 for Example 1.13.9 in Example 1.13.10. MS (ESI) m/e 899 (M+H).sup.+ , 897 (M-H).sup.-.

[1409] 1.33.4 tert-butyl 6-(3-(benzo[d]thiazol-2-ylcarbamoyl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-indazol-5-yl)-3-(1-((3-(2-((tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinate

[1410] The title compound was prepared by substituting Example 1.33.3 for Example 1.13.10 in Example 1.13.11. MS (ESI) m/e 1030 (M+NH.sub.4—H.sub.2O).sup.+ , 1029 (M-H).sup.-.

[1411] 1.33.5 6-[3-(1,3-benzothiazol-2-ylcarbamoyl)-1H-indazol-5-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-

carboxylic acid

[1412] Example 1.33.4 (83 mg) was dissolved in dichloromethane (0.5 mL). Trifluoroacetic acid (740 mg) was added, and the solution was stirred at room temperature for 16 hours. The solvents were removed under reduced pressure. The residue was dissolved in 1,4-dioxane (1 mL), and 1 M aqueous sodium hydroxide (0.5 mL) was added. The solution was stirred at room temperature for 60 minutes. The reaction was quenched with trifluoroacetic acid (0.1 mL) and purified by reverse-phase HPLC using 10-85% acetonitrile in water (w/0.1% trifluoroacetic acid) over 30 minutes on a Grace Reveleris® equipped with a Phenomenex® Luna® column: C.sub.18(2), 100 Å, 150×30 mm. Product fractions were combined, frozen, and lyophilized to yield the title compound as the bis trifluoroacetic acid salt. ¹H NMR (400 MHz, dimethyl sulfoxide-d₆) δ ppm 14.23 (s, 1H), 12.58 (bs, 1H), 8.97 (s, 1H), 8.34-8.29 (m, 3H), 8.22 (d, 1H), 8.04 (d, 1H), 7.91 (d, 1H), 7.87-7.81 (m, 2H), 7.51-7.45 (m, 2H), 7.36 (t, 1H), 3.92 (s, 3H), 3.58 (m, 2H), 3.04 (m, 2H), 2.58-2.56 (m, 2H), 2.26 (s, 3H), 1.47 (s, 2H), 1.34 (q, 4H), 1.22-1.14 (m, 4H), 1.07 (q, 2H), 0.89 (m, 6H). MS (ESI) m/e 745 (M+H).sup.+, 743 (M-H).sup.-.

[1413] 1.34 Synthesis of 6-[3-(1,3-benzothiazol-2-ylcarbamoyl)-1H-indol-5-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid (W3.34)

[1414] 1.34.1 5-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-1-(2-trimethylsilyl-ethoxymethyl)-1H-indole-3-carboxylic acid methyl ester

[1415] The title compound was prepared by substituting methyl 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-indole-3-carboxylate for ethyl 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-indazole-3-carboxylate in Example 1.33.1. MS (ESI) m/e 432 (M+H).sup.+, 430 (M-H).sup.-.

[1416] 1.34.2 methyl 5-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-((tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-indole-3-carboxylate

[1417] The title compound was prepared by substituting Example 1.34.1 for Example 1.33.1 in Example 1.33.2. MS (ESI) m/e 912 (M+H).sup.+, 910 (M-H).sup.-.

[1418] 1.34.3 5-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-((tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-indole-3-carboxylic acid

[1419] The title compound was prepared by substituting Example 1.34.2 for Example 1.13.9 in Example 1.13.10. MS (ESI) m/e 898 (M+H).sup.+, 896 (M-H).sup.-.

[1420] 1.34.4 tert-butyl 6-(3-(benzo[d]thiazol-2-ylcarbamoyl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-indol-5-yl)-3-(1-((3-(2-((tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinate

[1421] The title compound was prepared by substituting Example 1.34.3 for Example 1.13.10 in Example 1.13.11. MS (ESI) m/e 1030 (M+H).sup.+, 1028 (M-H).sup.-.

[1422] 1.34.5 6-[3-(1,3-benzothiazol-2-ylcarbamoyl)-1H-indol-5-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid

[1423] The title compound was prepared by substituting Example 1.34.4 for Example 1.33.4 in Example 1.33.5. ¹H NMR (400 MHz, dimethyl sulfoxide-d₆) δ ppm 12.47 (bs, 1H), 12.18 (s, 1H), 9.01 (s, 1H), 8.70 (d, 1H), 8.28 (bs, 3H), 8.12 (d, 1H), 8.05 (dd, 1H), 7.99 (d, 1H), 7.86 (d, 1H), 7.76 (d, 1H), 7.64 (d, 1H), 7.50 (s, 1H), 7.46 (td, 1H), 7.32 (t, 1H), 3.92 (s, 3H), 3.58 (m, 2H), 3.04 (m, 2H), 2.57 (m, 2H), 2.26 (s, 3H), 1.47 (s, 2H), 1.34 (q, 4H), 1.24-1.14 (m, 4H), 1.08 (m, 2H), 0.90 (s, 6H). MS (ESI) m/e 744 (M+H).sup.+, 742 (M-H).sup.-.

[1424] 1.35 Synthesis of 6-[3-(1,3-benzothiazol-2-ylcarbamoyl)-1H-pyrrolo[2,3-b]pyridin-5-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid (W3.35)

[1425] 1.35.1 5-bromo-1-(2-trimethylsilyl-ethoxymethyl)-1H-pyrrolo[2,3-b]pyridine-3-

carboxylic acid methyl ester

[1426] The title compound was prepared by substituting methyl 5-bromo-1H-pyrrolo[2,3-b]pyridine-3-carboxylate for ethyl 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-indazole-3-carboxylate in Example 1.33.1. MS (ESI) m/e 385, 387 (M+H).sup.+.

[1427] 1.35.2 5-(4,4,5,5-tetramethyl-[1,3,2]dioxaborolan-2-yl)-1-(2-trimethylsilanyl-ethoxymethyl)-1H-pyrrolo[2,3-b]pyridine-3-carboxylic acid methyl ester

[1428] The title compound was prepared by substituting Example 1.35.1 for Example 1.13.7 in Example 1.13.8. MS (ESI) m/e 433(M+H).sup.+.

[1429] 1.35.3 methyl 5-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-((tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrrolo[2,3-b]pyridine-3-carboxylate

[1430] The title compound was prepared by substituting Example 1.35.2 for Example 1.33.1 in Example 1.33.2. MS (ESI) m/e 913 (M+H).sup.+.

[1431] 1.35.4 5-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-((tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrrolo[2,3-b]pyridine-3-carboxylic acid

[1432] The title compound was prepared by substituting Example 1.35.3 for Example 1.13.9 in Example 1.13.10. MS (ESI) m/e 899 (M+H).sup.+, 897 (M-H).sup.-.

[1433] 1.35.5 tert-butyl 6-(3-(benzo[d]thiazol-2-ylcarbamoyl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrrolo[2,3-b]pyridin-5-yl)-3-(1-((3-(2-((tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinate

[1434] The title compound was prepared by substituting Example 1.35.4 for Example 1.13.10 in Example 1.13.11. MS (ESI) m/e 1031 (M+H).sup.+, 1029 (M-H).sup.-.

[1435] 1.35.6 6-[3-(1,3-benzothiazol-2-ylcarbamoyl)-1H-pyrrolo[2,3-b]pyridin-5-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid

[1436] The title compound was prepared by substituting Example 1.35.5 for Example 1.33.4 in Example 1.33.5. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 12.74 (d, 1H), 12.62 (bs, 1H), 9.26 (d, 1H), 9.13 (d, 1H), 8.83 (d, 1H), 8.28 (bs, 2H), 8.25 (d, 1H), 7.99 (d, 1H), 7.91 (d, 1H), 7.78 (d, 1H), 7.51 (s, 1H), 7.47 (t, 1H), 7.33 (t, 1H), 3.92 (s, 3H), 3.58 (t, 2H), 3.04 (m, 2H), 2.57 (t, 2H), 2.26 (s, 3H), 1.47 (s, 2H), 1.34 (q, 4H), 1.20 (t, 4H), 1.08 (q, 2H), 0.90 (s, 6H). MS (ESI) m/e 745 (M+H).sup.+, 743 (M-H).sup.-.

[1437] 1.36 Synthesis of 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-((3-(2-((2-(N,N-dimethylsulfamoyl)ethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid (W3.36)

[1438] To a solution of Example 1.18.18 (69.8 mg) in N,N-dimethylformamide (6 mL) was added N,N-dimethylethanesulfonamide (118 mg), N,N-diisopropylethylamine (0.2 mL) and H.sub.2O (0.2 mL).

[1439] The mixture was stirred at room temperature 4 days. The reaction mixture was diluted with ethyl acetate (200 mL), washed with water and brine, and dried over anhydrous sodium sulfate. After evaporation of the solvent, the residue was dissolved in dichloromethane and trifluoroacetic acid (10 mL, 1:1), and the resulting solution was stirred overnight. The solvents were removed under reduced pressure. The residue was diluted with N,N-dimethylformamide (2 mL), filtered and purified by reverse-phase HPLC on a Gilson system (C18 column), eluting with 20-80% acetonitrile in water containing 0.1% trifluoroacetic acid, to give the title compound. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 12.82 (s, 1H), 8.53 (s, 2H), 8.00 (dd, 1H), 7.76 (d, 1H), 7.59 (dd, 1H), 7.53-7.37 (m, 4H), 7.37-7.28 (m, 2H), 7.26 (s, 1H), 6.92 (d, 1H), 4.92 (s, 2H), 3.80 (s, 2H), 3.54 (t, 2H), 3.44-3.34 (m, 2H), 3.30 (s, 2H), 3.11 (s, 2H), 2.98 (t, 2H), 2.77 (s, 6H), 2.07 (s, 3H), 1.39 (s, 2H), 1.27 (q, 4H), 1.11 (s, 4H), 1.06-0.93 (m, 2H), 0.83 (s, 7H). MS (ESI) m/e 881.2 (M+H).sup.+.

[1440] 1.37 Synthesis of 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)naphthalen-2-yl]-3-{1-[(3-{2-[(3-hydroxypropyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid (W3.37)

[1441] 1.37.1 2-((3,5-dimethyl-7-((5-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazol-1-yl)methyl)adamantan-1-yl)oxy)ethanol

[1442] To a solution of Example 1.1.6 (8.9 g) and [1,1'-

bis(diphenylphosphino)ferrocene]dichloropalladium(II) dichloromethane (818 mg) in acetonitrile (120 mL) was added triethylamine (10 mL) and pinacolborane (12.8 mL). The mixture was stirred at reflux overnight. The mixture was cooled to room temperature and used in the next reaction directly. MS (ESI) m/e 467.3 (M+Na).sup.+.

[1443] 1.37.2 tert-butyl 6-chloro-3-(1-((3-(2-hydroxyethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinate

[1444] To a solution of tert-butyl 3-bromo-6-chloropicolinate (6.52 g) in tetrahydrofuran (100 mL) and water (20 mL) was added Example 1.37.1 (9.90 g), (1S,3R,5R,7S)-1,3,5,7-tetramethyl-8-tetradecyl-2,4,6-trioxa-8-phosphaadamantane (0.732 g), tris(dibenzylideneacetone)dipalladium(0) (1.02 g), and potassium phosphate (23.64 g), and the mixture was stirred at reflux overnight. The solvents were removed under vacuum. The residue was dissolved in ethyl acetate (500 mL), washed with water and brine, and dried over anhydrous sodium sulfate. Filtration and evaporation of the solvent gave a residue that purified by silica gel chromatography, eluting with 20% ethyl acetate in heptane, to give the title compound. MS (ESI) m/e 530.3 (M+H).sup.+.

[1445] 1.37.3 tert-butyl 3-{1-[(3-{2-[bis(tert-butoxycarbonyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]decan-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}-6-chloropyridine-2-carboxylate tert-butyl 6-chloro-3-(1-((3,5-dimethyl-7-(2-((methylsulfonyl)oxy)ethoxy)adamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinate

[1446] To a cooled (0° C.) stirring solution of Example 1.37.2 (3.88 g) in dichloromethane (30 mL) and triethylamine (6 mL) was added methanesulfonyl chloride (2.52 g). The mixture was stirred at room temperature for 4 hours. The reaction mixture was diluted with ethyl acetate (400 mL), washed with water and brine, and dried over anhydrous sodium sulfate. Filtration and evaporation of the solvent gave the title compound, which was used in the next reaction without further purification. MS (ESI) m/e 608.1 (M+H).sup.+.

[1447] 1.37.4 tert-butyl 3-{1-[(3-{2-[bis(tert-butoxycarbonyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}-6-chloropyridine-2-carboxylate

[1448] To a solution of Example 1.37.3 (151 mg) in N,N-dimethylformamide (3 mL) was added di-tert-butyl iminodicarboxylate (54 mg). The mixture was stirred at room temperature overnight. The reaction mixture was diluted with ethyl acetate (200 mL), washed with water and brine, and dried over anhydrous sodium sulfate. Filtration and evaporation of the solvent gave the title compound, which was used in the next step without further purification. MS (ESI) m/e 729.4 (M+H).sup.+.

[1449] 1.37.5 7-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-((tert-butoxycarbonyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-1-naphthoic acid

[1450] To a solution of methyl 7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-naphthoate (257 mg) in 1,4-dioxane (10 mL) and water (5 mL) was added Example 1.37.4 (600 mg), bis(triphenylphosphine)palladium(II) dichloride (57.8 mg), and cesium fluoride (375 mg). The mixture was stirred at 120° C. for 30 minutes under microwave conditions (Biotage Initiator). The mixture was diluted with ethyl acetate (200 mL), washed with water and brine, dried over anhydrous sodium sulfate, filtered and concentrated. Evaporation of the solvent gave a residue that purified by silica gel chromatography, eluting with 20% ethyl acetate in heptane, to give an intermediate di-ester. The residue was dissolved in tetrahydrofuran (10 mL), methanol (5 mL) and water (5 mL) and LiOH H.sub.2O (500 mg) was added. The mixture was stirred at room temperature overnight. The mixture was acidified with aqueous 2N HCl, dissolved in 400 mL of

ethyl acetate, washed with water and brine and dried over anhydrous sodium sulfate. Filtration and evaporation of the solvent gave the title compound. MS (APCI) m/e 765.3 (M+H).sup.+.

[1451] 1.37.6 3-(1-((3-(2-aminoethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)naphthalen-2-yl)picolinic acid

[1452] To a solution of Example 1.37.5 (500 mg) in dichloromethane (10 mL) was added benzo[d]thiazol-2-amine (98 mg), 1-ethyl-3-[3-(dimethylamino)propyl]-carbodiimide hydrochloride (251 mg) and 4-(dimethylamino)pyridine (160 mg). The mixture was stirred at room temperature overnight. The reaction mixture was diluted with ethyl acetate (400 mL), washed with water and brine, dried over anhydrous sodium sulfate, filtered and concentrated. The residue was dissolved in dichloromethane and trifluoroacetic acid (10 mL, 1:1), and the solution was stirred overnight. The solvents were removed, and the residue was dissolved in N,N-dimethylformamide (12 mL) and purified by reverse-phase HPLC on a Gilson system (C18 column), eluting with 20-80% acetonitrile in water containing 0.1% trifluoroacetic acid, to give the title compound. MS (ESI) m/e 741.2 (M+H).sup.+.

[1453] 1.37.7 3-((tert-butyldimethylsilyl)oxy)propanal

[1454] To a solution of dimethyl sulfoxide (2.5 mL) in dichloromethane (40 mL) at -78° C. was added oxalyl chloride (1.5 mL). The mixture was stirred 20 minutes at -78° C., and a solution of (3-((tert-butyldimethylsilyl)oxy)propan-1-ol (1.9 g) in dichloromethane (10 mL) was added by syringe.

[1455] After 1 hour, triethylamine (5 mL) was added. The cooling bath was removed, and the reaction was stirred overnight. The reaction mixture was diluted with ethyl acetate (300 mL), washed with water and brine, and dried over anhydrous sodium sulfate. Filtration and evaporation of solvent gave the title compound. MS (DCI) m/e 206.0(M+NH.sub.4).sup.+.

[1456] 1.37.8 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)naphthalen-2-yl]-3-{1-[(3-{2-[(3-hydroxypropyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid

[1457] To a solution of Example 1.37.6 (125 mg) in dichloromethane (10 mL) was added Example 1.37.7 (32 mg). The mixture was stirred at room temperature for 1 hour, and NaBH(OAc).sub.3 (107 mg) was added to the reaction mixture. The mixture was stirred at room temperature overnight. To the reaction mixture was added 2N aqueous sodium hydroxide (5 mL), and the reaction stirred for 4 hours. The mixture was neutralized with aqueous 2N HCl and extracted with ethyl acetate (100 mL×3). The combined organic layers were washed with aqueous 2% HCl, water and brine and dried over anhydrous sodium sulfate. Filtration and evaporation of the solvent gave a residue that was purified by reverse-phase HPLC on a Gilson system (C18 column), eluting with 20-80% acetonitrile in water containing 0.1% trifluoroacetic acid, to give a solid. The residue was dissolved in tetrahydrofuran (6 mL) and tetrabutyl ammonium fluoride (1 M in tetrahydrofuran, 4 mL) was added. The mixture was stirred at room temperature for 2 hours, and the solvents were removed under vacuum. The residue was dissolved in dimethyl sulfoxide/methanol (1:1, 12 mL) and was purified by reverse-phase HPLC on a Gilson system (C18 column), eluting with 20-80% acetonitrile in water containing 0.1% trifluoroacetic acid, to give the title compound. .sup.1H NMR (501 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 13.09 (s, 1H), 9.01 (s, 1H), 8.36 (dd, 1H), 8.20 (ddd, 5H), 8.09-8.02 (m, 1H), 8.03-7.95 (m, 1H), 7.92 (d, 1H), 7.80 (d, 1H), 7.69 (dd, 1H), 7.53-7.43 (m, 2H), 7.36 (ddd, 1H), 3.89 (s, 2H), 3.56 (t, 2H), 3.47 (t, 2H), 3.10-2.93 (m, 4H), 2.22 (s, 3H), 1.78-1.68 (m, 2H), 1.44 (s, 2H), 1.30 (q, 4H), 1.20-1.11 (m, 4H), 1.04 (q, 2H), 0.87 (s, 7H). MS (ESI) m/e 799.2 (M+H).sup.+.

[1458] 1.38 Synthesis of 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[3-(2-[[3-(dimethylamino)-3-oxopropyl]amino]ethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid (W3.38)

[1459] To a solution of Example 1.18.18 (55 mg) in N,N-dimethylformamide (6 mL) was added

N,N-dimethylacrylamide (73.4 mg), N,N-diisopropylethylamine (0.2 mL) and water (0.2 mL). The mixture was stirred at room temperature 4 days. The reaction mixture was diluted with ethyl acetate (200 mL), washed with water and brine, and dried over anhydrous sodium sulfate. After filtration and evaporation of the solvent, the residue was dissolved in dichloromethane and trifluoroacetic acid (10 mL, 1:1). After stirring for 16 hours, the mixture was concentrated under reduced pressure. The residue was dissolved in N,N-dimethylformamide (8 mL) and purified by reverse-phase HPLC on a Gilson system (C18 column), eluting with 20-80% acetonitrile in water containing 0.1% trifluoroacetic acid, to give the title compound. ¹H NMR (400 MHz, dimethyl sulfoxide-*d*₆) δ ppm 12.84 (s, 1H), 8.22 (s, 3H), 8.02 (d, 1H), 7.78 (d, 1H), 7.60 (d, 1H), 7.55-7.39 (m, 3H), 7.39-7.30 (m, 2H), 7.27 (s, 1H), 6.94 (d, 1H), 4.94 (s, 2H), 3.87 (t, 2H), 3.81 (s, 2H), 3.55 (t, 2H), 3.20-2.95 (m, 6H), 2.92 (s, 3H), 2.82 (s, 3H), 2.69 (q, 3H), 2.09 (s, 3H), 1.40 (s, 2H), 1.28 (q, 4H), 1.14 (d, 4H), 1.07-0.94 (m, 2H), 0.85 (s, 8H). MS (ESI) *m/e* 845.3 (M+H).⁺

[1460] 1.39 Synthesis of 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[3,5-dimethyl-7-(2-{[3-(methylamino)-3-oxopropyl]amino}ethoxy)tricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid (W3.39)

[1461] The title compound was prepared as described in Example 1.38, by replacing N,N-dimethylacrylamide with N-methylacrylamide. ¹H NMR (501 MHz, dimethyl sulfoxide-*d*₆) δ ppm 12.84 (s, 1H), 8.32 (s, 2H), 8.08-7.96 (m, 2H), 7.78 (d, 1H), 7.60 (d, 1H), 7.52-7.40 (m, 3H), 7.39-7.30 (m, 2H), 7.27 (s, 1H), 6.94 (d, 1H), 4.94 (s, 2H), 3.87 (t, 2H), 3.81 (s, 2H), 3.12 (p, 2H), 3.01 (dt, 4H), 2.57 (d, 3H), 2.09 (s, 3H), 1.40 (s, 2H), 1.28 (q, 5H), 1.18-1.07 (m, 4H), 1.02 (q, 2H), 0.85 (s, 7H). MS (ESI) *m/e* 831.3 (M+H).⁺

[1462] 1.40 Synthesis of 3-(1-{[3-(2-aminoacetamido)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]decan-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-{8-[(1,3-benzothiazol-2-yl)carbamoyl]-3,4-dihydroisoquinolin-2(1H)-yl}pyridine-2-carboxylic acid (W3.40)

[1463] 1.40.1 1-((3-bromo-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazole

[1464] To a cooled (-30° C.) solution of Example 1.1.3 (500 mg) in tetrahydrofuran (30 mL) was added *n*-butyllithium (9.67 mL), and the mixture was stirred at -30° C. for 2 hours. Methyl iodide (1.934 mL) was added dropwise at -30° C. After completion of the addition, the mixture was stirred at -30° C. for additional 2 hours. 1N aqueous HCl in ice water was added slowly, such that the temperature was maintained below 0° C., until the pH reached 6. The mixture was stirred at room temperature for 10 minutes, and was diluted with ice-water (10 mL) and ethyl acetate (20 mL). The layers were separated, and the aqueous was extracted twice with ethyl acetate. The combined organic phases were washed with brine, dried over MgSO₄, filtered and concentrated. The residue was purified by flash silica gel chromatography, eluting with 15/1 to 10/1 petroleum/ethyl acetate, to give the title compound. MS (LC-MS) *m/e* 337, 339 (M+H).⁺

[1465] 1.40.2 1-(3,5-dimethyl-7-((5-methyl-1H-pyrazol-1-yl)methyl)adamantan-1-yl)urea

[1466] Example 1.40.1 (2.7 g) and urea (4.81 g) were mixed and stirred at 140° C. for 16 hours. The mixture was cooled to room temperature and suspended in methanol (200 mL×2). The insoluble material was removed by filtration. The filtrate was concentrated to give the title compound. MS (LC-MS) *m/e* 317.3 (M+H).⁺

[1467] 1.40.3 3,5-dimethyl-7-((5-methyl-1H-pyrazol-1-yl)methyl)adamantan-1-amine

[1468] To a solution of Example 1.40.2 (2.53 g) in 20% ethanol in water (20 mL) was added sodium hydroxide (12.79 g). The mixture was stirred at 120° C. for 16 hours and at 140° C. for another 16 hours. 6N Aqueous HCl was added until the pH reached 6. The mixture was concentrated, and the residue was suspended in methanol (200 mL). The insoluble material was filtered off. The filtrate was concentrated to give the title compound as an HCl salt. MS (LC-MS) *m/e* 273.9 (M+H).⁺

[1469] 1.40.4 *tert*-butyl (2-((3,5-dimethyl-7-((5-methyl-1H-pyrazol-1-yl)methyl)adamantan-1-yl)amino)-2-oxoethyl)carbamate

[1470] To a solution of Example 1.40.3 (2.16 g) in N,N-dimethylformamide (100 mL) was added triethylamine (3.30 mL), 2-((tert-butoxycarbonyl)amino)acetic acid (1.799 g) and O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (3.90 g). The mixture was stirred at room temperature for 2 hours. Water (40 mL) was added, and the mixture was extracted with ethyl acetate (70 mL×2). The combined organic phases were washed with brine, dried over sodium sulfate, filtered and concentrated. The residue was purified by silica gel chromatography, eluting with 3/1 to 2/1 petroleum/ethyl acetate, to give the title compound. MS (LC-MS) m/e 430.8 (M+H).sup.+.

[1471] 1.40.5 tert-butyl (2-((3-((4-iodo-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)amino)-2-oxoethyl)carbamate

[1472] To an ambient solution of Example 1.40.4 (1.7 g) in N,N-dimethylformamide (20 mL) was added N-iodosuccinimide (1.066 g) in portions, and the mixture was stirred at room temperature for 16 hours. Ice-water (10 mL) and saturated aqueous Na.sub.2S.sub.2O.sub.3 solution (10 mL) were added. The mixture was extracted with ethyl acetate (30 mL×2). The combined organic phases were washed with brine, dried over sodium sulfate, filtered and concentrated. The residue was purified by silica gel chromatography, eluting with 3/1 to 2/1 petroleum/ethyl acetate, to give the title compound. MS (LC-MS) m/e 556.6 (M+H).sup.+.

[1473] 1.40.6 methyl 2-(5-bromo-6-(tert-butoxycarbonyl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1474] To a solution of methyl 1,2,3,4-tetrahydroisoquinoline-8-carboxylate hydrochloride (12.37 g) and Example 1.4.4 (15 g) in dimethyl sulfoxide (100 mL) was added N,N-diisopropylethylamine (12 mL), and the mixture was stirred at 50° C. for 24 hours. The mixture was then diluted with ethyl acetate (500 mL) and washed with water and brine. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluting with 20% ethyl acetate in hexane, to give the title compound. MS (ESI) m/e 448.4 (M+H).sup.+.

[1475] 1.40.7 methyl 2-(6-(tert-butoxycarbonyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1476] To a solution of Example 1.40.6 (2.25 g) and [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (205 mg) in acetonitrile (30 mL) was added triethylamine (3 mL) and pinacolborane (2 mL), and the mixture was stirred at reflux for 3 hours. The mixture was diluted with ethyl acetate (200 mL) and washed with water and brine. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. Purification of the residue by flash chromatography, eluting with 20% ethyl acetate in hexane, provided the title compound.

[1477] 1.40.8 methyl 2-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-((tert-butoxycarbonyl)amino)acetamido)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1478] The title compound was prepared using the procedure in Example 1.4.7, replacing Example 1.4.6 and Example 1.4.2 with Example 1.40.7 and Example 1.40.5, respectively. MS (ESI) m/e 797.4 (M+H).sup.+.

[1479] 1.40.9 2-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-((tert-butoxycarbonyl)amino)acetamido)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylic acid

[1480] The title compound was prepared using the procedure in Example 1.26.8, replacing Example 1.26.7 with Example 1.40.8. MS (ESI) m/e 783.4 (M+H).sup.+.

[1481] 1.40.10 tert-butyl 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-((3-(2-((tert-butoxycarbonyl)amino)acetamido)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinate

[1482] The title compound was prepared using the procedure in Example 1.26.9, replacing

Example 1.26.8 with Example 1.40.9. MS (ESI) m/e 915.3 (M+H).sup.+.

[1483] 1.40.11 3-(1-{[3-(2-aminoacetamido)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]decan-1-yl)methyl}-5-methyl-1H-pyrazol-4-yl)-6-{8-[(1,3-benzothiazol-2-yl)carbamoyl]-3,4-dihydroisoquinolin-2(1H)-yl}pyridine-2-carboxylic acid

[1484] The title compound was prepared using the procedure in Example 1.26.10, replacing Example 1.26.9 with Example 1.40.10. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ 12.82 (s, 1H), 8.00 (dd, 1H), 7.90-7.79 (m, 4H), 7.76 (d, 1H), 7.59 (dd, 1H), 7.49-7.38 (m, 3H), 7.37-7.29 (m, 2H), 7.25 (s, 1H), 6.92 (d, 1H), 4.92 (s, 2H), 3.85 (t, 2H), 3.77 (s, 2H), 3.40 (q, 2H), 2.98 (t, 2H), 2.07 (s, 3H), 1.63 (s, 2H), 1.57-1.38 (m, 4H), 1.15-0.93 (m, 6H), 0.80 (s, 6H). MS (ESI) m/e 759.2 (M+H).sup.+.

[1485] 1.41 Synthesis of 3-[1-({3-[(2-aminoethyl)sulfanyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-yl)carbamoyl]-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid (W3.41)

[1486] 1.41.1 3-bromo-5,7-dimethyladamantane-1-carboxylic acid

[1487] To a solution of bromine (18.75 mL) was added iron (10.19 g) at 0° C., and the mixture was stirred for 30 minutes. 3,5-Dimethyladamantane-1-carboxylic acid (19 g) was added to the above mixture portionwise. The mixture was stirred at room temperature for 36 hours. After adding ice-water (50 mL) and 6N aqueous HCl (100 mL), the mixture was treated with Na.sub.2SO.sub.3 (100 g dissolved in 500 mL water). The aqueous layer was extracted with dichloromethane (300 mL \times 4). The combined organic layers were washed with 1N aqueous HCl (300 mL) and brine, dried over magnesium sulfate, filtered and concentrated to give the title compound, which was used in the next step without additional purification. .sup.1H NMR: (400 MHz, CDCl.sub.3) δ ppm 2.23 (s, 2H), 2.01-1.74 (m, 4H), 1.61-1.47 (m, 6H), 0.93 (s, 6H). LC-MS (ESI) m/e 285.0 (M+H).sup.+.

[1488] 1.41.2 3-bromo-5,7-dimethyladamantan-1-yl)methanol

[1489] To a solution of Example 1.41.1 (10 g) in tetrahydrofuran (20 mL) was added BH.sub.3.THF (69.6 mL). The mixture was stirred at room temperature for 16 hours. Upon the completion of the reaction, methanol (20 mL) was added dropwise, and the resulting mixture was stirred for 30 minutes. The mixture was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel, eluting with petroleum ether/ethyl acetate (from 8/1 to 5/1), to give the title compound. .sup.1H NMR: (400 MHz, CDCl.sub.3) δ ppm 3.28 (s, 2H), 1.98-1.95 (m, 6H), 1.38-1.18 (m, 7H), 0.93 (s, 6H).

[1490] 1.41.3 1-((3-bromo-5,7-dimethyladamantan-1-yl)methyl)-1H-pyrazole

[1491] A mixture of 2-(tributylphosphoranylidene)acetonitrile (919 mg), 1H-pyrazole (259 mg) and Example 1.41.2 (800 mg) in toluene (8 mL) was stirred at 90° C. for 16 hours. The mixture was concentrated, and the residue was diluted with ethyl acetate (50 mL). The mixture was washed with brine, dried over magnesium sulfate, filtered and concentrated. The residue was purified by silica gel chromatography, eluting with petroleum ether/ethyl acetate, to give the title compound. LC-MS (ESI) m/e 325.1 (M+H).sup.+.

[1492] 1.41.4 3-((1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantane-1-thiol

[1493] A mixture of Example 1.41.3 (2.8 g) and thiourea (15.82 g) in 33% (w/w) HBr in acetic acid (50 mL) was stirred at 110° C. for 16 hours and concentrated under reduced pressure to give a residue. The residue was dissolved in 20% ethanol in water (v/v: 200 mL), and sodium hydroxide (19.06 g) was added. The resulting solution was stirred at room temperature for 16 hours and concentrated. The residue was dissolved in water (60 mL), and acidified with 6N aqueous HCl to pH 5-pH 6. The mixture was extracted with ethyl acetate (200 mL \times 2). The combined organic layers were washed with brine, dried over MgSO.sub.4, filtered and concentrated to give the title compound. MS (ESI) m/e 319.1 (M+H).sup.+.

[1494] 1.41.5 2-((-3-((1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)thio)ethanol

[1495] To a solution of Example 1.41.4 (3.3 g) in ethanol (120 mL) was added sodium ethoxide (2.437 g). The mixture was stirred for 10 minutes, and 2-chloroethanol (1.80 mL) was added

dropwise. The mixture was stirred at room temperature for 6 hours and neutralized with 1N aqueous HCl to pH 7. The mixture was concentrated, and the residue was extracted with ethyl acetate (200 mL×2). The combined organic layers were washed with brine, dried over MgSO₄, filtered and concentrated. The residue was purified by column chromatography on silica gel, eluting with petroleum ether/ethyl acetate from 6/1 to 2/1, to give the title compound. MS (ESI) m/e 321.2 (M+H)⁺.

[1496] 1.41.6 2-((-3,5-dimethyl-7-((5-methyl-1H-pyrazol-1-yl)methyl)adamantan-1-yl)thio)ethanol

[1497] To a solution of Example 1.41.5 (2.3 g) in tetrahydrofuran (60 mL) was added n-butyllithium (14.35 mL, 2M in hexane) at -20° C. dropwise under nitrogen. The mixture was stirred for 2 hours. Methyl iodide (4.49 mL) was added to the resulting mixture at -20° C., and the mixture was stirred at -20° C. for 2 hours. The reaction was quenched by the dropwise addition of saturated aqueous NH₄Cl solution at -20° C. The resulting mixture was stirred for 10 minutes and acidified with 1N aqueous HCl to pH 5. The mixture was extracted with ethyl acetate twice. The combined organic layers were washed with brine, dried over MgSO₄, filtered and concentrated to give the title compound. MS (ESI) m/e 335.3 (M+H)⁺.

[1498] 1.41.7 2-((-3-((4-iodo-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)thio)ethanol

[1499] To a solution of Example 1.41.6 (3.65 g) in N,N-dimethylformamide (90 mL) was added N-iodosuccinimide (3.68 g). The mixture was stirred at room temperature for 16 hours. The reaction was quenched by the addition of ice-water (8 mL) and saturated aqueous Na₂S₂O₃ solution (8 mL). The mixture was stirred for an additional 10 minutes and extracted with ethyl acetate (30 mL×2). The combined organic layers were washed with brine, dried over MgSO₄, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluting with petroleum ether/ethyl acetate (6/1 to 3/1), to give the title compound. MS (ESI) m/e 461.2 (M+H)⁺.

[1500] 1.41.8 di-tert-butyl[2-({3-[(4-iodo-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]decan-1-yl}sulfanyl)ethyl]-2-imidodicarbonate

[1501] To a cold solution (0° C. bath) of Example 1.41.7 (3 g) in dichloromethane (100 mL) was added triethylamine (1.181 mL) and mesyl chloride (0.559 mL). The mixture was stirred at room temperature for 4 hours, and the reaction was quenched by the addition of ice-water (30 mL). The mixture was stirred for an additional 10 minutes and was extracted with dichloromethane (50 mL×2). The combined organic layers were washed with brine, dried over MgSO₄, filtered and concentrated under reduced pressure. The residue was dissolved in acetonitrile (100 mL) and NH(Boc)₂ (1.695 g) and Cs₂CO₃ (4.24 g) were added. The mixture was stirred at 85° C. for 16 hours, and the reaction was quenched by the addition of water (20 mL). The mixture was stirred for 10 minutes and was extracted with ethyl acetate (40 mL×2). The combined organic layers were washed with brine, dried over MgSO₄, filtered and concentrated. The residue was purified by silica gel chromatography, eluting with petroleum ether/ethyl acetate from 10/1 to 6/1, to give the title compound. MS (ESI) m/e 660.1 (M+H)⁺.

[1502] 1.41.9 methyl 2-[5-(1-({3-({2-[bis(tert-butoxycarbonyl)amino]ethyl}sulfanyl)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]decan-1-yl)methyl}-5-methyl-1H-pyrazol-4-yl)-6-(tert-butoxycarbonyl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1503] The title compound was prepared using the procedure in Example 1.4.7, replacing Example 1.4.6 and Example 1.4.2 with Example 1.40.7 and Example 1.41.8, respectively. LC-MS (ESI) m/e 900.6 (M+H)⁺.

[1504] 1.41.10 2-(6-(tert-butoxycarbonyl)-5-(1-((3-((2-((tert-butoxycarbonyl)amino)ethyl)thio)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylic acid

[1505] A slurry of lithium hydroxide (553 mg) in water (4.03 mL) and methanol (4 mL) was cooled to 15° C. A solution of Example 1.41.9 (800 mg) in tetrahydrofuran (3.23 mL) and methanol (4

mL) was added slowly, and the reaction was stirred at room temperature. After 18 hours the reaction was cooled in an ice-bath and 1.8 g of phosphoric acid in water (4 mL) was added. The biphasic mixture was transferred to a separatory funnel and extracted with ethyl acetate to give the title compound. LC-MS (ESI) m/e 786.2 (M+H).sup.+.

[1506] 1.41.11 tert-butyl 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-((3-((2-((tert-butoxycarbonyl)amino)ethyl)thio)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinate

[1507] A 4 mL amber vial containing Example 1.41.10 (699 mg) was charged with ethyl acetate (5 mL) and 1,1'-carbonyldiimidazole (231 mg) and was stirred for 7 hours at room temperature. A solution of benzo[d]thiazol-2-amine (227 mg) and 1,8-diazabicyclo[5.4.0]undec-7-ene (0.228 mL) in acetonitrile (3 mL) was added, and the reaction was heated to 70° C. After stirring for 18 hours, the reaction was quenched by the addition of 10 mL 1N aqueous HCl and was extracted with ethyl acetate to give the title compound, which was used in the subsequent step without further purification. MS (ESI) m/e 818.2 (M+H).sup.+.

[1508] 1.41.12 3-[1-({3-[(2-aminoethyl)sulfanyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl}-5-methyl-1H-pyrazol-4-yl]-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid

[1509] To a solution of Example 1.41.11 (510 mg) in dichloromethane (10 mL) was added trifluoroacetic acid (10 mL), and the reaction was stirred at room temperature for 30 minutes. The reaction was quenched with aqueous saturated NaHCO₃ solution and extracted with dichloromethane. The product was purified by reverse-phase HPLC on a Gilson system (C18 column), eluting with 5-80% acetonitrile in water containing 0.1% trifluoroacetic acid, to give the title compound. ¹H NMR (400 MHz, DMSO-d₆) δ ppm 12.86 (bs, 1H), 8.03 (d, 1H), 7.76 (m, 2H), 7.62 (d, 1H), 7.39 (m, 6H), 6.95 (t, 1H), 5.07 (s, 1H), 4.96 (s, 1H), 3.85 (m, 4H), 3.01 (t, 2H), 2.97 (t, 2H), 2.90 (m, 2H), 2.69 (m, 2H), 2.11 (s, 3H), 1.54 (s, 2H), 1.36 (m, 4H), 1.17 (m, 4H), 1.08 (m, 2H), 0.84 (s, 6H). MS (ESI) m/e 762.2 (M+H).sup.+.

[1510] 1.42 Synthesis of 3-(1-{[3-(3-aminopropyl)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl}-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid (W3.42) 1.42.1 1-((3-allyl-5,7-dimethyladamantan-1-yl)methyl)-1H-pyrazole

[1511] To a solution of Example 1.41.3 (0.825 g) in toluene (5 mL) was added N, N'-azoisobutyronitrile (AIBN, 0.419 g) and allyltributylstannane (2.039 mL). The mixture was purged with N₂ stream for 15 minutes, heated at 80° C. for 8 hours and concentrated. The residue was purified by silica gel chromatography, eluting with 5% ethyl acetate in petroleum ether, to provide the title compound. MS (ESI) m/e 285.2 (M+H).sup.+.

[1512] 1.42.2 1-((3-allyl-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazole

[1513] To a solution of Example 1.42.1 (200 mg) in tetrahydrofuran (5 mL) at -78° C. under N₂ was added n-butyllithium (2.81 mL, 2.5 M in hexane). The mixture was stirred for 2 hours while the temperature increased to -20° C. and was stirred at -20° C. for 1 hour. Iodomethane (0.659 mL) was added, and the resulting mixture was stirred for 0.5 hour at -20° C. The reaction was quenched with saturated aqueous NH₄Cl solution and extracted with ethyl acetate twice. The organic layer was washed with brine to give the title compound. MS (ESI) m/e 299.2 (M+H).sup.+.

[1514] 1.42.3 3-(3,5-dimethyl-7-((5-methyl-1H-pyrazol-1-yl)methyl)adamantan-1-yl)propan-1-ol

[1515] Under a nitrogen atmosphere, a solution of Example 1.42.2 (2.175 g, 7.29 mmol) in anhydrous tetrahydrofuran (42.5 mL) was cooled to 0° C. BH₃.sub.3.Math.THF (15.30 mL) was added dropwise. The reaction mixture was stirred at room temperature for 2 hours and cooled to 0° C. To the reaction mixture was added 10N aqueous NaOH (5.03 mL) dropwise, followed by 30 percent H₂O.sub.2(16.52 mL) water solution. The resulting mixture was warmed to room temperature and stirred for 90 minutes. The reaction was quenched with 10 percent aqueous

hydrochloric acid (35 mL). The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (2×60 mL). The combined organic layers were washed with brine (3×60 mL) and cooled in an ice bath. A saturated aqueous solution of sodium sulfite (15 mL) was carefully added and the mixture was stirred for a few minutes. The organic layer was dried over sodium sulfate, filtered, and concentrated in vacuo. The residue was purified by silica gel chromatography, eluting with petroleum ether/ethyl acetate (3:1 to 1:1), to provide the title compound. MS (ESI) m/e 317.3 (M+H).sup.+.

[1516] 1.42.4 3-(3-((4-iodo-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)propan-1-ol

[1517] A mixture of Example 1.42.3 (1.19 g) and 1-iodopyrrolidine-2,5-dione (1.015 g) in N,N-dimethylformamide (7.5 mL) was stirred for 16 hours at room temperature. The reaction was quenched with saturated aqueous Na.sub.2SO.sub.3 solution. The mixture was diluted with ethyl acetate and washed with saturated aqueous Na.sub.2SO.sub.3, saturated aqueous Na.sub.2CO.sub.3 solution, water and brine. The organic layer was dried over anhydrous Na.sub.2SO.sub.4, filtered, and concentrated. The residue was purified by silica gel chromatography, eluting with petroleum ether/ethyl acetate (3:1 to 1:1), to provide the title compound. MS (ESI) m/e 443.1 (M+H).sup.+.

[1518] 1.42.5 3-(3-((4-iodo-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyladamantan-1-yl)propyl methanesulfonate

[1519] To a solution of Example 1.42.4 (1.55 g, 3.50 mmol) in dichloromethane (20 mL) at 0° C. were added triethylamine (0.693 mL) and mesyl chloride (0.374 mL) slowly. The mixture was stirred for 3.5 hours at 20° C. and was diluted with dichloromethane. The organic layer was washed with saturated aqueous NH.sub.4Cl, saturated aqueous NaHCO.sub.3 solution and brine. The organic layer was dried over Na.sub.2SO.sub.4, filtered and concentrated to provide the title compound. MS (ESI) m/e 521.1 (M+H).sup.+.

[1520] 1.42.6 di-tert-butyl (3-13-[(4-iodo-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]decan-1-yl}propyl)-2-imidodicarbonate

[1521] To a solution of Example 1.42.5 (1.92 g) in acetonitrile (40 mL) at 20° C. were added di-tert-butyl iminodicarbonate (0.962 g) and Cs.sub.2CO.sub.3 (2.404 g). The mixture was stirred for 16 hours at 80° C. and diluted with ethyl acetate, washed with water and brine. The organic layer was dried over Na.sub.2SO.sub.4, filtered and concentrated. The residue was purified by silica gel chromatography, eluting with petroleum ether/ethyl acetate (10:1), to provide the title compound. MS (ESI) m/e 642.3 (M+H).sup.+.

[1522] 1.42.7 methyl 2-[5-{1-[(3-{3-[bis(tert-butoxycarbonyl)amino]propyl})-5,7-dimethyltricyclo[3.3.1.1^{3,7}]decan-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}-6-(tert-butoxycarbonyl)pyridin-2-yl]-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1523] The title compound was prepared using the procedure in Example 1.4.7, replacing Example 1.4.6 and Example 1.4.2 with Example 1.40.7 and Example 1.42.6, respectively. LC-MS (ESI) m/e 882.6 (M+H).sup.+.

[1524] 1.42.8 2-(6-(tert-butoxycarbonyl)-5-(1-((3-(3-((tert-butoxycarbonyl)amino)propyl)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylic acid

[1525] The title compound was prepared using the procedure in Example 1.41.10 substituting Example 1.42.7 for Example 1.41.9. LC-MS (ESI) m/e 468.5 (M+H).sup.+.

[1526] 1.42.9 tert-butyl 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-((3-(3-((tert-butoxycarbonyl)amino)propyl)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinate

[1527] The title compound was prepared using the procedure in Example 1.41.11 substituting Example 1.42.8 for Example 1.41.10.

[1528] 1.42.10 3-(1-{[3-(3-aminopropyl)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl)-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-

yl]pyridine-2-carboxylic acid

[1529] The title compound was prepared using the procedure in Example 1.41.12 substituting Example 1.42.9 for Example 1.41.11. ¹H NMR (500 MHz, DMSO-d₆) δ ppm 12.86 (s, 1H), 8.03 (d, 1H), 7.79 (d, 1H), 7.62 (d, 4H), 7.47 (dt, 3H), 7.36 (q, 2H), 7.27 (s, 1H), 6.95 (d, 1H), 4.95 (s, 2H), 3.77 (s, 2H), 3.01 (t, 2H), 2.72 (q, 2H), 2.09 (s, 3H), 1.45 (t, 2H), 1.18-1.05 (m, 9H), 1.00 (d, 6H), 0.80 (s, 6H). MS (ESI) m/e 468.5 (M+H)⁺.

[1530] 1.43 Synthesis of 3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]decan-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-{5-[(1,3-benzothiazol-2-yl)carbamoyl]quinolin-3-yl}pyridine-2-carboxylic acid (W3.43)

[1531] 1.43.1 methyl 3-bromoquinoline-5-carboxylate

[1532] To a solution of 3-bromoquinoline-5-carboxylic acid (2 g) in methanol (30 mL) was added concentrated H₂SO₄ (5 mL). The solution was stirred at reflux overnight. The mixture was concentrated under reduced pressure. The residue was dissolved in ethyl acetate (300 mL) and washed with aqueous Na₂CO₃ solution, water and brine. After drying over anhydrous sodium sulfate, filtration and evaporation of the solvent gave the title product. MS (ESI) m/e 266 (M+H)⁺.

[1533] 1.43.2 methyl 3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)quinoline-5-carboxylate

[1534] To a solution of Example 1.43.1 (356 mg) in N,N-dimethylformamide (5 mL) was added [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (55 mg), potassium acetate (197 mg) and bis(pinacolato)diboron (510 mg). The mixture was stirred at 60° C. overnight. The mixture was cooled to room temperature and used in the next reaction without further work up. MS (ESI) m/e 339.2 (M+Na)⁺.

[1535] 1.43.3 methyl 3-[5-{1-[(3-{2-[bis(tert-butoxycarbonyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]decan-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}-6-(tert-butoxycarbonyl)pyridin-2-yl]quinoline-5-carboxylate

[1536] To a solution of Example 1.43.2 (626 mg) in 1,4-dioxane (10 mL) and water (5 mL) was added Example 1.23.3 (1.46 g), bis(triphenylphosphine)palladium(II) dichloride (140 mg), and CsF (911 mg). The mixture was stirred at 120° C. for 30 minutes under microwave conditions (Biotage Initiator). The mixture was diluted with ethyl acetate (200 mL), washed with water and brine, dried over anhydrous sodium sulfate, filtered and concentrated. The residue was purified by silica gel chromatography, eluting with 20% ethyl acetate in heptane (1 L) to give the title product. MS (ESI) m/e 880.3 (M+H)⁺.

[1537] 1.43.4 3-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-((tert-butoxycarbonyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)quinoline-5-carboxylic acid

[1538] To a solution of Example 1.43.3 (1.34 g) in tetrahydrofuran (10 mL), methanol (5 mL) and water (5 mL) was added LiOH H₂O (120 mg), and the mixture was stirred at room temperature overnight. The mixture was acidified with 2N aqueous HCl, diluted with ethyl acetate (400 mL), washed with water and brine and dried over anhydrous sodium sulfate. Filtration and evaporation of solvent gave the title product. MS (APCI) m/e 766.3 (M+H)⁺.

[1539] 1.43.5 3-(1-{[3-(2-aminoethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]decan-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)-6-{5-[(1,3-benzothiazol-2-yl)carbamoyl]quinolin-3-yl}pyridine-2-carboxylic acid

[1540] To a solution of Example 1.43.4 (200 mg) in dichloromethane (10 mL) was added benzo[d]thiazol-2-amine (39.2 mg), 1-ethyl-3-[3-(dimethylamino)propyl]-carbodiimide hydrochloride (50 mg) and 4-dimethylaminopyridine (32 mg). The mixture was stirred at room temperature overnight. The reaction mixture was diluted with ethyl acetate (200 mL), washed with water and brine, dried over anhydrous sodium sulfate, filtered and concentrated. The residue was dissolved in dichloromethane and trifluoroacetic acid (10 mL, 1:1), and the reaction was stirred overnight. The mixture was concentrated, and the residue was dissolved in N,N-

dimethylformamide (12 mL) and purified by reverse-phase HPLC on a Gilson system (C18 column), eluting with 20-80% acetonitrile in water containing 0.1% trifluoroacetic acid, to give the title product. MS (ESI) m/e 742.1 (M+H).sup.+.

[1541] 1.44 Synthesis of 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3,5-dimethyl-7-{2-[(2-sulfoethyl)amino]ethoxy}tricyclo[3.3.1.1^{sup}.3,7]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid (W3.44)

[1542] 1.44.1 tert-butyl 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-[1-({3,5-dimethyl-7-[(2,2,7,7-tetramethyl-10,10-dioxido-3,3-diphenyl-4,9-dioxa-10⁰⁶-thia-13-aza-3-silapentadecan-15-yl)oxy]tricyclo[3.3.1.1^{sup}.3,7]dec-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylate

[1543] To a solution of Example 1.18.18 (500 mg) in N,N-dimethylformamide (8 mL) was added 4-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylbutyl ethenesulfonate (334 mg). The reaction was stirred at room temperature overnight and methylamine (0.3 mL) was added to quench the reaction. The resulting mixture was stirred for 20 minutes and purified by reverse-phase chromatography using an Analogix system (C18 column), eluting with 50-100% acetonitrile in water containing 0.1% v/v trifluoroacetic acid, to provide the title compound.

[1544] 1.44.2 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3,5-dimethyl-7-{2-[(2-sulfoethyl)amino]ethoxy}tricyclo[3.3.1.1^{sup}.3,7]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid

[1545] Example 1.44.1 (200 mg) in dichloromethane (5 mL) was treated with trifluoroacetic acid (2.5 mL) overnight. The reaction mixture was concentrated and purified by reverse phase chromatography (C18 column), eluting with 20-60% acetonitrile in water containing 0.1% v/v trifluoroacetic acid, to provide the title compound. .sup.1H NMR (500 MHz, dimethylsulfoxide-d.sub.6) δ ppm 12.86 (s, 1H), 8.32 (s, 2H), 8.02 (d, 1H), 7.78 (d, 1H), 7.60 (d, 1H), 7.51 (d, 1H), 7.40-7.49 (m, 2H), 7.31-7.39 (m, 2H), 7.27 (s, 1H), 6.95 (d, 1H), 4.94 (s, 2H), 3.87 (t, 2H), 3.81 (s, 2H), 3.15-3.25 (m, 2H), 3.03-3.13 (m, 2H), 3.00 (t, 2H), 2.79 (t, 2H), 2.09 (s, 3H), 1.39 (s, 2H), 1.22-1.34 (m, 4H), 0.94-1.18 (m, 6H), 0.85 (s, 6H). MS (ESI) m/e 854.1 (M+H).sup.+.

[1546] Example 2. Synthesis of Exemplary Synthons

[1547] This example provides synthetic methods for exemplary synthons useful more making ADCs.

[1548] 2.1 Synthesis of N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[(2-((3-[(4-{6-[1-(1,3-benzothiazol-2-ylcarbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyltricyclo[3.3.1.1^{sup}.3,7]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]phenyl}-N^{sup}.5-carbamoyl-L-ornithinamide (Synthon BS)

[1549] Example 1.1.14 (72 mg) and 4-((S)-2-((S)-2-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl (4-nitrophenyl) carbonate (91 mg) in N,N-dimethylformamide (3 mL) was cooled in a water-ice bath and N,N-diisopropylethylamine (0.12 mL) was added. The mixture was stirred at 0° C. for 2 hours and acetic acid (0.057 mL) was added. After concentration of the solvents, the residue was purified via HPLC (20-80% acetonitrile in 0.1% TFA/water) to provide the title compound. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 9.98 (s, 1H), 8.40 (s, 1H), 8.06 (d, 1H), 8.00 (d, 1H), 7.74-7.89 (m, 4H), 7.59 (d, 2H), 7.46 (s, 2H), 7.37 (t, 1H), 7.18-7.32 (m, 4H), 6.99 (s, 2H), 6.01 (s, 1H), 4.98 (s, 3H), 4.38 (d, 2H), 3.47 (d, 2H), 3.36 (t, 2H), 3.28 (t, 2H), 2.91-3.10 (m, 2H), 2.79-2.91 (m, 4H), 2.19-2.25 (m, 3H), 2.06-2.20 (m, 2H), 1.89-2.02 (m, 3H), 1.53-1.74 (m, 2H), 1.30-1.55 (m, 8H), 1.06-1.29 (m, 10H), 0.91-1.06 (m, 2H), 0.76-0.89 (m, 12H). MS (ESI) m/e 1356.3 (M+H).sup.+.

[1550] 2.2 Synthesis of N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[(2-((3-[(4-{6-[4-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydro-2H-1,4-benzoxazin-6-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyltricyclo[3.3.1.1^{sup}.13,7]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]phenyl}-N^{sup}5-carbamoyl-L-ornithinamide (Synthon

(DK)

[1551] To a solution of 4-((S)-2-((S)-2-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl 4-nitrophenyl carbonate (57 mg) and Example 1.2.2 (57 mg) in N,N-dimethylformamide (6 mL) was added N,N-diisopropylethylamine (0.5 mL). The mixture was stirred overnight. The mixture was concentrated under vacuum and the residue was diluted with methanol (3 mL) and acetic acid (0.3 mL), loaded onto a 300 g reverse-phase column, and eluted with 30-70% acetonitrile in 0.1% aqueous TFA solution to provide the title compound. ¹H NMR (400 MHz, dimethyl sulfoxide-d₆) δ ppm 9.97 (s, 1H), 8.73 (d, 1H), 8.07 (d, 1H), 7.90-7.98 (m, 1H), 7.71-7.87 (m, 4H), 7.54-7.63 (m, 2H), 7.45 (d, 1H), 7.32-7.42 (m, 2H), 7.17-7.31 (m, 3H), 6.92-7.03 (m, 3H), 5.88-6.08 (m, 1H), 4.97 (s, 3H), 4.29-4.46 (m, 4H), 4.12-4.26 (m, 4H), 3.86 (s, 3H), 3.21-3.41 (m, 8H), 2.78-3.10 (m, 6H), 2.20 (s, 3H), 1.90-2.18 (m, 3H), 0.92-1.77 (m, 24H), 0.75-0.88 (m, 6H). MS (ESI) m/e 1360.2 (M+H).⁺

[1552] 2.3 Synthesis of N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[(2-({3-[(4-{6-[4-(1,3-benzothiazol-2-ylcarbamoyl)-1-methyl-1,2,3,4-tetrahydroquinoxalin-6-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl)(methyl)carbamoyl}oxy)methyl]phenyl}-N5-carbamoyl-L-ornithinamide (Synthon DQ)

[1553] The title compound was prepared by substituting Example 1.3.2 for Example 1.2.2 in Example 2.2. ¹H NMR (500 MHz, dimethyl sulfoxide-d₆) δ ppm 9.99 (s, 1H), 8.17-8.35 (m, 1H), 8.07 (d, 1H), 7.89 (d, 1H), 7.71-7.84 (m, 4H), 7.55-7.65 (m, 2H), 7.43 (s, 1H), 7.36 (t, 1H), 7.28 (d, 2H), 7.21 (t, 1H), 6.99 (s, 2H), 6.83 (d, 1H), 5.97 (s, 1H), 5.28-5.51 (m, 2H), 4.98 (s, 2H), 4.32-4.44 (m, 1H), 4.19 (dd, 1H), 3.97-4.13 (m, 2H), 3.85 (s, 2H), 3.29 (d, 3H), 3.00 (s, 3H), 2.80-2.98 (m, 4H), 2.18-2.26 (m, 3H), 1.88-2.17 (m, 3H), 0.91-1.73 (m, 23H), 0.74-0.92 (m, 12H). MS (ESI) m/e 1373.3 (M+H).⁺

[1554] 2.4 Synthesis of 4-[(1E)-3-({2-({3-[(4-{6-[1-(1,3-benzothiazol-2-ylcarbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl)(methyl)carbamoyl}oxy)prop-1-en-1-yl]-2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid (Synthon DJ)

[1555] 2.4.1 (E)-tert-butyldimethyl((3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)allyl)oxy)silane

[1556] To a flask charged with tert-butyldimethyl(prop-2-yn-1-yloxy)silane (5 g) and dichloromethane (14.7 mL) under nitrogen atmosphere was added dropwise 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (3.94 g). The mixture was stirred at room temperature for one minute then transferred via cannula to a nitrogen-sparged flask containing Cp₂ZrClH (chloridobis(η⁵-cyclopentadienyl)hydrido zirconium, Schwartz's Reagent) (379 mg). The resulting reaction mixture was stirred at room temperature for 16 hours. The mixture was carefully quenched with water (15 mL), and then extracted with diethyl ether (3×30 mL). The combined organic phases were washed with water (15 mL), dried over MgSO₄, filtered, concentrated and purified by silica gel chromatography, eluting with a gradient from 0-8% ethyl acetate/heptanes to give the title compound. MS (ESI) m/z 316.0 (M+NH₄).⁺

[1557] 2.4.2 (2S,3R,4S,5S,6S)-2-(4-bromo-2-nitrophenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

[1558] (2R,3R,4S,5S,6S)-2-Bromo-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (5 g) was dissolved in acetonitrile (100 mL). Ag₂O (2.92 g) was added to the solution and the reaction was stirred for 5 minutes at room temperature. 4-Bromo-2-nitrophenol (2.74 g) was added and the reaction mixture was stirred at room temperature for 4 hours. The silver salt residue was filtered through diatomaceous earth and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel chromatography eluting with a gradient of 10-70% ethyl acetate in heptanes to provide the title compound. MS (ESI⁺) m/z 550.9 (M+NH₄).⁺

[1559] 2.4.3 (2S,3R,4S,5S,6S)-2-(4-(((E)-3-((tert-butyldimethylsilyl)oxy)prop-1-en-1-yl)-2-nitrophenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

[1560] Example 2.4.2 (1 g), sodium carbonate (0.595 g), tris(dibenzylideneacetone)dipalladium (Pd.sub.2(dba).sub.3) (0.086 g), and 1,3,5,7-tetramethyl-6-phenyl-2,4,8-trioxa-6-phosphaadamantane (0.055 g) were combined in a 3-neck 50-mL round bottom flask equipped with a reflux condenser and the system was degassed with nitrogen. Separately, a solution of Example 2.4.1 (0.726 g) in tetrahydrofuran (15 mL) was degassed with nitrogen for 30 minutes. This latter solution was transferred via cannula into the flask containing the solid reagents, followed by addition of degassed water (3 mL) via syringe. The reaction was heated to 60° C. for two hours. The reaction mixture was partitioned between ethyl acetate (3×30 mL) and water (30 mL). The combined organic phases were dried (Na.sub.2SO.sub.4), filtered, and concentrated. The residue was purified by silica gel chromatography, eluting with a gradient from 0-35% ethyl acetate/heptanes to provide the title compound. MS (ESI+) m/z 643.1 (M+NH.sub.4).sup.+.

[1561] 2.4.4 (2S,3R,4S,5S,6S)-2-(2-amino-4-((E)-3-hydroxyprop-1-en-1-yl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

[1562] A 500-mL three-neck, nitrogen-flushed flask equipped with a pressure-equalizing addition funnel was charged with zinc dust (8.77 g). A degassed solution of Example 2.4.3 (8.39 g) in tetrahydrofuran (67 mL) was added via cannula. The resulting suspension was chilled in an ice bath and then 6N aqueous HCl (22.3 mL) was added dropwise via addition funnel at such a rate that the internal temperature of the reaction did not exceed 35° C. After the addition was complete, the reaction mixture was stirred for two hours at room temperature and then filtered through a pad of diatomaceous earth, rinsing with water and ethyl acetate. The filtrate was treated with saturated aqueous NaHCO.sub.3 solution until the water layer was no longer acidic, and the mixture was filtered to remove the resulting solids. The filtrate was transferred to a separatory funnel and the layers were separated. The aqueous layer was extracted with ethyl acetate (3×75 mL) and the combined organic layers were washed with water (100 mL), dried over Na.sub.2SO.sub.4, filtered, and concentrated. The residue was triturated with diethyl ether and the solid was collected by filtration to give the title compound. MS (ESI+) m/z 482.0 (M+H).sup.+.

[1563] 2.4.5 (9H-fluoren-9-yl)methyl (3-chloro-3-oxopropyl)carbamate

[1564] To a solution of 3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanoic acid (5.0 g) in dichloromethane (53.5 mL) was added sulfurous dichloride (0.703 mL). The mixture was stirred at 60° C. for one hour. The mixture was cooled and concentrated to provide the title compound which was used in the next step without further purification.

[1565] 2.4.6 (2S,3R,4S,5S,6S)-2-(2-(3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-((E)-3-hydroxyprop-1-en-1-yl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

[1566] Example 2.4.4 (6.78 g) was dissolved in dichloromethane (50 mL) and the solution was chilled to 0° C. in an ice bath. N,N-Diisopropylethylamine (3.64 g) was added, followed by dropwise addition of a solution of Example 2.4.5 (4.88 g) in dichloromethane (50 mL). The reaction was stirred for 16 hours allowing the ice bath to come to room temperature. Saturated aqueous NaHCO.sub.3 solution (100 mL) was added and the layers were separated. The aqueous layer was further extracted with dichloromethane (2×50 mL). The extracts were dried over Na.sub.2SO.sub.4, filtered, concentrated and then purified by silica gel chromatography, eluting with a gradient of 5-95% ethyl acetate/heptane, to give an inseparable mixture of starting aniline and desired title compound. This mixture was partitioned between 1N aqueous HCl (40 mL) and a 1:1 mixture of diethyl ether and ethyl acetate (40 mL), and then the aqueous phase was further extracted with ethyl acetate (2×25 mL). The organic phases were combined, washed with water (2×25 mL), dried over Na.sub.2SO.sub.4, filtered, and concentrated to give the title compound. MS (ESI+) m/z 774.9 (M+H).sup.+.

[1567] 2.4.7 (2S,3R,4S,5S,6S)-2-(2-(3-((((9H-fluoren-9-

yl)methoxy)carbonyl)amino)propanamido)-4-((E)-3-(((4-nitrophenoxy)carbonyl)oxy)prop-1-en-1-yl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

[1568] Example 2.4.6 (3.57 g) was dissolved in dichloromethane (45 mL) and bis(4-nitrophenyl)carbonate (2.80 g) was added, followed by dropwise addition of N,N-diisopropylethylamine (0.896 g). The reaction was stirred at room temperature for two hours. Silica gel (20 g) was then added to the reaction solution and the mixture was concentrated to dryness under reduced pressure, keeping the bath temperature at or below 25° C. The silica residue was loaded atop a column and the crude material was purified by silica gel chromatography, eluting with a gradient from 0-100% ethyl acetate-heptane, providing partially purified title compound which was contaminated with nitrophenol. This material was triturated with methyl tert-butyl ether (250 mL) and the resulting slurry was allowed to sit for 1 hour. The title compound was collected by filtration. Three successive crops were collected in a similar fashion to give the title compound. MS (ESI+) m/z 939.8 (M+H).sup.+.

[1569] 2.4.8 3-(1-((3-(2-(((E)-3-(3-(3-aminopropanamido)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)phenyl)allyl)oxy)carbonyl(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(1-(benzo[d]thiazol-2-ylcarbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl)picolinic acid

[1570] Example 1.1.14 (31 mg) and Example 2.4.7 (33.3 mg) in N,N-dimethylformamide (3 mL) at 0° C. was added N,N-diisopropylethylamine (25 µL). The mixture was stirred overnight, diluted with ethyl acetate and washed with water and brine. The organic layer was dried over Na.sub.2SO.sub.4, filtered, and concentrated. The residue was dissolved in methanol (2 mL) and tetrahydrofuran (1 mL), cooled to 0° C., and 3 M lithium hydroxide aqueous solution (0.35 mL) was added. The mixture was stirred at 0° C. for 4 hours, concentrated and purified by a Gilson HPLC system (C18 column), eluting with 0-60% acetonitrile in 0.1% TFA/water to provide the title compound.

[1571] 2.4.9 4-[(1E)-3-({[2-({3-[(4-{6-[1-(1,3-benzothiazol-2-ylcarbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)prop-1-en-1-yl]-2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid

[1572] To a solution of Example 2.4.8 (19 mg) in N,N-dimethylformamide (2.5 mL) at 0° C. was added 2,5-dioxopyrrolidin-1-yl 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoate (10 mg) and N,N-diisopropylethylamine (11.08 µL). The mixture was stirred at 0° C. for 15 minutes and a few drops of acetic acid were added. The mixture was purified by a Gilson HPLC system (C18 column), eluting with 20-60% acetonitrile in 0.1% TFA/water to provide the title compound.

.sup.1H NMR (500 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 9.03 (s, 1H), 8.40 (s, 1H), 8.25 (d, 1H), 8.00 (d, 1H), 7.73-7.91 (m, 4H), 7.46 (s, 2H), 7.37 (t, 1H), 7.29 (d, 1H), 7.22 (t, 1H), 7.08-7.13 (m, 1H), 7.04 (d, 1H), 6.98 (s, 2H), 6.56 (d, 1H), 6.10-6.25 (m, 1H), 4.86 (s, 1H), 4.64 (d, 2H), 3.95 (d, 2H), 3.86 (d, 4H), 3.24-3.41 (m, 4H), 2.79-2.96 (m, 6H), 2.54 (t, 2H), 2.21 (s, 3H), 2.03 (t, 2H), 1.90-1.98 (m, 2H), 1.34-1.52 (m, 6H), 1.20-1.30 (m, 5H), 0.89-1.20 (m, 8H), 0.82 (d, 6H). MS (ESI) m/e 1391.2 (M+H).sup.+.

[1573] 2.5 Synthesis of 4-[(1E)-3-({[2-({3-[(4-{6-[4-(1,3-benzothiazol-2-ylcarbamoyl)-1-methyl-1,2,3,4-tetrahydroquinoxalin-6-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)prop-1-en-1-yl]-2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid (Synthon DO)

[1574] 2.5.1 3-(1-((3-(2-((E)-4-(3-(3-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-(((2S,3R,4S,5S,6S)-3,4,5-triacetoxy-6-(methoxycarbonyl)tetrahydro-2H-pyran-2-yl)oxy)phenyl)-N-methylbut-3-enamido)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(4-(benzo[d]thiazol-2-

ylcarbamoyl)-1-methyl-1,2,3,4-tetrahydroquinoxalin-6-yl)picolinic acid
[1575] To a cold (0° C.) solution of Example 2.4.7 (98 mg) and Example 1.3.2 (91 mg) was added N-ethyl-N-isopropylpropan-2-amine (0.054 mL). The reaction was slowly warmed to room temperature and stirred overnight. The reaction was quenched by the addition of water and ethyl acetate. The layers were separated, and the aqueous was extracted with additional ethyl acetate (2×). The combined organics were dried with anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was used in the subsequent step without further purification. MS (ESI) m/e 1576.8 (M+H).sup.+.

[1576] 2.5.2 3-(1-(((3-(2-((((E)-3-(3-(3-aminopropanamido)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)phenyl)allyl)oxy)carbonyl)

(methylamino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(4-(benzo[d]thiazol-2-ylcarbamoyl)-1-methyl-1,2,3,4-tetrahydroquinoxalin-6-yl)picolinic acid

[1577] To a solution of Example 2.5.1 (158 mg) in tetrahydrofuran/methanol/water (2:1:1, 4 mL) was added lithium hydroxide monohydrate (20 mg). The reaction mixture was stirred overnight. The mixture was concentrated under vacuum, acidified with TFA, and dissolved in dimethyl sulfoxide/methanol (9 mL) and loaded on an HPLC (Gilson system, eluting with 10-85% acetonitrile in 0.1% TFA in water) for purification to give the pure title compound. MS (ESI) m/e 1228.2 (M+NH.sub.4).sup.+.

[1578] 2.5.3 4-[(1E)-3-([2-([3-([4-{6-[4-(1,3-benzothiazol-2-ylcarbamoyl)-1-methyl-1,2,3,4-tetrahydroquinoxalin-6-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)prop-1-en-1-yl]-2-([N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid

[1579] To a solution of Example 2.5.2 (20 mg) and 2,5-dioxopyrrolidin-1-yl 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoate (6.5 mg) in N,N-dimethylformamide (2 mL) was added N,N-diisopropylethylamine (0.054 mL). The reaction was stirred overnight. The reaction mixture was diluted with methanol (2 mL) and acidified with TFA. The mixture was concentrated and purified on HPLC (Gilson system, eluting with 10-85% acetonitrile in 0.1% TFA in water) to give the pure title compound. .sup.1H NMR (500 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 9.03 (s, 1H), 8.25 (s, 2H), 7.85-7.95 (m, 2H), 7.72-7.83 (m, 3H), 7.43 (s, 2H), 7.32-7.37 (m, 1H), 7.17-7.25 (m, 1H), 7.08-7.14 (m, 1H), 7.04 (d, 1H), 6.98 (s, 2H), 6.82 (d, 1H), 6.56 (d, 1H), 6.08-6.25 (m, 1H), 4.82-4.92 (m, 1H), 4.64 (d, 3H), 4.00-4.11 (m, 4H), 3.81-3.94 (m, 6H), 3.27-3.50 (m, 17H), 3.00 (s, 3H), 2.83-2.96 (m, 3H), 2.53-2.59 (m, 2H), 2.20 (s, 3H), 2.03 (t, 2H), 1.37-1.55 (m, 4H), 0.90-1.29 (m, 10H), 0.82 (d, 6H). MS (ESI) m/e 1406.2 (M+H).sup.+.

[1580] 2.6 Synthesis of 4-[(1E)-3-([2-([3-([4-{6-[4-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydro-2H-1,4-benzoxazin-6-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)prop-1-en-1-yl]-2-([N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid (Synthon DP)

[1581] 2.6.1 3-(1-(((3-(2-((E)-4-(3-(3-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-(((2S,3R,4S,5S,6S)-3,4,5-triacetoxy-6-(methoxycarbonyl)tetrahydro-2H-pyran-2-yl)oxy)phenyl)-N-methylbut-3-enamido)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(4-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)picolinic acid

[1582] To a cold (0° C.) solution of Example 2.4.7 (98 mg) and Example 1.2.2 (91 mg) was added N-ethyl-N-isopropylpropan-2-amine (0.054 mL). The reaction was slowly warmed to room temperature and was stirred overnight. The reaction was quenched by the addition of water and ethyl acetate. The layers were separated, and the aqueous layer was extracted twice with additional ethyl acetate. The combined organics were dried with anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residue was used in the subsequent step without further

purification. MS (ESI) m/e 1547.7 (M+H).sup.+.

[1583] 2.6.2 3-(1-((3-(2-((((E)-3-(3-(3-aminopropanamido)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)phenyl)allyl)oxy)carbonyl)

(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(4-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydro-2H-benzo[b][1,4]oxazin-6-yl)picolinic acid

[1584] The title compound was prepared by substituting Example 2.6.1 for Example 2.5.1 in Example 2.5.2. MS (ESI) m/e 1200.1 (M+NH.sub.4).sup.+.

[1585] 2.6.3 4-[(1E)-3-({[2-({3-[4-{6-[4-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydro-2H-1,4-benzoxazin-6-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl)(methyl)carbamoyl}oxy)prop-1-en-1-yl]-2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid

[1586] The title compound was prepared by substituting Example 2.6.2 for Example 2.5.2 in Example 2.5.3. .sup.1H NMR (500 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 9.04 (s, 1H), 8.74 (s, 1H), 8.26 (s, 1H), 7.96 (d, 1H), 7.71-7.92 (m, 4H), 7.35-7.48 (m, 3H), 7.23 (t, 1H), 7.11 (d, 1H), 6.96-7.07 (m, 4H), 6.57 (d, 1H), 6.11-6.24 (m, 1H), 4.81-4.93 (m, 1H), 4.65 (d, 2H), 4.32-4.40 (m, 2H), 4.17 (s, 3H), 3.23-3.51 (m, 14H), 2.83-2.98 (m, 3H), 2.54 (t, 2H), 2.21 (s, 3H), 2.03 (t, 2H), 1.34-1.55 (m, 6H), 0.92-1.31 (m, 13H), 0.82 (d, 6H). MS (ESI) m/e 1415.2 (M+Na).sup.+.

[1587] 2.7 Synthesis of 4-[(1E)-3-({[2-({3-[4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)naphthalen-2-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl)(methyl)carbamoyl}oxy)prop-1-en-1-yl]-2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid (Synthon HO)

[1588] 2.7.1 3-(1-((3-(2-((((E)-3-(3-(3-aminopropanamido)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)phenyl)allyl)oxy)carbonyl)

(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)naphthalen-2-yl)picolinic acid

[1589] To a cold (0° C.) solution of Example 2.4.7 (22 mg) and Example 1.6.3 (20 mg) was added N-ethyl-N-isopropylpropan-2-amine (0.054 mL). The reaction was slowly warmed to room temperature and stirred overnight. The reaction was quenched by the addition of water and ethyl acetate. The layers were separated, and the aqueous layer was extracted twice with additional ethyl acetate. The combined organics were dried with anhydrous sodium sulfate, filtered and concentrated under reduced pressure to give the crude title compound which was dissolved in tetrahydrofuran/methanol/water (2:1:1, 4 mL). Lithium hydroxide monohydrate (40 mg) was added, and the reaction mixture stirred overnight. The mixture was then concentrated under vacuum, acidified with TFA, dissolved in dimethyl sulfoxide/methanol and purified on an HPLC (Gilson system, eluting with 10-85% acetonitrile in 0.1% TFA in water) to give the title compound.

[1590] 2.7.2 4-[(1E)-3-({[2-({3-[4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)naphthalen-2-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl)(methyl)carbamoyl}oxy)prop-1-en-1-yl]-2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid

[1591] The title compound was prepared by substituting Example 2.7.1 for Example 2.5.2 in Example 2.5.3. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 13.09 (s, 1H), 9.02 (s, 2H), 8.37 (d, 1H), 8.12-8.29 (m, 4H), 8.06 (s, 1H), 8.02 (d, 1H), 7.93 (d, 1H), 7.76-7.89 (m, 2H), 7.70 (t, 1H), 7.43-7.54 (m, 2H), 7.37 (t, 1H), 7.00-7.13 (m, 2H), 6.98 (s, 2H), 6.56 (d, 1H), 6.08-6.25 (m, 1H), 4.86 (s, 1H), 4.64 (d, 2H), 3.81-3.94 (m, 6H), 3.18-3.51 (m, 12H), 2.78-2.96 (m, 4H), 2.49-2.59 (m, 2H), 2.22 (s, 3H), 2.03 (t, 2H), 1.33-1.54 (m, 6H), 0.93-1.30 (m, 12H), 0.82 (d, 6H). MS (ESI) m/e 1408.3 (M+Na).sup.+.

[1592] 2.8 Synthesis of 4-[(1E)-3-({[2-({3-[4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-

dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](oxetan-3-yl)carbamoyl}oxy)prop-1-en-1-yl]-2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid (Synthon IT)

[1593] 2.8.1 3-(1-(((3-(2-((((E)-3-(3-(3-aminopropanamido)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)phenyl)allyl)oxy)carbonyl)(oxetan-3-yl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid, Trifluoroacetic Acid

[1594] To a solution of Example 1.16.7 (0.039 g) and Example 2.4.7 (0.048 g) in N,N-dimethylformamide (1 mL) was added N,N-diisopropylethylamine (0.037 mL), and the reaction was stirred at room temperature for 2 days. The reaction was concentrated, the residue was re-dissolved in a mixture of methanol (0.5 mL) and tetrahydrofuran (0.5 mL) and treated with lithium hydroxide monohydrate (0.027 g) in water (0.5 mL), and the solution was stirred at room temperature. After stirring for 1 hour, the reaction was quenched with trifluoroacetic acid (0.066 mL), diluted with N,N-dimethylformamide (1 mL), and purified by HPLC using a Gilson system eluting with 10-60% acetonitrile in water containing 0.1% v/v trifluoroacetic acid. The desired fractions were combined and freeze-dried to provide the title compound.

[1595] 2.8.2 4-[(1E)-3-({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](oxetan-3-yl)carbamoyl}oxy)prop-1-en-1-yl]-2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid

[1596] To a solution of Example 2.8.1 (0.024 g) and 2,5-dioxopyrrolidin-1-yl 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoate (8.95 mg) in N,N-dimethylformamide (0.5 mL) was added N-ethyl-N-isopropylpropan-2-amine (0.017 mL), and the reaction was stirred at room temperature for 2 hours. The reaction was diluted with N,N-dimethylformamide (1 mL) and water (1 mL) and was purified by HPLC using a Gilson system eluting with 10-60% acetonitrile in water containing 0.1% v/v trifluoroacetic acid. The desired fractions were combined and freeze-dried to provide the title compound. ¹H NMR (400 MHz, dimethyl sulfoxide-d₆) δ ppm 12.83 (s, 1H), 9.02 (s, 1H), 8.22 (d, 1H), 8.02 (d, 1H), 7.86 (t, 1H), 7.78 (d, 1H), 7.60 (d, 1H), 7.56-7.39 (m, 3H), 7.39-7.30 (m, 2H), 7.27 (s, 1H), 7.14-6.89 (m, 5H), 6.56 (d, 1H), 4.94 (s, 2H), 4.83 (t, 1H), 4.63 (t, 2H), 4.54 (t, 1H), 3.93-3.83 (m, 6H), 3.83-3.75 (m, 4H), 3.33 (dt, 10H), 2.99 (t, 2H), 2.54 (d, 2H), 2.08 (d, 3H), 2.02 (t, 2H), 1.54-0.72 (m, 26H). MS (ESI) m/e 1433.3 (M+H)⁺.

[1597] 2.9 Synthesis of 4-[(1E)-3-({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](2-methoxyethyl)carbamoyl}oxy)prop-1-en-1-yl]-2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid (Synthon KA)

[1598] 2.9.1 3-(1-(((3-(2-((((E)-3-(3-(3-aminopropanamido)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)phenyl)allyl)oxy)carbonyl)(2-methoxyethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

[1599] Example 1.12.10 (150 mg) was dissolved in N,N-dimethylformamide (0.5 mL), and Example 2.4.7 (190 mg) and N-ethyl-N-isopropylpropan-2-amine (0.30 mL) was added. The reaction was stirred at room temperature overnight. Additional Example 2.4.7 (70 mg) and N,N-diisopropylethylamine (0.10 mL) were added and the reaction was allowed to stir another day. The reaction was then concentrated and the residue was dissolved in tetrahydrofuran (2 mL) and methanol (2 mL), then 1.94N aqueous lithium hydroxide monohydrate (1.0 mL) was added and the mixture was stirred at room temperature for one hour. Purification by reverse phase chromatography (C18 column), eluting with 10-90% acetonitrile in 0.1% TFA/water, provided the

title compound as a trifluoroacetic acid salt. MS (ESI) m/e 1270.4 (M-H).sup.-.

[1600] 2.9.2 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-((3-(2-(((E)-3-(4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)-3-(3-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)propanamido)phenyl)allyl)oxy)carbonyl)(2-methoxyethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid

[1601] Example 2.9.1 (16 mg) was dissolved in N,N-dimethylformamide (0.3 mL), then 2,5-dioxopyrrolidin-1-yl 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoate (5 mg) and N-ethyl-N-isopropylpropan-2-amine (11 µL) were added. The reaction mixture was stirred for three hours at room temperature, and purification by reverse phase chromatography (C18 column), eluting with 10-90% acetonitrile in 0.1% TFA/water, provided the title compound. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 12.71 (v br s, 1H), 9.03 (s, 1H), 8.25 (s, 1H), 8.01 (d, 1H), 7.87 (br m, 1H), 7.76 (t, 2H), 7.50 (d, 1H), 7.46 (t, 1H), 7.33 (t, 1H), 7.28 (s, 1H), 7.08 (d, 1H), 7.03 (m, 2H), 6.98 (s, 2H), 6.56 (d, 1H), 6.17 (m, 1H), 5.00 (s, 2H), 4.86 (br m, 1H), 4.64 (d, 2H), 3.88 (m, 6H), 3.79 (br m, 2H), 3.43, 3.35 (m, m, total 16H), 3.22 (s, 3H), 2.80 (m, 2H), 2.54 (m, 2H), 2.09 (s, 3H), 2.03 (t, 2H), 1.45 (m, 6H), 1.37 (br m, 2H), 1.28-0.90 (m, 10H), 0.77-0.82 (m, 6H). MS (ESI) m/e 1463.5 (M-H).sup.-.

[1602] 2.10 Synthesis of 4-[(1E)-3-({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](2-methoxyethyl)carbamoyl}oxy)prop-1-en-1-yl]-2-({N-[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid (Synthon KB)

[1603] Example 2.9.1 (16 mg) was dissolved in N,N-dimethylformamide (0.3 mL), then 2,5-dioxopyrrolidin-1-yl 2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetate (4 mg) and N-ethyl-N-isopropylpropan-2-amine (11 µL) were added. The reaction mixture was stirred for three hours at room temperature, and purification by reverse phase chromatography (C18 column), eluting with 10-90% acetonitrile in 0.1% TFA/water, provided the title compound. 1H NMR (400 MHz, dimethyl sulfoxide-d₆) δ ppm 9.06 (s, 1H), 8.25 (br m, 2H), 8.01 (d, 1H), 7.76 (t, 2H), 7.49 (d, 1H), 7.47 (t, 1H), 7.33 (t, 1H), 7.28 (s, 1H), 7.11 (d, 1H), 7.08 (s, 2H), 7.03 (m, 2H), 6.56 (d, 1H), 6.17 (m, 1H), 5.00 (s, 2H), 4.86 (br m, 1H), 4.64 (d, 2H), 4.02 (s, 2H), 3.88 (m, 6H), 3.79 (br m, 2H), 3.43, 3.35 (m, m, total 14H), 3.22 (s, 3H), 2.80 (m, 2H), 2.57 (m, 2H), 2.09 (s, 3H), 1.37 (br m, 2H), 1.28-0.90 (m, 10H), 0.77-0.82 (m, 6H). MS (ESI) m/e 1407.4 (M-1).sup.-.

[1604] 2.11 Synthesis of 4-[({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](2-methoxyethyl)carbamoyl}oxy)methyl]-3-[2-(2-{[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid (Synthon KT)

[1605] 2.11.1 (2S,3R,4S,5S,6S)-2-(4-formyl-3-hydroxyphenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

[1606] 2,4-Dihydroxybenzaldehyde (15 g) and (2S,3R,4S,5S,6S)-2-bromo-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (10 g) were dissolved in acetonitrile followed by the addition of silver carbonate (10 g) and the reaction was heated to 49° C. After stirring for 4 hours, the reaction was cooled, filtered and concentrated. The crude title compound was suspended in dichloromethane and was filtered through diatomaceous earth and concentrated. The residue was purified by silica gel chromatography, eluting with ethyl acetate/heptane, to provide the title compound.

[1607] 2.11.2 (2S,3R,4S,5S,6S)-2-(3-hydroxy-4-(hydroxymethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

[1608] A solution of Example 2.11.1 (16.12 g) in tetrahydrofuran (200 mL) and methanol (200 mL) was cooled to 0° C. and sodium borohydride (1.476 g) was added portionwise. The reaction was

stirred for 20 minutes and quenched with a 1:1 mixture of water:aqueous saturated sodium bicarbonate solution (400 mL). The resulting solids were filtered off and rinsed with ethyl acetate. The phases were separated and the aqueous layer extracted four times with ethyl acetate. The combined organic layers were dried over magnesium sulfate, filtered, and concentrated. The crude title compound was purified via silica gel chromatography eluting with heptane/ethyl acetate to provide the title compound. MS (ESI) m/e 473.9 (M+NH₄).sup.+.

[1609] 2.11.3 (2S,3R,4S,5S,6S)-2-(4-(((tert-butyldimethylsilyl)oxy)methyl)-3-hydroxyphenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

[1610] To Example 2.11.2 (7.66 g) and tert-butyldimethylsilyl chloride (2.78 g) in dichloromethane (168 mL) at -5° C. was added imidazole (2.63 g) and the reaction was stirred overnight allowing the internal temperature of the reaction to warm to 12° C. The reaction mixture was poured into saturated aqueous ammonium chloride and extracted four times with dichloromethane. The combined organics were washed with brine, dried over magnesium sulfate, filtered and concentrated. The crude title compound was purified via silica gel chromatography eluting with heptane/ethyl acetate to provide the title compound. MS (ESI) m/e 593.0 (M+Na).sup.+.

[1611] 2.11.4 (2S,3R,4S,5S,6S)-2-(3-(2-(2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethoxy)ethoxy)-4-(((tert-butyldimethylsilyl)oxy)methyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

[1612] To Example 2.11.3 (5.03 g) and triphenylphosphine (4.62 g) in toluene (88 mL) was added di-tert-butyl-azodicarboxylate (4.06 g) and the reaction was stirred for 30 minutes. (9H-Fluoren-9-yl)methyl (2-(2-hydroxyethoxy)ethyl)carbamate was added and the reaction was stirred for an additional 1.5 hours. The reaction was loaded directly onto silica gel and was eluted with heptane/ethyl acetate to provide the title compound.

[1613] 2.11.5 (2S,3R,4S,5S,6S)-2-(3-(2-(2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethoxy)ethoxy)-4-(hydroxymethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

[1614] Example 2.11.4 (4.29 g) was stirred in a 3:1:1 solution of acetic acid:water:tetrahydrofuran (100 mL) overnight. The reaction was poured into saturated aqueous sodium bicarbonate and extracted with ethyl acetate. The organic layer was dried over magnesium sulfate, filtered and concentrated. The crude title compound was purified via silica gel chromatography, eluting with heptane/ethyl acetate, to provide the title compound.

[1615] 2.11.6 (2S,3R,4S,5S,6S)-2-(3-(2-(2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethoxy)ethoxy)-4-(((4-nitrophenoxy)carbonyl)oxy)methyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

[1616] To a solution of Example 2.11.5 (0.595 g) and bis(4-nitrophenyl) carbonate (0.492 g) in N,N-dimethylformamide (4 mL) was added N-ethyl-N-isopropylpropan-2-amine (0.212 mL). After 1.5 hours, the reaction was concentrated under high vacuum. The reaction was loaded directly onto silica gel and eluted using heptane/ethyl acetate to provide the title compound. MS (ESI) m/e 922.9 (M+Na).sup.+.

[1617] 2.11.7 3-(1-((3-(2-(((2-(2-(2-aminoethoxy)ethoxy)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(2-methoxyethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-yl)carbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

[1618] Example 1.12.10 (150 mg) was dissolved in dimethylformamide (0.5 mL). Example 2.11.6 (190 mg) and N,N-diisopropylethylamine (0.30 mL) were added. The reaction was stirred at room temperature overnight. Then more Example 2.11.6 (70 mg) and more N,N-diisopropylethylamine (0.10 mL) were added and the reaction was allowed to stir for another 24 hours. The reaction was then concentrated and the residue was dissolved in tetrahydrofuran (2 mL) and methanol (2 mL), then 1.94N aqueous lithium hydroxide monohydrate (1.0 mL) was added and the mixture stirred at

room temperature for one hour. Purification by reverse phase chromatography (C18 column), eluting with 10-90% acetonitrile in 0.1% TFA/water, provided the title compound as a trifluoroacetic acid salt. MS (ESI) m/e 1261.4 (M-H).sup.-.

[1619] 2.11.8 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-((3-(2-(((4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)-2-(2-(2-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)ethoxy)ethoxy)benzyl)oxy)carbonyl)(2-methoxyethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid

[1620] Example 2.11.7 (19 mg) was dissolved in dimethylformamide (0.3 mL), then 2,5-dioxopyrrolidin-1-yl 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoate (6 mg) and N-ethyl-N-isopropylpropan-2-amine (13 µL) were added. The reaction was stirred for three hours at room temperature, then purification by reverse phase chromatography (C18 column), eluting with 10-90% acetonitrile in 0.1% TFA/water, provided the title compound. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 12.70 (v br s, 1H), 8.00 (m, 2H), 7.76 (t, 2H), 7.50 (d, 1H), 7.46 (t, 1H), 7.34 (t, 1H), 7.28 (s, 1H), 7.19 (d, 1H), 7.00 (m, 2H), 6.97 (s, 2H), 6.66 (d, 1H), 6.60 (dd, 1H), 5.06 (br m, 1H), 5.00 (s, 2H), 4.96 (s, 2H), 4.09 (m, 2H), 3.88 (m, 6H), 3.80 (br m, 3H), 3.71 (m, 2H), 3.59 (t, 2H), 3.44, 3.38 (both m, total 8H), 3.28 (m, 4H), 3.18 (m, 4H), 2.82 (br m, 2H), 2.33 (t, 2H), 2.09 (s, 3H), 1.33 (br m, 2H), 1.28-0.90 (m, 10H), 0.82 (m, 6H). MS (ESI) m/e 1412.4 (M-H).sup.-.

[1621] 2.12 Synthesis of 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-{[3-(2-{[({(2E)-3-[4-{[(2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl]oxy}-3-({3-[(({(2E)-3-(4-{[(2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl]oxy}-3-[(3-{[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}propanoyl)amino]phenyl)prop-2-en-1-yl]oxy}carbonyl)amino]propanoyl}amino)phenyl]prop-2-en-1-yl}oxy)carbonyl](2-methoxyethyl)amino}ethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid (Synthon KU)

[1622] 2.12.1 3-(1-((3-(2-(((E)-3-(3-(3-(((E)-3-(3-(3-aminopropanamido)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)phenyl)allyl)oxy)carbonyl)amino)propanamido)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)phenyl)allyl)oxy)carbonyl)(2-methoxyethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

[1623] The title compound was isolated as a by-product during the synthesis of Example 2.9.1. MS (ESI) m/e 1708.5 (M-H).sup.-.

[1624] 2.12.2 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-((3-(2-(((E)-3-(4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)-3-(3-(((E)-3-(4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)-3-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)propanamido)phenyl)allyl)oxy)carbonyl)amin o)propanamido)phenyl)allyl)oxy)carbonyl)(2-methoxyethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid

[1625] The title compound was prepared by substituting Example 2.12.1 for Example 2.11.7 in Example 2.11.8. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 8.99 (s, 1H), 8.97 (s, 1H), 8.17 (br s, 2H), 8.00 (br t, 1H), 7.94 (d, 1H), 7.70 (dd, 2H), 7.41 (m, 2H), 7.27 (t, 1H), 7.04 (br d, 2H), 6.97 (d, 2H), 6.93 (m, 2H), 6.89 (s, 2H), 6.52 (d, 1H), 6.49 (d, 1H), 6.11 (m, 2H), 4.93 (s, 2H), 4.80 (m, 2H), 4.56 (m, 4H), 3.83 (m, 7H), 3.72 (br d, 2H), 3.53 (m, 2H), 3.45-3.28 (m, 28H), 3.15 (s, 3H), 2.74 (m, 2H), 2.48 (m, 4H), 2.26 (t, 2H), 2.02 (s, 3H), 1.28 (br d, 2H), 1.17 (m, 4H), 1.02 (m, 4H), 0.89 (m, 2H), 0.2 (m, 6H). MS (ESI-) m/e 1859.5 (M-H).sup.-.

[1626] 2.13 Synthesis of 4-[(2-(2-{2-[(2-[(3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-

methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](2-methoxyethyl)carbamoyl}oxy)methyl]-5-(beta-D-glucopyranuronosyloxy)phenoxy}ethoxy)ethyl]carbamoyl}oxy)methyl]-3-[2-(2-{[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid (Synthon KV)

[1627] 2.13.1 3-(1-((3-(2-(((2-(2-(2-(((2-(2-(2-aminoethoxy)ethoxy)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)amino)ethoxy)ethoxy)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(2-methoxyethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid [1628] The title compound was isolated as a by-product during the synthesis of Example 2.11.7. MS (ESI) m/e 1690.5 (M-H).sup.-.

[1629] 2.13.2 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-((3-(2-(((4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)-2-(2-(2-(((4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)-2-(2-(2-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)ethoxy)ethoxy)benzyl)oxy)carbonyl)amino)ethoxy)ethoxy)benzyl)oxy)carbonyl)(2-methoxyethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid

[1630] The title compound was prepared by substituting Example 2.13.1 for Example 2.11.7 in Example 2.11.8. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 8.00 (m, 2H), 7.76 (t, 2H), 7.50 (d, 1H), 7.46 (m, 1H), 7.34 (m, 1H), 7.28 (s, 1H), 7.19 (m, 3H), 6.99 (m, 2H), 6.97 (s, 2H), 6.66 (m, 2H), 6.60 (m, 2H), 5.07 (m, 2H) 5.00 (s, 2H), 4.96 (s, 2H), 4.93 (s, 2H), 4.09 (m, 4H), 3.90 (m, 7H), 3.80 (br d, 4H), 3.71 (m, 4H), 3.59 (t, 2H), 3.48, 3.44, 3.38 (all m, total 14H), 3.28 (m, 7H), 3.16 (m, 7H), 2.81 (br m, 2H), 2.33 (t, 2H), 2.09 (s, 3H), 1.35 (br d, 2H), 1.28-0.90 (m, 10H), 0.82 (m, 6H). MS (ESI) m/e 1842.5 (M-H).sup.-.

[1631] 2.14 Synthesis of 4-[(2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](2-methoxyethyl)carbamoyl}oxy)methyl]-3-[2-(2-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid (Synthon KW)

[1632] The title compound was prepared by substituting 2,5-dioxopyrrolidin-1-yl 2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetate for 2,5-dioxopyrrolidin-1-yl 3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoate in Example 2.11.8. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 12.73 (v br s, 1H), 8.21 (br t, 1H), 8.01 (d, 1H), 7.76 (t, 2H), 7.50 (d, 1H), 7.46 (t, 1H), 7.34 (t, 1H), 7.28 (s, 1H), 7.19 (d, 1H), 7.07 (s, 2H), 6.99 (t, 2H), 6.66 (d, 1H), 6.60 (dd, 1H), 5.06 (br m, 1H), 5.00 (s, 2H), 4.96 (s, 2H), 4.09 (m, 2H), 4.02 (s, 2H), 3.88 (m, 6H), 3.80 (br m, 3H), 3.71 (m, 2H), 3.48 (t, 2H), 3.39 (m, 6H), 3.28, 3.21 (both m, 8H), 2.82 (br m, 2H), 2.09 (s, 3H), 1.33 (br m, 2H), 1.28-0.90 (m, 10H), 0.831 (m, 6H). MS (ESI) m/e 1398.4 (M-H).sup.-.

[1633] 2.15 Synthesis of 6-[1-(1,3-benzothiazol-2-ylcarbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl]-3-{1-[(3-{[34-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3-methyl-4,32-dioxo-7,10,13,16,19,22,25,28-octaoxa-3,31-diazatetatriacont-1-yl]oxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylic acid (Synthon DC)

[1634] To a mixture of Example 1.1.14 (30 mg) and 2,5-dioxopyrrolidin-1-yl 1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3-oxo-7,10,13,16,19,22,25,28-octaoxa-4-azahentriacontan-31-oate (MAL-dPEG8-NHS-Ester) (40.8 mg) in N,N-dimethylformamide (3 mL) at 0° C. was added N,N-diisopropylethylamine (48 μL). The mixture was stirred at 0° C. for 20 minutes and at room temperature for 10 minutes. Acetic acid (23 μL) was added and the mixture was purified by reverse

phase chromatography (C18 column), eluting with 20-60% acetonitrile in 0.1% TFA/water, to provide the title compound. MS (ESI) m/e 1332.5 (M+H).sup.+.

[1635] 2.16 Synthesis of 4-[(2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-cyano-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl]carbamoyl}oxy)methyl]-3-[2-(2-({3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl}amino)ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid (Synthon KZ)

[1636] 2.16.1 3-(1-((3-(2-(((2-(2-(2-aminoethoxy)ethoxy)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-5-cyano-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

[1637] The title compound was prepared by substituting Example 1.13.12 for Example 1.12.10 in Example 2.11.7. MS (ESI) m/e 1200 (M+H).sup.+ , 1198 (M-H).sup.-.

[1638] 2.16.2 4-[(2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-cyano-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl]carbamoyl}oxy)methyl]-3-[2-(2-({3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl}amino)ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid

[1639] The title compound was prepared by substituting Example 2.16.1 for Example 2.11.7 in Example 2.11.8. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 13.06 (bs, 2H), 8.04 (d, 1H), 8.01 (t, 1H), 7.92 (d, 1H), 7.78 (dd, 2H), 7.53 (d, 1H), 7.48 (t, 1H), 7.37 (t, 1H), 7.29 (s, 1H), 7.19 (d, 1H), 7.06 (t, 1H), 7.03 (d, 1H), 6.98 (s, 1H), 6.65 (d, 1H), 6.59 (dd, 1H), 5.07 (d, 1H), 4.98 (s, 1H), 4.92 (1H), 4.09 (m, 2H), 3.96 (t, 2H), 3.90 (d, 2H), 3.80 (s, 2H), 3.70 (m, 6H), 3.60 (m, 6H), 3.43 (t, 2H), 3.39 (t, 2H), 3.33 (t, 1H), 3.28 (dd, 1H), 3.16 (m, 4H), 3.03 (q, 2H), 2.33 (t, 2H), 2.09 (s, 3H), 1.37 (s, 2H), 1.25 (q, 4H), 1.11 (q, 4H), 1.00 (dd, 2H), 0.83 (s, 6H). MS (ESI) m/e 1351 (M+H).sup.+ , 1349 (M-H).sup.-.

[1640] 2.17 Synthesis of 4-[(1E)-3-({2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl)(2-methoxyethyl)carbamoyl}oxy)prop-1-en-1-yl]-2-({N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid (Synthon LW)

[1641] The title compound was prepared by substituting Example 2.9.1 for Example 2.11.7 in Example 2.11.8. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 9.03 (s, 1H), 8.25 (br m, 1H), 8.05 (br t, 1H), 8.01 (d, 1H), 7.76 (t, 2H), 7.49 (d, 1H), 7.47 (t, 1H), 7.33 (t, 1H), 7.28 (s, 1H), 7.10 (d, 1H), 7.05 (m, 1H), 7.00 (m, 2H), 6.96 (s, 2H), 6.56 (d, 1H), 6.17 (m, 1H), 5.00 (s, 2H), 4.86 (br m, 1H), 4.64 (d, 2H), 3.88 (m, 6H), 3.79 (br m, 2H), 3.60 (t, 2H), 3.43, 3.35 (m, m, total 14H), 3.22 (s, 3H), 2.80 (m, 2H), 2.53 (m, 2H), 2.33 (t, 2H), 2.09 (s, 3H), 1.37 (br m, 2H), 1.28-0.90 (m, 10H), 0.82, 0.77 (both s, total 6H). MS (ESI-) m/e 1421.5 (M-H).sup.-.

[1642] 2.18 Synthesis of N-[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-3-sulfo-L-alanyl-N-{5-[(1E)-3-({2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl)(2-methoxyethyl)carbamoyl}oxy)prop-1-en-1-yl]-2-(beta-D-glucopyranuronosyloxy)phenyl}-beta-alaninamide (Synthon LY)

[1643] 2.18.1 3-(1-((3-(2-(((E)-3-(3-(3-((R)-2-amino-3-sulfopropanamido)propanamido)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)phenyl)allyl)oxy)carbonyl)(2-methoxyethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

[1644] To a solution of (R)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-sulfopropanoic acid (29 mg) and 2-(3H-[1,2,3]triazolo[4,5-b]pyridin-3-yl)-1,1,3,3-tetramethylisouronium

hexafluorophosphate(V) (28 mg) in N,N-dimethylformamide (0.7 mL) was added N,N-diisopropylethylamine (0.013 mL). After stirring for 2 minutes, the reaction was added to a solution of Example 2.9.1 (70 mg) and N-ethyl-N-isopropylpropan-2-amine (0.035 mL) in N,N-dimethylformamide (0.5 mL) at room temperature, and the mixture was stirred for 3 hours. Diethylamine (0.035 mL) was added to the reaction and stirring was continued for an additional 2 hours. The reaction was diluted with water (1 mL), and purified by prep HPLC using a Gilson system eluting with 10-85% acetonitrile in water containing 0.1% v/v trifluoroacetic acid. The desired fractions were combined and freeze-dried to provide the title compound. MS (ESI) m/e 1421.4 (M-H).

[1645] 2.18.2 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-((3-(2-((((E)-3-(4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)-3-(3-((R)-2-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetamido)-3-sulfopropanamido)propanamido)phenyl)allyl)oxy)carbonyl)(2-methoxyethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid

[1646] The title compound was prepared by substituting Example 2.18.1 for Example 2.9.1 in Example 2.10. ¹H NMR (400 MHz, dimethyl sulfoxide-d₆) δ ppm 9.12 (s, 1H), 8.32 (d, 1H), 8.22 (br m, 1H), 8.01 (d, 1H), 7.97 (br t, 1H), 7.76 (t, 2H), 7.49 (d, 1H), 7.47 (t, 1H), 7.33 (t, 1H), 7.28 (s, 1H), 7.10 (d, 1H), 7.07 (s, 2H), 7.05 (m, 1H), 7.00 (m, 2H), 6.56 (d, 1H), 6.17 (m, 1H), 5.00 (s, 2H), 4.86 (br m, 1H), 4.64 (d, 2H), 4.32 (m, 1H), 4.07 (s, 2H), 3.88 (m, 6H), 3.79 (br m, 2H), 3.43, 3.35 (m, m, total 14H), 3.22 (s, 3H), 2.80 (m, 4H), 2.53 (m, 2H), 2.09 (s, 3H), 1.37 (br m, 2H), 1.28-0.90 (m, 10H), 0.82, 0.77 (both s, total 6H). MS (ESI-) m/e 1558.4 (M-H).

[1647] 2.19 Synthesis of N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-3-sulfo-L-alanyl-N-{5-[(1E)-3-({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](2-methoxyethyl)carbamoyl}oxy)prop-1-en-1-yl]-2-(beta-D-glucopyranuronosyloxy)phenyl}-beta-alaninamide (Synthon LZ)

[1648] The title compound was prepared by substituting Example 2.18.1 for Example 2.11.7 in Example 2.11.8. ¹H NMR (400 MHz, dimethyl sulfoxide-d₆) δ ppm 9.12 (s, 1H), 8.22 (br m, 1H), 8.07 (br d, 1H), 8.01 (d, 1H), 7.89 (br t, 1H), 7.76 (t, 2H), 7.49 (d, 1H), 7.47 (t, 1H), 7.33 (t, 1H), 7.28 (s, 1H), 7.10 (d, 1H), 7.05 (m, 1H), 7.00 (m, 2H), 6.96 (s, 2H), 6.56 (d, 1H), 6.17 (m, 1H), 5.00 (s, 2H), 4.86 (br m, 1H), 4.64 (d, 2H), 4.32 (m, 1H), 3.88 (m, 6H), 3.79 (br m, 2H), 3.60 (t, 2H), 3.43, 3.35 (m, m, total 14H), 3.22 (s, 3H), 2.80 (m, 4H), 2.53 (m, 2H), 2.37 (m, 2H), 2.09 (s, 3H), 1.37 (br m, 2H), 1.28-0.90 (m, 10H), 0.82, 0.77 (both s, total 6H). MS (ESI-) m/e 1572.5 (M-H).^{sup.}-.

[1649] 2.20 Synthesis of N-[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-beta-alanyl-N-{5-[(1E)-3-({[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](2-methoxyethyl)carbamoyl}oxy)prop-1-en-1-yl]-2-(beta-D-glucopyranuronosyloxy)phenyl}-beta-alaninamide (Synthon MB)

[1650] 2.20.1 3-(1-((3-(2-((((E)-3-(3-(3-(3-aminopropanamido)propanamido)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)phenyl)allyl)oxy)carbonyl)(2-methoxyethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

[1651] The title compound was prepared by substituting 3-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanoic acid for (R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-sulfopropanoic acid in Example 2.18.1. MS (ESI-) m/e 1341.5 (M-H).^{sup.}-.

[1652] 2.20.2 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-((3-(2-((((E)-3-(4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-

yl)oxy)-3-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetamido)propanamido)propanamido)phenyl)allyl)oxy)carbonyl)(2-methoxyethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid

[1653] The title compound was prepared by substituting Example 2.20.1 for Example 2.9.1 in Example 2.10. ¹H NMR (400 MHz, dimethyl sulfoxide-d₆) δ ppm 9.06 (s, 1H), 8.25 (br m, 1H), 8.14 (br t 1H), 8.01 (d, 1H), 7.99 (br m, 1H), 7.76 (t, 2H), 7.49 (d, 1H), 7.47 (t, 1H), 7.33 (t, 1H), 7.28 (s, 1H), 7.10 (d, 1H), 7.07 (s, 2H), 7.05 (m, 1H), 7.00 (m, 2H), 6.56 (d, 1H), 6.17 (m, 1H), 5.00 (s, 2H), 4.86 (br m, 1H), 4.64 (d, 2H), 3.99 (s, 2H), 3.88 (m, 6H), 3.79 (br m, 2H), 3.43, 3.35 (m, m, total 14H), 3.25 (m, 2H), 3.22 (s, 3H), 2.80 (m, 2H), 2.55 (m, 2H), 2.23 (t, 2H), 2.09 (s, 3H), 1.37 (br m, 2H), 1.28-0.90 (m, 10H), 0.82, 0.77 (both s, total 6H). MS (ESI⁻) m/e 1478.5 (M-H).^{sup.}-.

[1654] 2.21 Synthesis of N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-beta-alanyl-N-{5-[(1E)-3-({2-({3-[(4-{6-[8-(1,3-benzothiazol-2-yl)carbonyl]-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](2-methoxyethyl)carbonyl}oxy)prop-1-en-1-yl]-2-(beta-D-glucopyranuronosyloxy)phenyl}-beta-alaninamide (Synthon MC)

[1655] The title compound was prepared by substituting Example 2.20.1 for Example 2.11.7 in Example 2.11.8. ¹H NMR (400 MHz, dimethyl sulfoxide-d₆) δ ppm 9.06 (s, 1H), 8.25 (br m, 1H), 8.01 (d, 1H), 7.94 (br m, 2H), 7.76 (t, 2H), 7.49 (d, 1H), 7.47 (t, 1H), 7.33 (t, 1H), 7.28 (s, 1H), 7.10 (d, 1H), 7.05 (m, 1H), 7.00 (m, 2H), 6.97 (s, 2H), 6.56 (d, 1H), 6.17 (m, 1H), 5.00 (s, 2H), 4.86 (br m, 1H), 4.64 (d, 2H), 3.88 (m, 6H), 3.79 (br m, 2H), 3.60 (t, 2H), 3.43, 3.35 (m, m, total 14H), 3.22 (s, 3H), 3.18 (m, 2H), 2.80 (m, 2H), 2.55 (m, 2H), 2.29 (t, 2H), 2.20 (t, 2H), 2.09 (s, 3H), 1.37 (br m, 2H), 1.28-0.90 (m, 10H), 0.82, 0.77 (both s, total 6H). MS (ESI⁻) m/e 1492.5 (M-H).^{sup.}-.

[1656] 2.22 Synthesis of 4-[(2-({3-[(4-{6-[8-(1,3-benzothiazol-2-yl)carbonyl]-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](2-methoxyethyl)carbonyl}oxy)methyl]-3-{2-[2-({N-[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-3-sulfo-L-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid (Synthon ME)

[1657] 2.22.1 3-(1-((3-(2-(((2-(2-(2-((R)-2-amino-3-sulfopropanamido)ethoxy)ethoxy)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(2-methoxyethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-yl)carbonyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

[1658] The title compound was prepared by substituting Example 2.11.7 for Example 2.9.1 in Example 2.18.1. MS (ESI⁻) m/e 1412.4 (M-H).^{sup.}-.

[1659] 2.22.2 6-(8-(benzo[d]thiazol-2-yl)carbonyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-((3-(2-(((4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)-2-(2-(2-((R)-2-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetamido)-3-sulfopropanamido)ethoxy)ethoxy)benzyl)oxy)carbonyl)(2-methoxyethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid

[1660] The title compound was prepared by substituting Example 2.22.1 for Example 2.9.1 in Example 2.10. ¹H NMR (400 MHz, dimethyl sulfoxide-d₆) δ ppm 8.32 (d, 1H), 8.02 (d, 1H), 7.76 (m, 3H), 7.52 (d, 1H), 7.46 (t, 1H), 7.34 (t, 1H), 7.30 (s, 1H), 7.19 (d, 1H), 7.06 (s, 2H), 7.00 (m, 2H), 6.66 (d, 1H), 6.58 (dd, 1H), 5.06 (br m, 1H), 5.00 (s, 2H), 4.96 (s, 2H), 4.31 (m, 1H), 4.09 (m, 2H), 4.08 (s, 2H), 3.88 (m, 6H), 3.80 (br m, 4H), 3.71 (m, 2H), 3.44, 3.38 (both m, total 8H), 3.28 (m, 4H), 3.18 (m, 4H), 2.82 (br m, 3H), 2.72 (m, 1H), 2.09 (s, 3H), 1.33 (br m, 2H), 1.28-0.90 (m, 10H), 0.84, 0.81 (both s, total 6H). MS (ESI⁻) m/e 1549.5 (M-H).^{sup.}-.

[1661] 2.23 Synthesis of 4-[(2-({3-[(4-{6-[8-(1,3-benzothiazol-2-yl)carbonyl]-5-methoxy-3,4-

dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl]-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](2-methoxyethyl)carbamoyl}oxy)methyl]-3-{2-[2-({N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-3-sulfo-L-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid (Synthon MF)

[1662] The title compound was prepared by substituting Example 2.22.1 for Example 2.11.7 in Example 2.11.8. ¹H NMR (400 MHz, dimethyl sulfoxide-d₆) δ ppm 12.70 (v br s, 1H), 8.06 (d, 1H), 8.02 (d, 1H), 7.76 (m, 3H), 7.52 (d, 1H), 7.46 (t, 1H), 7.34 (t, 1H), 7.30 (s, 1H), 7.19 (d, 1H), 7.00 (m, 2H), 6.95 (s, 2H), 6.66 (d, 1H), 6.58 (dd, 1H), 5.06 (br m, 1H), 5.00 (s, 2H), 4.96 (s, 2H), 4.31 (m, 1H), 4.09 (m, 2H), 3.88 (m, 6H), 3.80 (br m, 4H), 3.71 (m, 2H), 3.59 (t, 2H), 3.44, 3.38 (both m, total 8H), 3.28 (m, 4H), 3.18 (m, 4H), 2.82 (br m, 3H), 2.72 (m, 1H), 2.33 (m, 2H), 2.09 (s, 3H), 1.33 (br m, 2H), 1.28-0.90 (m, 10H), 0.84, 0.81 (both s, total 6H). MS (ESI⁻) m/e 1563.5 (M-H)⁺.

[1663] 2.24 Synthesis of 4-[(2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl]-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](2-methoxyethyl)carbamoyl}oxy)methyl]-3-{2-[2-({N-[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-beta-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid (Synthon MH)

[1664] 2.24.1 3-(1-((3-(2-(((2-(2-(3-aminopropanamido)ethoxy)ethoxy)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(2-methoxyethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

[1665] The title compound was prepared by substituting 3-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanoic acid for (R)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-sulfopropanoic acid and Example 2.11.7 for Example 2.9.1 in Example 2.18.1. MS (ESI⁻) m/e 1332.5 (M-H)⁺.

[1666] 2.24.2 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-((3-(2-(((4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)-2-(2-(2-(3-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetamido)propanamido)ethoxy)ethoxy)benzyl)oxy)carbonyl)(2-methoxyethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid

[1667] The title compound was prepared by substituting Example 2.24.1 for Example 2.9.1 in Example 2.10. ¹H NMR (400 MHz, dimethyl sulfoxide-d₆) δ ppm 12.70 (v br s, 1H), 8.14 (t, 1H), 8.02 (d, 1H), 7.92 (t, 1H), 7.76 (t, 2H), 7.52 (d, 1H), 7.46 (t, 1H), 7.34 (t, 1H), 7.28 (s, 1H), 7.19 (d, 1H), 7.06 (s, 2H), 7.00 (m, 2H), 6.66 (d, 1H), 6.58 (dd, 1H), 5.06 (br m, 1H), 5.00 (s, 2H), 4.96 (s, 2H), 4.09 (m, 2H), 3.98 (s, 2H), 3.88 (m, 6H), 3.80 (br m, 4H), 3.71 (m, 2H), 3.44, 3.38 (both m, total 8H), 3.28 (m, 4H), 3.18 (m, 6H), 2.82 (br m, 2H), 2.24 (t, 2H), 2.09 (s, 3H), 1.33 (br m, 2H), 1.28-0.90 (m, 10H), 0.84, 0.81 (both s, total 6H). MS (ESI⁻) m/e 1469.5 (M-H)⁺.

[1668] 2.25 Synthesis of 4-[(2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl]-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](2-methoxyethyl)carbamoyl}oxy)methyl]-3-{2-[2-({N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-beta-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid (Synthon MI)

[1669] The title compound was prepared by substituting Example 2.24.1 for Example 2.11.7 in Example 2.11.8. ¹H NMR (400 MHz, dimethyl sulfoxide-d₆) δ ppm 12.70 (v br s, 1H), 8.02 (d, 1H), 7.94 (t, 1H), 7.88 (t, 1H), 7.76 (t, 2H), 7.52 (d, 1H), 7.46 (t, 1H), 7.34 (t, 1H), 7.28 (s, 1H), 7.19 (d, 1H), 7.00 (m, 2H), 6.95 (s, 2H), 6.66 (d, 1H), 6.58 (dd, 1H), 5.06 (br m, 1H), 5.00 (s, 2H), 4.96 (s, 2H), 4.09 (m, 2H), 3.88 (m, 6H), 3.80 (br m, 4H), 3.71 (m, 2H), 3.59 (t, 2H), 3.44,

3.38 (both m, total 8H), 3.28 (m, 4H), 3.18 (m, 6H), 2.82 (br m, 2H), 2.30 (t, 2H), 2.20 (t, 2H), 2.09 (s, 3H), 1.33 (br m, 2H), 1.28-0.90 (m, 10H), 0.84, 0.81 (both s, total 6H). MS (ESI-) m/e 1483.5 (M-H).sup.-.

[1670] 2.26 Synthesis of 2-[(2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl)(2-methoxyethyl)carbamoyl}oxy)methyl]-5-{2-[2-({N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-3-sulfo-L-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid (Synthon NJ)

[1671] 2.26.1 4-(2-(2-bromoethoxy)ethoxy)-2-hydroxybenzaldehyde

[1672] A solution of 2,4-dihydroxybenzaldehyde (1.0 g), 1-bromo-2-(2-bromoethoxy)ethane (3.4 g) and potassium carbonate (1.0 g) were stirred together in acetonitrile (30 mL) and heated to 75° C. After stirring for 2 days, the reaction was cooled, diluted with ethyl acetate (100 mL), washed with water (50 mL) and brine (50 mL), dried over magnesium sulfate, filtered and concentrated. Purification via silica gel chromatography, eluting using a gradient of 5-30% ethyl acetate/heptane, provided the title compound. MS (ELSD) m/e 290.4 (M+H).sup.+.

[1673] 2.26.2 4-(2-(2-azidoethoxy)ethoxy)-2-hydroxybenzaldehyde

[1674] To a solution of Example 2.26.1 (1.26 g) in N,N-dimethylformamide (10 mL) was added sodium azide (0.43 g) and the reaction was stirred at room temperature overnight. The reaction was diluted with diethyl ether (100 mL), washed with water (50 mL) and brine (50 mL), dried over magnesium sulfate, filtered, and concentrated. Purification via silica gel chromatography, eluting with a gradient of 5-30% ethyl acetate/heptane, gave the title compound. MS (ELSD) m/e 251.4 (M+H).sup.+.

[1675] 2.26.3 (2S,3R,4S,5S,6S)-2-(5-(2-(2-azidoethoxy)ethoxy)-2-formylphenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

[1676] A solution of Example 2.26.2 (0.84 g), (3R,4S,5S,6S)-2-bromo-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (1.99 g) and silver (I) oxide (1.16 g) were stirred together in acetonitrile (15 mL). After stirring overnight, the reaction was diluted with dichloromethane (20 mL), diatomaceous earth was added and the reaction filtered and concentrated. Purification via silica gel chromatography, eluting with a gradient of 5-75% ethyl acetate/heptane, gave the title compound.

[1677] 2.26.4 (2S,3R,4S,5S,6S)-2-(5-(2-(2-azidoethoxy)ethoxy)-2-(hydroxymethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

[1678] A solution of Example 2.26.3 (0.695 g) in methanol (5 mL) and tetrahydrofuran (2 mL) was cooled to 0° C. Sodium borohydride (0.023 g) was added, and the reaction was warmed to room temperature. After stirring for a total of 1 hour, the reaction was poured into a mixture of ethyl acetate (75 mL) and water (25 mL) and saturated aqueous sodium bicarbonate (10 mL) was added. The organic layer was separated, washed with brine (50 mL), dried over magnesium sulfate, filtered, and concentrated. Purification via silica gel chromatography, eluting with a gradient of 5-85% ethyl acetate/heptane, gave the title compound. MS (ELSD) m/e 551.8 (M-H.sub.2O).sup.-.

[1679] 2.26.5 (2S,3R,4S,5S,6S)-2-(5-(2-(2-aminoethoxy)ethoxy)-2-(hydroxymethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

[1680] To Example 2.26.4 (0.465 g) in tetrahydrofuran (20 mL) was added 5% Pd/C (0.1 g) in a 50 mL pressure bottle and the mixture shaken for 16 hours at 30 psi hydrogen. The reaction was then filtered and concentrated to give the title compound which was used without further purification. MS (ELSD) m/e 544.1 (M+H).sup.+.

[1681] 2.26.6 (2S,3R,4S,5S,6S)-2-(5-(2-(2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethoxy)ethoxy)-2-(hydroxymethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

[1682] A solution of Example 2.26.5 (0.443 g) in dichloromethane (8 mL) was cooled to 0° C., then N,N-diisopropylethylamine (0.214 mL) and (9H-fluoren-9-yl)methyl carbonochloridate (0.190 g)

were added. After 1 hour, the reaction was concentrated and purified via column chromatography, eluting with 5-95% ethyl acetate/heptane, to give the title compound. MS (ELSD) m/e 748.15 (M-OH)-.

[1683] 2.26.7 (2S,3R,4S,5S,6S)-2-(5-(2-(2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)ethoxy)ethoxy)-2-(((4-nitrophenoxy)carbonyl)oxy)methyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

[1684] To a solution of Example 2.26.6 (0.444 g) in N,N-dimethylformamide (5 mL) was added N,N-diisopropylethylamine (0.152 mL) and bis(4-nitrophenyl) carbonate (0.353 g) and the reaction was stirred at room temperature. After 5 hours, the reaction was concentrated and the residue was purified via column chromatography, eluting with 5-90% ethyl acetate/heptane, to give the title compound.

[1685] 2.26.8 3-(1-((3-(2-(((4-(2-(2-aminoethoxy)ethoxy)-2-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(2-methoxyethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

[1686] Example 1.12.10 (360 mg) was dissolved in dimethylformamide (2.5 mL). Example 2.26.7 (450 mg) and N,N-diisopropylethylamine (0.35 mL) were added. The reaction was stirred at room temperature overnight. The reaction was then concentrated and the residue dissolved in tetrahydrofuran (2.5 mL) and methanol (2.5 mL). Aqueous lithium hydroxide monohydrate (1.94N, 2.2 mL) was added, and the mixture was stirred at room temperature for one hour. Purification by reverse phase chromatography (C18 column), eluting with 10-90% acetonitrile in 0.1% TFA/water, provided the title compound as a trifluoroacetic acid salt. MS (ESI) m/e 1261.4 (M-H).sup.-.

[1687] 2.26.9 3-(1-((3-(2-(((4-(2-(2-((R)-2-amino-3-sulfopropanamido)ethoxy)ethoxy)-2-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(2-methoxyethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

[1688] The title compound was prepared by substituting Example 2.26.8 for Example 2.9.1 in Example 2.18.1. MS (ESI-) m/e 1412.4 (M-H).sup.-.

[1689] 2.26.10 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-((3-(2-(((2-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)-4-(2-(2-((R)-2-(3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanamido)-3-sulfopropanamido)ethoxy)ethoxy)benzyl)oxy)carbonyl)(2-methoxyethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid

[1690] The title compound was prepared by substituting Example 2.26.9 for Example 2.11.7 in Example 2.11.8. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 12.70 (v br s, 1H), 8.06 (d, 1H), 8.02 (d, 1H), 7.76 (t, 3H), 7.52 (d, 1H), 7.46 (t, 1H), 7.34 (t, 1H), 7.30 (s, 1H), 7.19 (d, 1H), 7.00 (m, 2H), 6.95 (s, 2H), 6.70 (d, 1H), 6.58 (dd, 1H), 5.06 (br m, 1H), 5.00 (s, 2H), 4.96 (s, 2H), 4.31 (m, 1H), 4.09 (m, 2H), 3.88 (m, 6H), 3.80 (br m, 4H), 3.71 (m, 2H), 3.59 (t, 2H), 3.44, 3.38 (both m, total 8H), 3.28 (m, 4H), 3.18 (m, 4H), 2.82 (br m, 3H), 2.72 (m, 1H), 2.33 (m, 2H), 2.09 (s, 3H), 1.33 (br m, 2H), 1.28-0.90 (m, 10H), 0.84, 0.81 (both s, total 6H). MS (ESI-) m/e 1563.5 (M-H).sup.-.

[1691] 2.27 Synthesis of 2-[(2-((3-((4-((6-((1,3-benzothiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)oxy)ethyl)(2-methoxyethyl)carbonyl)oxy)methyl]-5-{2-[2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid (Synthon NK)

[1692] The title compound was prepared by substituting Example 2.26.9 for Example 2.9.1 in Example 2.9.2. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 12.70 (v br s, 1H),

8.06 (d, 1H), 8.02 (d, 1H), 7.76 (t, 3H), 7.52 (d, 1H), 7.46 (t, 1H), 7.34 (t, 1H), 7.30 (s, 1H), 7.19 (d, 1H), 7.00 (m, 2H), 6.95 (s, 2H), 6.70 (d, 1H), 6.58 (dd, 1H), 5.06 (br m, 1H), 5.00 (s, 2H), 4.96 (s, 2H), 4.31 (m, 1H), 4.09 (m, 2H), 3.88 (m, 6H), 3.80 (br m, 4H), 3.71 (m, 2H), 3.59 (t, 2H), 3.44, 3.38 (both m, total 8H), 3.28 (m, 4H), 3.18 (m, 4H), 2.82 (br m, 3H), 2.72 (m, 1H), 2.33 (m, 2H), 2.09 (s, 3H), 1.46 (br m, 4H) 1.33 (br m, 2H), 1.28-0.90 (m, 12H), 0.84, 0.81 (both s, total 6H). MS (ESI-) m/e 1605.4 (M-H).sup.-.

[1693] 2.28 Synthesis of 4-[(2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl)(2-methoxyethyl)carbamoyl}oxy)methyl]-3-[3-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)propoxy]phenyl beta-D-glucopyranosiduronic acid (Synthon NL)

[1694] 2.28.1 (2S,3R,4S,5S,6S)-2-(3-(3-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)propoxy)-4-formylphenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

[1695] To a solution of (9H-fluoren-9-yl)methyl (3-hydroxypropyl)carbamate (0.245 g) and triphenylphosphine (0.216 g) in tetrahydrofuran (2 mL) at 0° C. was added diisopropyl azodicarboxylate (0.160 mL) dropwise. After stirring for 15 minutes, Example 2.11.1 (0.250 g) was added, the ice bath was removed, and the reaction was allowed to warm to room temperature. After 2 hours, the reaction was concentrated, loaded onto silica gel, and eluted using a gradient of 5-70% ethyl acetate/hexanes to give the title compound. MS (APCI) m/e 512.0 (M-FMOC).sup.-.

[1696] 2.28.2 (2S,3R,4S,5S,6S)-2-(3-(3-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)propoxy)-4-(hydroxymethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

[1697] To a suspension of Example 2.28.1 (0.233 g) in methanol (3 mL) and tetrahydrofuran (1 mL) was added sodium borohydride (6 mg). After 30 minutes, the reaction was poured into ethyl acetate (50 mL) and water (25 mL), followed by the addition of sodium bicarbonate (5 mL). The organic layer was separated, washed with brine (25 mL), dried over magnesium sulfate, filtered, and concentrated. Silica gel chromatography, eluting with a gradient of 5-80% ethyl acetate/heptane, gave the title compound. MS (APCI) m/e 718.1 (M-OH).sup.-.

[1698] 2.28.3 (2S,3R,4S,5S,6S)-2-(3-(3-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)propoxy)-4-(((4-nitrophenoxy)carbonyl)oxy)methyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

[1699] To a solution of Example 2.28.2 (0.140 g) and bis(4-nitrophenyl) carbonate (0.116 g) in N,N-dimethylformamide (1 mL) was added N-ethyl-N-isopropylpropan-2-amine (0.050 mL). After 1.5 hours, the reaction was concentrated under high vacuum, loaded onto silica gel, and eluted using a gradient of 10-70% ethyl acetate/heptane to give the title compound.

[1700] 2.28.4 3-(1-((3-(2-(((2-(3-aminopropoxy)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(2-methoxyethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

[1701] The title compound was prepared by substituting Example 2.28.3 for Example 2.26.7 in Example 2.26.8. MS (ESI-) m/e 1231.3 (M-H).sup.-.

[1702] 2.28.5 3-(1-((3-(2-(((2-(3-((R)-2-amino-3-sulfopropanamido)propoxy)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(2-methoxyethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

[1703] The title compound was prepared by substituting Example 2.28.4 for Example 2.9.1 in Example 2.18.1. MS (ESI-) m/e 1382.4 (M-H).sup.-.

[1704] 2.28.6 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-((3-(2-(((4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)-2-(3-((R)-2-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-3-

sulfopropanamido)propoxy)benzyl)oxy)carbonyl)(2-methoxyethyl)amino)ethoxy)-5,7-

dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid

[1705] The title compound was prepared by substituting Example 2.28.5 for Example 2.9.1 in Example 2.9.2. ¹H NMR (400 MHz, dimethyl sulfoxide-d₆) δ ppm 8.01 (d, 1H), 7.85 (m, 2H), 7.76 (m, 2H), 7.52 (d, 1H), 7.46 (t, 1H), 7.34 (m, 1H), 7.30 (s, 1H), 7.16 (d, 1H), 7.00 (m, 3H), 6.97 (s, 2H), 6.64 (d, 1H), 6.56 (dd, 1H), 5.04 (br m, 1H), 5.00 (s, 2H), 4.96 (s, 2H), 4.28 (m, 1H), 3.97 (m, 2H), 3.88 (m, 6H), 3.80 (m, 2H), 3.71 (m, 2H), 3.37 (m, 8H), 3.27 (m, 4H), 3.17 (m, 4H), 2.90-2.65 (m, 4H), 2.09 (s, 3H), 2.05 (t, 2H), 1.81 (m, 2H), 1.46 (br m, 4H), 1.33 (br m, 2H), 1.28-0.90 (m, 12H), 0.84, 0.81 (both s, total 6H). MS (ESI⁻) m/e 1575.5 (M-H).sup.-.

[1706] 2.29 Synthesis of 4-[(2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl)(methyl)carbamoyl}oxy)methyl]-3-[3-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)propoxy]phenyl beta-D-glucopyranosiduronic acid (Synthon NM)

[1707] 2.29.1 3-(1-((3-(2-(((2-(3-aminopropoxy)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

[1708] The title compound was prepared by substituting Example 2.28.3 for Example 2.26.7 and Example 1.9.11 for Example 1.12.10 in Example 2.26.8. MS (ESI⁻) m/e 1187.4 (M-H).sup.-.

[1709] 2.29.2 3-(1-((3-(2-(((2-(3-((R)-2-amino-3-sulfopropanamido)propoxy)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

[1710] The title compound was prepared by substituting Example 2.29.1 for Example 2.9.1 in Example 2.18.1. MS (ESI⁻) m/e 1338.3 (M-H).sup.-.

[1711] 2.29.3 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-((3-(2-(((4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)-2-(3-((R)-2-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-3-sulfopropanamido)propoxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid

[1712] The title compound was prepared by substituting Example 2.29.2 for Example 2.9.1 in Example 2.9.2. ¹H NMR (400 MHz, dimethyl sulfoxide-d₆) δ ppm 8.01 (d, 1H), 7.85 (m, 2H), 7.76 (m, 2H), 7.52 (d, 1H), 7.46 (t, 1H), 7.34 (m, 1H), 7.30 (s, 1H), 7.16 (d, 1H), 7.00 (m, 3H), 6.97 (s, 2H), 6.64 (d, 1H), 6.56 (dd, 1H), 5.04 (br m, 1H), 5.00 (s, 2H), 4.96 (s, 2H), 4.28 (m, 1H), 3.97 (m, 2H), 3.88 (m, 6H), 3.80 (m, 2H), 3.44 (m, 6H), 3.28 (m, 4H), 3.17 (m, 2H), 2.90-2.65 (m, 4H), 2.09 (s, 3H), 2.05 (t, 2H), 1.81 (m, 2H), 1.46 (br m, 4H), 1.33 (br m, 2H), 1.28-0.90 (m, 12H), 0.84, 0.81 (both s, total 6H). MS (ESI⁻) m/e 1531.5 (M-H).sup.-.

[1713] 2.30 Synthesis of N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[(3S)-1-{8-(1,3-benzothiazol-2-ylcarbamoyl)-2-[6-carboxy-5-(1-{3-(2-methoxyethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl]-1,2,3,4-tetrahydroisoquinolin-6-yl}pyrrolidin-3-yl]carbamoyl}oxy)methyl]phenyl}-L-alaninamide (Synthon NR)

[1714] Example 1.17.10 (40 mg) was dissolved in dimethyl sulfoxide (0.3 mL), and 4-((S)-2-((S)-2-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanamido)-3-methylbutanamido)propanamido)benzyl (4-nitrophenyl) carbonate (31 mg) and triethylamine (33 μL) were added. The reaction mixture was stirred for 72 hours at room temperature, and purification by reverse phase chromatography (C18 column), eluting with 10-90% acetonitrile in 0.1% TFA water, provided the title compound. MS (ESI) m/e 1357.4 (M+H).sup.+, 1355.5

(M-H).sup.-.

[1715] 2.31 Synthesis of N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[(2-({3-[(4-{6-[8-(1,3-benzothiazol-2-yl)carbamoyl]-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](2-sulfamoyl)ethyl)carbamoyl}oxy)methyl]phenyl}-N5-carbamoyl-L-ornithinamide (Synthon EB)

[1716] The title compound was prepared as described in previous examples. ¹H NMR (500 MHz, dimethyl sulfoxide-d₆) δ ppm 12.85 (s, 1H), 9.98 (s, 1H), 8.00-8.09 (m, 2H), 7.78 (t, 2H), 7.61 (t, 3H), 7.40-7.53 (m, 3H), 7.33-7.39 (m, 2H), 7.25-7.30 (m, 3H), 6.86-7.00 (m, 5H), 5.99 (s, 1H), 4.86-5.10 (m, 4H), 4.38 (s, 1H), 4.10-4.26 (m, 1H), 3.88 (t, 2H), 3.80 (d, 2H), 3.33-3.39 (m, 2H), 3.30 (d, 2H), 3.18-3.26 (m, 2H), 2.88-3.06 (m, 5H), 2.04-2.24 (m, 5H), 1.87-2.00 (m, 1H), 1.28-1.74 (m, 10H), 0.89-1.27 (m, 12H), 0.74-0.87 (m, 12H). MS (ESI) m/e 1451.3 (M+H).sup.+.

[1717] 2.32 Synthesis of Control Synthon 4-[(2-({3-[(4-{6-[8-(1,3-benzothiazol-2-yl)carbamoyl]-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl)(methyl)carbamoyl}oxy)methyl]-2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid (Synthon H)

[1718] 2.32.1 (2S,3R,4S,5S,6S)-2-(4-formyl-2-nitrophenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

[1719] To a solution of (2R,3R,4S,5S,6S)-2-bromo-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (4 g) in acetonitrile (100 mL) was added silver(I) oxide (10.04 g) and 4-hydroxy-3-nitrobenzaldehyde (1.683 g). The reaction mixture was stirred for 4 hours at room temperature and filtered. The filtrate was concentrated, and the residue was purified by silica gel chromatography, eluting with 5-50% ethyl acetate in heptanes, to provide the title compound. MS (ESI) m/e (M+18).sup.+.

[1720] 2.32.2 (2S,3R,4S,5S,6S)-2-(4-(hydroxymethyl)-2-nitrophenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

[1721] To a solution of Example 2.32.1 (6 g) in a mixture of chloroform (75 mL) and isopropanol (18.75 mL) was added 0.87 g of silica gel. The resulting mixture was cooled to 0° C., NaBH₄ (0.470 g) was added, and the resulting suspension was stirred at 0° C. for 45 minutes. The reaction mixture was diluted with dichloromethane (100 mL) and filtered through diatomaceous earth. The filtrate was washed with water and brine and concentrated to give the crude product, which was used without further purification. MS (ESI) m/e (M+NH₄).sup.+:

[1722] 2.32.3 (2S,3R,4S,5S,6S)-2-(2-amino-4-(hydroxymethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

[1723] A stirred solution of Example 2.32.2 (7 g) in ethyl acetate (81 mL) was hydrogenated at 20° C. under 1 atmosphere H₂, using 10% Pd/C (1.535 g) as a catalyst for 12 hours. The reaction mixture was filtered through diatomaceous earth, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel chromatography, eluting with 95/5 dichloromethane/methanol, to give the title compound.

[1724] 2.32.4 3-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanoic acid

[1725] 3-Aminopropanoic acid (4.99 g) was dissolved in 10% aqueous Na₂CO₃ solution (120 mL) in a 500 mL flask and cooled with an ice bath. To the resulting solution, (9H-fluoren-9-yl)methyl carbonochloridate (14.5 g) in 1,4-dioxane (100 mL) was gradually added. The reaction mixture was stirred at room temperature for 4 hours, and water (800 mL) was then added. The aqueous phase layer was separated from the reaction mixture and washed with diethyl ether (3×750 mL). The aqueous layer was acidified with 2N HCl aqueous solution to a pH value of 2 and extracted with ethyl acetate (3×750 mL). The organic layers were combined and concentrated to obtain crude product. The crude product was recrystallized in a mixed solvent of ethyl acetate:

hexane 1:2 (300 mL) to give the title compound.

[1726] 2.32.5 (9H-fluoren-9-yl)methyl (3-chloro-3-oxopropyl)carbamate

[1727] To a solution of Example 2.32.4 in dichloromethane (160 mL) was added sulfurous dichloride (50 mL). The mixture was stirred at 60° C. for 1 hour. The mixture was cooled and concentrated to give the title compound.

[1728] 2.32.6 (2S,3R,4S,5S,6S)-2-(2-(3-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-(hydroxymethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

[1729] To a solution of Example 2.32.3 (6 g) in dichloromethane (480 mL) was added N,N-diisopropylethylamine (4.60 mL). Example 2.32.5 (5.34 g) was added, and the mixture was stirred at room temperature for 30 minutes. The mixture was poured into saturated aqueous sodium bicarbonate and was extracted with ethyl acetate. The combined extracts were washed with water and brine and were dried over sodium sulfate. Filtration and concentration gave a residue that was purified via radial chromatography, using 0-100% ethyl acetate in petroleum ether as mobile phase, to give the title compound.

[1730] 2.32.7 (2S,3R,4S,5S,6S)-2-(2-(3-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)propanamido)-4-(((4-nitrophenoxy)carbonyl)oxy)methyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

[1731] To a mixture of Example 2.32.6 (5.1 g) in N,N-dimethylformamide (200 mL) was added bis(4-nitrophenyl) carbonate (4.14 g) and N,N-diisopropylethylamine (1.784 mL). The mixture was stirred for 16 hours at room temperature and concentrated under reduced pressure. The crude material was dissolved in dichloromethane and aspirated directly onto a 1 mm radial Chromatotron plate and eluted with 50-100% ethyl acetate in hexanes to give the title compound. MS (ESI) m/e (M+H).sup.+.

[1732] 2.32.8 3-bromo-5,7-dimethyladamantanecarboxylic acid

[1733] In a 50 mL round-bottomed flask at 0° C. was added bromine (16 mL). Iron powder (7 g) was then added, and the reaction was stirred at 0° C. for 30 minutes. 3,5-Dimethyladamantane-1-carboxylic acid (12 g) was then added. The mixture was warmed up to room temperature and stirred for 3 days. A mixture of ice and concentrated HCl was poured into the reaction mixture. The resulting suspension was treated twice with Na.sub.2SO.sub.3 (50 g in 200 mL water) to destroy bromine and was extracted three times with dichloromethane. The combined organics were washed with 1N aqueous HCl, dried over Na.sub.2SO.sub.4, filtered, and concentrated to give the crude title compound.

[1734] 2.32.9 3-bromo-5,7-dimethyladamantanemethanol

[1735] To a solution of Example 2.32.8 (15.4 g) in tetrahydrofuran (200 mL) was added BH.sub.3 (1M in tetrahydrofuran, 150 mL). The mixture was stirred at room temperature overnight. The reaction mixture was then carefully quenched by adding methanol dropwise. The mixture was then concentrated under vacuum, and the residue was balanced between ethyl acetate (500 mL) and 2N aqueous HCl (100 mL). The aqueous layer was further extracted twice with ethyl acetate, and the combined organic extracts were washed with water and brine, dried over Na.sub.2SO.sub.4, and filtered. Evaporation of the solvent gave the title compound.

[1736] 2.32.10 1-((3-bromo-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl)-1H-pyrazole

[1737] To a solution of Example 2.32.9 (8.0 g) in toluene (60 mL) was added 1H-pyrazole (1.55 g) and cyanomethylenetriethylphosphorane (2.0 g). The mixture was stirred at 90° C. overnight. The reaction mixture was then concentrated and the residue was purified by silica gel column chromatography (10:1 heptane:ethyl acetate) to give the title compound. MS (ESI) m/e 324.2 (M+H).sup.+.

[1738] 2.32.11 2-{[3,5-dimethyl-7-(1H-pyrazol-1-yl)methyl]tricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy}ethanol

[1739] To a solution of Example 2.32.10 (4.0 g) in ethane-1,2-diol (12 mL) was added

triethylamine (3 mL). The mixture was stirred at 150° C. under microwave conditions (Biotage Initiator) for 45 minutes. The mixture was poured into water (100 mL) and extracted three times with ethyl acetate. The combined organic extracts were washed with water and brine, dried over Na.sub.2SO.sub.4, and filtered. Evaporation of the solvent gave the crude product, which was purified by silica gel chromatography, eluting with 20% ethyl acetate in heptane, followed by 5% methanol in dichloromethane, to give the title compound. MS (ESI) m/e 305.2 (M+H).sup.+.

[1740] 2.32.12 2-({3,5-dimethyl-7-[(5-methyl-1H-pyrazol-1-yl)methyl]tricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethanol

[1741] To a cooled (-78° C.) solution of Example 2.32.11 (6.05 g) in tetrahydrofuran (100 mL) was added n-BuLi (40 mL, 2.5M in hexane). The mixture was stirred at -78° C. for 1.5 hours. Iodomethane (10 mL) was added through a syringe, and the mixture was stirred at -78° C. for 3 hours. The reaction mixture was then quenched with aqueous NH.sub.4Cl and extracted twice with ethyl acetate, and the combined organic extracts were washed with water and brine. After drying over Na.sub.2SO.sub.4, the solution was filtered and concentrated, and the residue was purified by silica gel column chromatography, eluting with 5% methanol in dichloromethane, to give the title compound. MS (ESI) m/e 319.5 (M+H).sup.+.

[1742] 2.32.13 1-({3,5-dimethyl-7-[2-(hydroxy)ethoxy]tricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-4-iodo-5-methyl-1H-pyrazole

[1743] To a solution of Example 2.32.12 (3.5 g) in N,N-dimethylformamide (30 mL) was added N-iodosuccinimide (3.2 g). The mixture was stirred at room temperature for 1.5 hours. The reaction mixture was then diluted with ethyl acetate (600 mL) and washed with aqueous NaHSO.sub.3, water, and brine. After drying over Na.sub.2SO.sub.4, the solution was filtered and concentrated and the residue was purified by silica gel chromatography (20% ethyl acetate in dichloromethane) to give the title compound. MS (ESI) m/e 445.3 (M+H).sup.+.

[1744] 2.32.14 2-({3-[(4-iodo-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl methanesulfonate

[1745] To a cooled solution of Example 2.32.13 (6.16 g) in dichloromethane (100 mL) was added triethylamine (4.21 g) followed by methanesulfonyl chloride (1.6 g). The mixture was stirred at room temperature for 1.5 hours. The reaction mixture was then diluted with ethyl acetate (600 mL) and washed with water and brine. After drying over Na.sub.2SO.sub.4, the solution was filtered and concentrated, and the residue was used in the next reaction without further purification. MS (ESI) m/e 523.4 (M+H).sup.+.

[1746] 2.32.15 1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-4-iodo-5-methyl-1H-pyrazole

[1747] A solution of Example 2.32.14 (2.5 g) in 2M methylamine in methanol (15 mL) was stirred at 100° C. for 20 minutes under microwave conditions (Biotage Initiator). The reaction mixture was concentrated under vacuum. The residue was then diluted with ethyl acetate (400 mL) and washed with aqueous NaHCO.sub.3, water and brine. After drying over Na.sub.2SO.sub.4, the solution was filtered and concentrated, and the residue was used in the next reaction without further purification. MS (ESI) m/e 458.4 (M+H).sup.+.

[1748] 2.32.16 tert-butyl[2-({3-[(4-iodo-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl]methylcarbamate

[1749] To a solution of Example 2.32.15 (2.2 g) in tetrahydrofuran (30 mL) was added di-tert-butyl dicarbonate (1.26 g) and a catalytic amount of 4-dimethylaminopyridine. The mixture was stirred at room temperature for 1.5 hours and diluted with ethyl acetate (300 mL). The solution was washed with saturated aqueous NaHCO.sub.3, water (60 mL), and brine (60 mL). The organic layer was dried with Na.sub.2SO.sub.4, filtered, and concentrated. The residue was purified by silica gel chromatography, eluting with 20% ethyl acetate in dichloromethane, to give the title compound. MS (ESI) m/e 558.5 (M+H).sup.+.

[1750] 2.32.17 6-fluoro-3-bromopicolinic acid

[1751] A slurry of 6-amino-3-bromopicolinic acid (25 g) in 400 mL 1:1 dichloromethane/chloroform was added to nitrosonium tetrafluoroborate (18.2 g) in dichloromethane (100 mL) at 5° C. over 1 hour, and the resulting mixture was stirred for another 30 minutes, then warmed to 35° C. and stirred overnight. The reaction was cooled to room temperature, and then adjusted to pH 4 with aqueous NaH₂PO₄ solution. The resulting solution was extracted three times with dichloromethane, and the combined extracts were washed with brine, dried over sodium sulfate, filtered and concentrated to provide the title compound.

[1752] 2.32.18 Tert-butyl 3-bromo-6-fluoropicolinate

[1753] Para-toluenesulfonyl chloride (27.6 g) was added to a solution of Example 2.32.17 (14.5 g) and pyridine (26.7 mL) in dichloromethane (100 mL) and tert-butanol (80 mL) at 0° C. The reaction was stirred for 15 minutes, warmed to room temperature, and stirred overnight. The solution was concentrated and partitioned between ethyl acetate and aqueous Na₂CO₃ solution. The layers were separated, and the aqueous layer extracted with ethyl acetate. The organic layers were combined, rinsed with aqueous Na₂CO₃ solution and brine, dried over sodium sulfate, filtered, and concentrated to provide the title compound.

[1754] 2.32.19 methyl 2-(5-bromo-6-(tert-butoxycarbonyl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1755] To a solution of methyl 1,2,3,4-tetrahydroisoquinoline-8-carboxylate hydrochloride (12.37 g) and Example 2.32.18 (15 g) in dimethyl sulfoxide (100 mL) was added N,N-diisopropylethylamine (12 mL). The mixture was stirred at 50° C. for 24 hours. The mixture was then diluted with ethyl acetate (500 mL), washed with water and brine, and dried over Na₂SO₄. Filtration and evaporation of the solvent gave a residue that was purified by silica gel chromatography, eluting with 20% ethyl acetate in heptane, to give the title compound. MS (ESI) m/e 448.4 (M+H)⁺.

[1756] 2.32.20 methyl 2-(6-(tert-butoxycarbonyl)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1757] To a solution of Example 2.32.19 (2.25 g) and [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II) (205 mg) in acetonitrile (30 mL) was added triethylamine (3 mL) and pinacolborane (2 mL). The mixture was stirred at reflux for 3 hours. The mixture was diluted with ethyl acetate (200 mL) and washed with water and brine, and dried over Na₂SO₄. Filtration, evaporation of the solvent, and silica gel chromatography (eluted with 20% ethyl acetate in heptane) gave the title compound. MS (ESI) m/e 495.4 (M+H)⁺.

[1758] 2.32.21 methyl 2-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-(tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylate

[1759] To a solution of Example 2.32.20 (4.94 g) in tetrahydrofuran (60 mL) and water (20 mL) was added Example 2.32.16 (5.57 g), 1,3,5,7-tetramethyl-8-tetradecyl-2,4,6-trioxa-8-phosphaadamantane (412 mg), tris(dibenzylideneacetone)dipalladium(0) (457 mg), and K₃PO₄ (11 g). The mixture was stirred at reflux for 24 hours. The reaction mixture was cooled, diluted with ethyl acetate (500 mL), washed with water and brine, and dried over Na₂SO₄. Filtration and evaporation of the solvent gave a residue that was purified by silica gel chromatography, eluting with 20% ethyl acetate in heptane, to give the title compound. MS (ESI) m/e 799.1 (M+H)⁺.

[1760] 2.32.22 2-(6-(tert-butoxycarbonyl)-5-(1-((3-(2-(tert-butoxycarbonyl)(methyl)amino)ethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl)-1,2,3,4-tetrahydroisoquinoline-8-carboxylic acid

[1761] To a solution of Example 2.32.21 (10 g) in tetrahydrofuran (60 mL), methanol (30 mL) and water (30 mL) was added lithium hydroxide monohydrate (1.2 g). The mixture was stirred at room temperature for 24 hours. The reaction mixture was neutralized with 2% aqueous HCl and

concentrated under vacuum. The residue was diluted with ethyl acetate (800 mL) and washed with water and brine, and dried over Na.sub.2SO.sub.4. Filtration and evaporation of the solvent gave the title compound. MS (ESI) m/e 785.1 (M+H).sup.+.

[1762] 2.32.23 tert-butyl 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-{1-[(3-{2-[(tert-butoxycarbonyl)(methyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}pyridine-2-carboxylate

[1763] To a solution of Example 2.32.22 (10 g) in N,N-dimethylformamide (20 mL) was added benzo[d]thiazol-2-amine (3.24 g), fluoro-N,N,N',N'-tetramethylformamidinium hexafluorophosphate (5.69 g) and N,N-diisopropylethylamine (5.57 g). The mixture was stirred at 60° C. for 3 hours. The reaction mixture was diluted with ethyl acetate (800 mL) and washed with water and brine, and dried over Na.sub.2SO.sub.4. Filtration and evaporation of the solvent gave a residue that was purified by silica gel chromatography, eluting with 20% ethyl acetate in dichloromethane, to give the title compound. MS (ESI) m/e 915.5 (M+H).sup.+.

[1764] 2.32.24 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-[1-({3,5-dimethyl-7-[2-(methylamino)ethoxy]tricyclo[3.3.1.1^{3,7}]dec-1-yl}methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid

[1765] To a solution of Example 2.32.23 (5 g) in dichloromethane (20 mL) was added trifluoroacetic acid (10 mL). The mixture was stirred overnight. The solvent was evaporated under vacuum, and the residue was dissolved in dimethyl sulfoxide/methanol (1:1, 10 mL), and chromatographed via reverse-phase using an Analogix system and a C.sub.18 cartridge (300 g), eluting with 10-85% acetonitrile and 0.1% trifluoroacetic acid in water, to give the title compound as a TFA salt. .sup.1H NMR (300 MHz, dimethyl sulfoxide d.sub.6) δ ppm 12.85 (s, 1H), 8.13-8.30 (m, 2H), 8.03 (d, 1H), 7.79 (d, 1H), 7.62 (d, 1H), 7.32-7.54 (m, 3H), 7.28 (d, 1H), 6.96 (d, 1H), 4.96 (dd, 1H), 3.80-3.92 (m, 4H), 3.48-3.59 (m, 1H), 2.91-3.11 (m, 2H), 2.51-2.59 (m, 4H), 2.03-2.16 (m, 2H), 1.21-1.49 (m, 6H), 0.97-1.20 (m, 4H), 0.87 (s, 6H). MS (ESI) m/e 760.4 (M+H).sup.+.

[1766] 2.32.25 3-(1-((3-(2-(((3-(3-aminopropanamido)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

[1767] To a solution of Example 2.32.24 (325 mg) and Example 2.32.7 (382 mg) in N,N-dimethylformamide (9 mL) at 0° C. was added N,N-diisopropylamine (49.1 mg). The reaction mixture was stirred at 0° C. for 5 hours, and acetic acid (22.8 mg) was added. The resulting mixture was diluted with ethyl acetate and washed with water and brine. The organic layer was dried over Na.sub.2SO.sub.4, filtered and concentrated. The residue was dissolved in a mixture of tetrahydrofuran (10 mL) and methanol (5 mL). To this solution at 0° C. was added 1 M aqueous lithium hydroxide solution (3.8 mL). The resulting mixture was stirred at 0° C. for 1 hour, acidified with acetic acid and concentrated. The concentrate was lyophilized to provide a powder. The powder was dissolved in N,N-dimethylformamide (10 mL), cooled in an ice-bath, and piperidine (1 mL) at 0° C. was added. The mixture was stirred at 0° C. for 15 minutes and 1.5 mL of acetic acid was added. The solution was purified by reverse-phase HPLC using a Gilson system, eluting with 30-80% acetonitrile in water containing 0.1% v/v trifluoroacetic acid, to provide the title compound. MS (ESI) m/e 1172.2 (M+H).sup.+.

[1768] 2.32.26 4-[(2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl]-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl)(methyl)carbamoyl}oxy)methyl]-2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid

[1769] To Example 2.32.25 (200 mg) in N,N-dimethylformamide (5 mL) at 0° C. was added 2,5-dioxopyrrolidin-1-yl 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoate (105 mg) and N,N-

diisopropylethylamine (0.12 mL). The mixture was stirred at 0° C. for 15 minutes, warmed to room temperature and purified by reverse-phase HPLC on a Gilson system using a 100 g C18 column, eluting with 30-80% acetonitrile in water containing 0.1% v/v trifluoroacetic acid, to provide the title compound. ¹H NMR (500 MHz, dimethyl sulfoxide-*d*.₆) δ ppm 12.85 (s, 2H) 9.07 (s, 1H) 8.18 (s, 1H) 8.03 (d, 1H) 7.87 (t, 1H) 7.79 (d, 1H) 7.61 (d, 1H) 7.41-7.53 (m, 3H) 7.36 (q, 2H) 7.28 (s, 1H) 7.03-7.09 (m, 1H) 6.96-7.03 (m, 3H) 6.94 (d, 1H) 4.95 (s, 4H) 4.82 (t, 1H) 3.88 (t, 3H) 3.80 (d, 2H) 3.01 (t, 2H) 2.86 (d, 3H) 2.54 (t, 2H) 2.08 (s, 3H) 2.03 (t, 2H) 1.40-1.53 (m, 4H) 1.34 (d, 2H) 0.90-1.28 (m, 12H) 0.82 (d, 6H). MS (ESI) *m/e* 1365.3 (M+H).⁺

[1770] 2.33 Synthesis of Control Synthon 4-[(2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl)(methyl)carbamoyl}oxy)methyl]-2-({N-[19-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-17-oxo-4,7,10,13-tetraoxa-16-azanonadecan-1-oyl]-beta-alanyl}amino)phenyl beta-D-glucopyranosiduronic acid (Synthon I)

[1771] The title compound was prepared using the procedure in Example 2.32.26, replacing 2,5-dioxopyrrolidin-1-yl 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoate with 2,5-dioxopyrrolidin-1-yl 1-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-3-oxo-7,10,13,16-tetraoxa-4-azanonadecan-19-oate. ¹H NMR (500 MHz, dimethyl sulfoxide-*d*.₆) δ ppm 8.95 (s, 1H) 8.16 (s, 1H) 7.99 (d, 1H) 7.57-7.81 (m, 4H) 7.38-7.50 (m, 3H) 7.34 (q, 2H) 7.27 (s, 1H) 7.10 (d, 1H) 7.00 (d, 1H) 6.88-6.95 (m, 2H) 4.97 (d, 4H) 4.76 (d, 2H) 3.89 (t, 2H) 3.84 (d, 2H) 3.80 (s, 2H) 3.57-3.63 (m, 4H) 3.44-3.50 (m, 4H) 3.32-3.43 (m, 6H) 3.29 (t, 2H) 3.16 (q, 2H) 3.02 (t, 2H) 2.87 (s, 3H) 2.52-2.60 (m, 2H) 2.29-2.39 (m, 3H) 2.09 (s, 3H) 1.37 (s, 2H) 1.20-1.29 (m, 4H) 1.06-1.18 (m, 4H) 0.92-1.05 (m, 2H) 0.83 (s, 6H). MS (ESI) *m/e* 1568.6 (M-H).⁻

[1772] 2.34 Synthesis of 4-[(2-({3-[(4-{6-[1-(1,3-benzothiazol-2-ylcarbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl)(2-methoxyethyl)carbamoyl}oxy)methyl]-3-{2-[2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid (Synthon OG)

[1773] 2.34.1 3-(1-((3-(2-(((2-(2-(2-aminoethoxy)ethoxy)-4-(((2R,3S,4R,5R,6R)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(2-methoxyethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(1-(benzo[d]thiazol-2-ylcarbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl)picolinic acid

[1774] To a cold (0° C.) solution of Example 2.11.6 (279 mg) and Example 1.14.9 (240 mg) in N,N-dimethylformamide (10 mL) was added N,N-diisopropylethylamine (0.157 mL). The reaction was slowly warmed to room temperature and was stirred overnight. To the reaction was added water (2 mL) and LiOH H.sub.2O (50 mg), and the mixture was stirred at room temperature for 3 hours. The mixture was acidified with trifluoroacetic acid, filtered and purified by reverse-phase HPLC on a Gilson system (C18 column), eluting with 20-80% acetonitrile in water containing 0.1% trifluoroacetic acid, to provide the title compound. MS (ESI) *m/e* 1233.0 (M-H).⁻

[1775] 2.34.2 3-(1-((3-(2-(((2-(2-(2-((R)-2-amino-3-sulfopropanamido)ethoxy)ethoxy)-4-(((2R,3S,4R,5R,6R)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(2-methoxyethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(1-(benzo[d]thiazol-2-ylcarbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl)picolinic acid

[1776] To a solution of (R)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-sulfopropanoic acid (45.7 mg) in N,N-dimethylformamide (1 mL) was added O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (45 mg) and N,N-diisopropylethylamine (0.02 mL). The mixture was stirred at room temperature for 10 minutes, and a solution of Example 2.34.1 (96 mg) and N,N-diisopropylethylamine (0.1 mL) in N,N-dimethylformamide (2 mL) was added. The reaction mixture was stirred at room temperature for 3 hours. To the reaction mixture was added diethylamine (0.1 mL), and the reaction was stirred at room temperature overnight. The mixture

was diluted with N,N-dimethylformamide (2 mL), filtered and purified by reverse-phase HPLC on a Gilson system (C18 column), eluting with 20-80% acetonitrile in water containing 0.1% trifluoroacetic acid, to provide the title compound. MS (ESI) m/e 1382.2 (M-H).sup.-.

[1777] 2.34.3 4-[(2-((3-((4-((6-((1-(1,3-benzothiazol-2-ylcarbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)oxy)ethyl)(methyl)carbamoyl)oxy)methyl]-3-{2-[2-((N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)ethoxy)ethoxy}phenyl beta-D-glucopyranosiduronic acid

[1778] The title compound was prepared as described in Example 2.5.3, substituting Example 2.5.2 with Example 2.34.2. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 8.38 (s, 1H), 7.99 (d, 1H), 7.90-7.70 (m, 6H), 7.44 (s, 1H), 7.35 (t, 1H), 7.28 (d, 1H), 7.24-7.14 (m, 2H), 6.96 (s, 1H), 6.66 (s, 1H), 5.04 (s, 1H), 4.95 (s, 2H), 4.28 (q, 1H), 4.07 (d, 2H), 3.89 (dd, 3H), 3.22 (ddd, 6H), 2.87-2.61 (m, 4H), 2.20 (s, 3H), 2.04 (t, 2H), 1.93 (p, 2H), 1.54-0.90 (m, 20H), 0.83 (d, 7H). MS (ESI) m/e 1575.2 (M-H).sup.-.

[1779] 2.35 Synthesis of 2-[(2-((3-((4-((6-((5-(1,3-benzothiazol-2-ylcarbamoyl)quinolin-3-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)oxy)ethyl)(methyl)carbamoyl)oxy)methyl]-5-{2-[2-((N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)ethoxy)ethoxy}phenyl beta-D-glucopyranosiduronic acid (Synthon OH)

[1780] 2.35.1 3-(1-((3-(2-(((4-(2-(2-aminoethoxy)ethoxy)-2-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(5-(benzo[d]thiazol-2-ylcarbamoyl)quinolin-3-yl)picolinic acid

[1781] To a cold (0° C.) solution of Example 2.26.7 (76 mg) and 6-(5-(benzo[d]thiazol-2-ylcarbamoyl)quinolin-3-yl)-3-(1-((3,5-dimethyl-7-(2-(methylamino)ethoxy)adamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid (62 mg) in N,N-dimethylformamide (2 mL) was added N,N-diisopropylethylamine (0.043 mL). The reaction was slowly warmed to room temperature and stirred overnight. To the reaction was added water (2 mL) and LiOH H.sub.2O (50 mg), and the mixture was stirred at room temperature for 3 hours. The mixture was acidified with trifluoroacetic acid, filtered and purified by reverse-phase HPLC on a Gilson system (C18 column), eluting with 20-80% acetonitrile in water containing 0.1% trifluoroacetic acid, to provide the title compound. MS (ESI) m/e 1183.3 (M-H).sup.-.

[1782] 2.35.2 3-(1-((3-(2-(((4-(2-(2-((R)-2-amino-3-sulfopropanamido)ethoxy)ethoxy)-2-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(5-(benzo[d]thiazol-2-ylcarbamoyl)quinolin-3-yl)picolinic acid

[1783] To a solution of (R)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-sulfopropanoic acid (22.3 mg) in N,N-dimethylformamide (1 mL) was added O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (22 mg) and N,N-diisopropylethylamine (0.02 mL). The mixture was stirred at room temperature for 10 minutes, and a solution of Example 2.35.1 (45 mg) and N,N-diisopropylethylamine (0.1 mL) in N,N-dimethylformamide (2 mL) was added. The reaction was stirred at room temperature for 3 hours. To the reaction mixture was added diethylamine (0.1 mL), and the reaction was stirred at room temperature overnight. The mixture was diluted with N,N-dimethylformamide (2 mL), filtered and purified by reverse-phase HPLC on a Gilson system (C18 column), eluting with 20-80% acetonitrile in water containing 0.1% trifluoroacetic acid, to provide the title compound. MS (ESI) m/e 1334.5 (M-H).sup.-.

[1784] 2.35.3 2-[(2-((3-((4-((6-((5-(1,3-benzothiazol-2-ylcarbamoyl)quinolin-3-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)oxy)ethyl)(methyl)carbamoyl)oxy)methyl]-5-{2-[2-((N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)ethoxy)ethoxy}phenyl beta-D-glucopyranosiduronic acid

[1785] The title compound was prepared as described in Example 2.34.1, substituting Example 2.5.2 with Example 2.35.2. ¹H NMR (400 MHz, dimethyl sulfoxide-d₆) δ ppm 9.72 (d, 1H), 9.43 (s, 1H), 8.32 (dd, 2H), 8.17 (d, 1H), 8.06 (d, 1H), 8.02-7.92 (m, 2H), 7.86 (d, 1H), 7.82-7.71 (m, 2H), 7.52-7.43 (m, 2H), 7.36 (t, 1H), 7.17 (d, 1H), 6.96 (s, 2H), 6.69 (d, 1H), 6.58 (dd, 1H), 5.03 (dd, 3H), 4.28 (q, 1H), 4.02 (d, 3H), 3.93 (d, 1H), 3.47-3.21 (m, 8H), 3.16 (p, 1H), 2.85 (d, 3H), 2.80-2.63 (m, 2H), 2.22 (s, 3H), 2.04 (t, 2H), 1.53-1.30 (m, 6H), 1.32-0.90 (m, 12H), 0.83 (d, 6H). MS (ESI) m/e 1527.4 (M-H).⁺

[1786] 2.36 Synthesis of 2-[(2-({3-[(4-{6-[1-(1,3-benzothiazol-2-ylcarbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl)(methyl)carbamoyl}oxy)methyl]-5-[2-(2-{[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid (Synthon ON)

[1787] 2.36.1 3-(1-((3-(2-(((4-(2-(2-aminoethoxy)ethoxy)-2-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(1-(benzo[d]thiazol-2-ylcarbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl)picolinic acid, Trifluoroacetic Acid

[1788] To a solution of Example 1.1.14 (157 mg) and Example 2.26.7 (167 mg) in N,N-dimethylformamide (3 mL) at 0° C. was added N,N-diisopropylethylamine (188 μL). The mixture was warmed to room temperature, stirred overnight and concentrated. The residue was dissolved in methanol (2 mL) and tetrahydrofuran (3 mL). The solution was cooled in an ice water bath and 1M aqueous lithium hydroxide solution (1.14 mL) was added. The mixture was stirred 0° C. at room temperature for 2 hours and concentrated. The residue was dissolved in dimethyl sulfoxide and purified by reverse-phase HPLC on a Gilson system (C18 column), eluting with 20-80% acetonitrile in water containing 0.1% trifluoroacetic acid, to provide the title compound.

[1789] 2.36.2 2-[(2-({3-[(4-{6-[1-(1,3-benzothiazol-2-ylcarbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl)(methyl)carbamoyl}oxy)methyl]-5-[2-(2-{[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid

[1790] To a solution of Example 2.36.1 (18 mg) and 2,5-dioxopyrrolidin-1-yl 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoate (6.39 mg) in N,N-dimethylformamide (3 mL) was added N,N-diisopropylethylamine (24 μL). The resulting mixture was stirred for 1 hour and was purified by reverse-phase HPLC on a Gilson system (C18 column), eluting with 20-75% acetonitrile in water containing 0.1% trifluoroacetic acid, to provide the title compound. ¹H NMR (400 MHz, dimethyl sulfoxide-d₆) δ ppm 8.36 (s, 1H), 7.97 (d, 1H), 7.85-7.70 (m, 4H), 7.43 (s, 1H), 7.38-7.30 (m, 1H), 7.26 (d, 1H), 7.23-7.10 (m, 2H), 6.95 (s, 2H), 6.65 (d, 1H), 6.56 (dd, 1H), 5.08-4.94 (m, 3H), 4.02 (dd, 2H), 3.92 (dd, 3H), 3.84 (s, 2H), 3.67 (t, 2H), 3.31-3.20 (m, 2H), 3.16 (q, 2H), 2.91-2.74 (m, 6H), 2.18 (s, 3H), 1.99 (t, 2H), 1.91 (p, 2H), 1.51-1.29 (m, 5H), 1.29-0.88 (m, 9H), 0.81 (d, 6H). MS (ESI) m/e 1380.2 (M-H).⁺

[1791] 2.37 Synthesis of 4-[(2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)naphthalen-2-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl)(methyl)carbamoyl}oxy)methyl]-3-{2-[2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid (Synthon OT)

[1792] 2.37.1 3-(1-((3-(2-(((2-(2-(2-aminoethoxy)ethoxy)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)naphthalen-2-yl)picolinic acid

[1793] The title compound was prepared by substituting Example 1.6.3 for Example 1.12.10 and Example 2.11.6 for Example 2.26.7 in Example 2.26.8. MS (ESI) m/e 1182.3 (M-H).⁺

[1794] 2.37.2 3-(1-(((3-(2-(((2-(2-(2-((R)-2-amino-3-sulfopropylamino)ethoxy)ethoxy)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-

yl)oxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)naphthalen-2-yl)picolinic acid [1795] The title compound was prepared by substituting Example 2.37.1 for Example 2.9.1 in Example 2.18.1. MS (ESI) m/e 1333.3 (M-H).sup.-.

[1796] 2.37.3 4-[(1[2-(13-[(4-16-[8-(1,3-benzothiazol-2-ylcarbamoyl)naphthalen-2-yl]-2-carboxypyridin-3-yl]-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-{2-[2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid

[1797] The title compound was prepared by substituting Example 2.37.2 for Example 2.9.1 in Example 2.9.2. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 9.02 (s, 1H), 8.37 (d, 1H), 8.23 (d, 1H), 8.20 (d, 1H), 8.18 (d, 1H), 8.06 (d, 1H), 8.01 (d, 1H), 7.94 (d, 1H), 7.87 (br d, 1H), 7.81 (d, 1H), 7.77 (br t, 1H), 7.70 (dd, 1H), 7.48 (dd, 1H), 7.48 (s, 1H), 7.37 (dd, 1H), 7.19 (d, 1H), 6.97 (s, 2H), 6.68 (d, 1H), 6.59 (dd, 1H), 5.06 (br m, 1H), 4.97 (s, 2H), 4.31 (m, 1H), 4.09 (m, 2H), 3.90 (m, 5H), 3.71 (m, 2H), 3.45 (m, 5H), 3.36 (m, 3H), 3.28 (m, 4H), 3.19 (m, 2H), 2.82 (br d, 2H), 2.76 (dd, 2H), 2.23 (s, 3H), 2.06 (t, 2H), 1.52-1.32 (m, 6H), 1.32-0.92 (m, 10H), 0.85 (br s, 6H). MS (ESI) m/e 1526.4 (M-H).sup.-.

[1798] 2.38 Synthesis of 2-[(1[2-({3-[(4-{6-[4-(1,3-benzothiazol-2-ylcarbamoyl)quinolin-6-yl]-2-carboxypyridin-3-yl]-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-[2-(2-{{6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid (Synthon OP)

[1799] 2.38.1 3-(1-(((3-(2-(((4-(2-(2-aminoethoxy)ethoxy)-2-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(4-(benzo[d]thiazol-2-ylcarbamoyl)quinolin-6-yl)picolinic acid

[1800] The title compound was prepared as described in Example 2.36.1, substituting Example 1.1.14 with Example 1.11.4.

[1801] 2.38.2 2-[(1[2-({3-[(4-{6-[4-(1,3-benzothiazol-2-ylcarbamoyl)quinolin-6-yl]-2-carboxypyridin-3-yl]-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-5-[2-(2-{{6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid

[1802] The title compound was prepared as described in Example 2.36.2, substituting Example 2.36.1 with Example 2.38.1. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 9.12 (d, 1H), 8.93 (s, 1H), 8.60 (dd, 1H), 8.27 (d, 1H), 8.21 (d, 1H), 8.07 (d, 1H), 7.97-7.90 (m, 2H), 7.81 (d, 2H), 7.47 (d, 2H), 7.37 (t, 1H), 7.17 (d, 1H), 6.96 (s, 2H), 6.67 (d, 1H), 6.58 (dd, 1H), 5.11-4.96 (m, 3H), 4.04 (dd, 2H), 3.92 (d, 1H), 3.86 (s, 2H), 3.40 (q, 5H), 3.34 (t, 2H), 3.31-3.22 (m, 4H), 3.17 (q, 2H), 2.85 (d, 3H), 2.20 (s, 3H), 2.00 (t, 2H), 1.51-1.31 (m, 6H), 1.30-0.88 (m, 13H), 0.82 (d, 6H). MS (ESI) m/e 1400.3 (M+Na).sup.+.

[1803] 2.39 Synthesis of 4-[(1[2-({3-[(4-{6-[1-(1,3-benzothiazol-2-ylcarbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl]-2-carboxypyridin-3-yl]-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-{2-[2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid (Synthon OU)

[1804] 2.39.1 3-(1-(((3-(2-(((2-(2-(2-aminoethoxy)ethoxy)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(1-(benzo[d]thiazol-2-ylcarbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl)picolinic acid

[1805] The title compound was prepared by substituting Example 1.1.14 for Example 1.12.10 and Example 2.11.6 for Example 2.26.7 in Example 2.26.8. MS (ESI-) m/e 1187.2 (M-H).sup.-.

[1806] 2.39.2 3-(1-((3-(2-(((2-(2-(2-((R)-2-amino-3-sulfopropanamido)ethoxy)ethoxy)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(1-(benzo[d]thiazol-2-ylcarbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl)picolinic acid

[1807] The title compound was prepared by substituting Example 2.39.1 for Example 2.9.1 in Example 2.18.1. MS (ESI⁻) m/e 1338.2 (M-H).sup.-.

[1808] 2.39.3 4-[(2-((3-((4-((6-((1-(1,3-benzothiazol-2-ylcarbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl)(methyl)carbamoyl}oxy)methyl]-3-{2-[2-((N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)ethoxy)ethoxy}phenyl beta-D-glucopyranosiduronic acid

[1809] The title compound was prepared by substituting Example 2.39.2 for Example 2.9.1 in Example 2.9.2. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 8.39 (br s 1H), 8.00 (d, 1H), 7.86 (d, 2H), 7.81 (d, 1H), 7.77 (d, 2H), 7.48 (v br s, 1H), 7.46 (s, 1H), 7.37 (t, 1H), 7.29 (d, 1H), 7.23 (d, 1H), 7.19 (d, 1H), 6.92 (s, 2H), 6.68 (d, 1H), 6.59 (dd, 1H), 5.06 (br m, 1H), 4.97 (s, 2H), 4.31 (m, 1H), 4.09 (m, 2H), 3.96 (br t, 2H), 3.88 (br m, 2H), 3.71 (m, 2H), 3.45 (m, 5H), 3.37 (m, 3H), 3.28 (m, 4H), 3.18 (m, 2H), 2.86 (br m, 5H), 2.75 (dd, 2H), 2.22 (s, 3H), 2.06 (t, 2H), 1.95 (m, 2H), 1.52-1.32 (m, 6H), 1.32-0.92 (m, 12H), 0.85 (br s, 6H). MS (ESI⁻) m/e 1531.2 (M-H).sup.-.

[1810] 2.40 Synthesis of 4-[(2-((3-((4-((6-((1-(1,3-benzothiazol-2-ylcarbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl)(methyl)carbamoyl}oxy)methyl]-3-(3-{[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]amino}propoxy)phenyl beta-D-glucopyranosiduronic acid (Synthon 00)

[1811] 2.40.1 3-(1-((3-(2-(((2-(3-aminopropoxy)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(1-(benzo[d]thiazol-2-ylcarbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl)picolinic acid

[1812] The title compound was prepared as described in Example 2.36.1, substituting Example 2.26.7 with Example 2.28.3. MS (ESI) m/e 1159.2 (M+H).sup.+.

[1813] 2.40.2 4-[(2-((3-((4-((6-((1-(1,3-benzothiazol-2-ylcarbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl)(methyl)carbamoyl}oxy)methyl]-3-(3-{[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]amino}propoxy)phenyl beta-D-glucopyranosiduronic acid

[1814] The title compound was prepared as described in Example 2.36.2, substituting Example 2.36.1 with Example 2.40.1. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 8.38 (s, 1H), 7.98 (d, 1H), 7.87-7.72 (m, 2H), 7.44 (s, 1H), 7.35 (t, 1H), 7.28 (d, 1H), 7.19 (dd, 2H), 6.96 (s, 2H), 6.62 (d, 1H), 6.57 (dd, 1H), 5.03 (s, 1H), 4.95 (s, 2H), 4.03-3.81 (m, 8H), 3.42-3.20 (m, 7H), 3.16 (q, 2H), 2.90-2.75 (m, 5H), 2.20 (s, 3H), 2.01 (t, 2H), 1.97-1.87 (m, 2H), 1.80 (t, 2H), 1.45 (td, 4H), 1.13 (d, 8H), 0.83 (d, 6H). MS (ESI) m/e 1350.2 (M-H).sup.-.

[1815] 2.41 Synthesis of 4-[(2-((3-((4-((6-((1-(1,3-benzothiazol-2-ylcarbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl)(methyl)carbamoyl}oxy)methyl]-3-[3-((N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)propoxy)phenyl beta-D-glucopyranosiduronic acid (Synthon OQ)

[1816] 2.41.1 3-(1-((3-(2-(((2-(3-((R)-2-amino-3-sulfopropanamido)propoxy)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-

methyl-1H-pyrazol-4-yl)-6-(1-(benzo[d]thiazol-2-ylcarbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl)picolinic acid

[1817] To a solution of (R)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-sulfopropionic acid (35.4 mg) and O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (29.8 mg) in N,N-dimethylformamide (1 mL) at 0° C. was added N,N-diisopropylethylamine (30 L). The resulting mixture was stirred for 15 minutes and added to a mixture of Example 2.40.1 (70 mg) and N,N-diisopropylethylamine (80 L) in N,N-dimethylformamide (2 mL). The resulting mixture was stirred for 1 hour. Diethylamine (62.2 µL) was added, and the mixture was stirred for 1 hour. The reaction was cooled in ice-bath and trifluoroacetic acid (93 µL) was added. The mixture was diluted with dimethyl sulfoxide (5.5 mL) and purified by reverse-phase HPLC on a Gilson system (C18 column), eluting with 20-75% acetonitrile in water containing 0.1% trifluoroacetic acid, to provide the title compound.

[1818] 2.41.2 4-[(2-((3-((4-((6-[1-(1,3-benzothiazol-2-ylcarbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl]-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl)(methyl)carbamoyl}oxy)methyl]-3-[(N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)propoxy]phenyl beta-D-glucopyranosiduronic acid

[1819] The title compound was prepared as described in Example 2.36.2, substituting Example 2.36.1 with Example 2.41.1. ¹H NMR (501 MHz, dimethyl sulfoxide-d₆) 6.8.37 (s, 1H), 7.98 (d, 1H), 7.87-7.72 (m, 5H), 7.44 (s, 1H), 7.35 (t, 1H), 7.27 (d, 1H), 7.20 (t, 1H), 7.16 (d, 1H), 6.96 (s, 2H), 6.63 (d, 1H), 6.55 (dd, 1H), 5.02 (s, 1H), 4.95 (s, 2H), 4.26 (q, 1H), 4.04-3.79 (m, 8H), 3.32-3.08 (m, 4H), 2.89-2.66 (m, 7H), 2.35 (q, OH), 2.20 (s, 3H), 2.03 (t, 2H), 1.93 (p, 2H), 1.80 (t, 2H), 1.52-1.30 (m, 6H), 1.30-0.89 (m, 13H), 0.83 (d, 6H). MS (ESI) m/e 1502.2 (M-H).sup.-.

[1820] 2.42 Synthesis of 2-[(2-((3-((4-((6-[1-(1,3-benzothiazol-2-ylcarbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl]-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl)(methyl)carbamoyl}oxy)methyl]-5-{2-[2-((N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)ethoxy)ethoxy}phenyl beta-D-glucopyranosiduronic acid (Synthon OR)

[1821] 2.42.1 3-(1-((3-(2-(((4-(2-(2-((R)-2-amino-3-sulfopropionamido)ethoxy)ethoxy)-2-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(1-(benzo[d]thiazol-2-ylcarbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl)picolinic acid

[1822] The title compound was prepared as described in Example 2.41.1, substituting Example 2.40.1 with Example 2.36.1. MS (ESI) m/e 1338.2 (M-H).sup.-.

[1823] 2.42.2 2-[(2-((3-((4-((6-[1-(1,3-benzothiazol-2-ylcarbamoyl)-1,2,3,4-tetrahydroquinolin-7-yl]-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl)(methyl)carbamoyl}oxy)methyl]-5-{2-[2-((N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)ethoxy)ethoxy}phenyl beta-D-glucopyranosiduronic acid

[1824] The title compound was prepared as described in Example 2.36.2, substituting Example 2.36.1 with Example 2.42.1. ¹H NMR (500 MHz, dimethyl sulfoxide-d₆) 6.8.39 (s, 1H), 8.00 (d, 1H), 7.86 (t, 2H), 7.83-7.73 (m, 3H), 7.45 (s, 1H), 7.40-7.32 (m, 1H), 7.29 (d, 1H), 7.26-7.13 (m, 2H), 6.97 (s, 2H), 6.70 (d, 1H), 6.59 (dd, 1H), 5.11-4.94 (m, 3H), 4.29 (dt, 1H), 4.04 (dd, 2H), 3.99-3.91 (m, 3H), 3.87 (d, 2H), 3.69 (t, 2H), 3.40-3.07 (m, 7H), 2.91-2.74 (m, 6H), 2.69 (dd, 1H), 2.21 (s, 3H), 2.05 (t, 2H), 1.94 (p, 2H), 1.53-1.32 (m, 5H), 1.31-0.90 (m, 7H), 0.84 (d, 6H). MS (ESI) m/e 1531.2 (M-H).sup.-.

[1825] 2.43 Synthesis of 4-[(2-((3-((4-((6-[8-(1,3-benzothiazol-2-ylcarbamoyl)naphthalen-2-yl]-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-

dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](2-methoxyethyl)carbamoyl}oxy)methyl]-3-{2-[2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid (Synthon OS)

[1826] 2.43.1 3-(1-((3-(2-(((2-(2-(2-aminoethoxy)ethoxy)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(2-methoxyethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)naphthalen-2-yl)picolinic acid

[1827] The title compound was prepared as described in Example 2.34.1, substituting Example 2.5.2 with Example 1.15.1. MS (ESI) m/e 1228.1 (M-H).sup.-.

[1828] 2.43.2 3-(1-((3-(2-(((2-(2-(2-((R)-2-amino-3-sulfopropanamido)ethoxy)ethoxy)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(2-methoxyethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)naphthalen-2-yl)picolinic acid

[1829] The title compound was prepared as described in Example 2.34.2, substituting Example 2.34.1 with Example 2.43.2. MS (ESI) m/e 1379.1.1 (M+H).sup.+.

[1830] 2.43.3 4-[(({2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)naphthalen-2-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](2-methoxyethyl)carbamoyl}oxy)methyl]-3-{2-[2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid

[1831] The title compound was prepared as described in Example 2.34, substituting Example 2.34.2 with Example 2.43.2. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 9.00 (s, 1H), 8.36 (d, 1H), 8.27-8.12 (m, 3H), 8.05 (d, 1H), 8.00 (d, 1H), 7.92 (d, 1H), 7.85 (d, 1H), 7.79 (d, 1H), 7.75 (t, 1H), 7.69 (t, 1H), 7.52-7.43 (m, 2H), 7.35 (t, 1H), 7.24-7.12 (m, 1H), 6.95 (s, 2H), 6.66 (s, 1H), 6.57 (d, 1H), 5.04 (d, 1H), 4.95 (s, 2H), 4.29 (q, 1H), 4.15-4.01 (m, 2H), 3.86 (d, 3H), 3.46-3.11 (m, 16H), 2.84-2.62 (m, 2H), 2.21 (d, 3H), 2.04 (t, 2H), 1.53-1.30 (m, 6H), 1.28-0.89 (m, 6H), 0.82 (d, 7H). MS (ESI) m/e 1570.4 (M-H).sup.-.

[1832] 2.44 Synthesis of N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-[4-((({2-({8-(1,3-benzothiazol-2-ylcarbamoyl)-2-[6-carboxy-5-(1-{3-(2-methoxyethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]methyl}-5-methyl-1H-pyrazol-4-yl)pyridin-2-yl]-1,2,3,4-tetrahydroisoquinolin-6-yl}(methyl)amino)ethyl}(methyl)carbamoyl}oxy)methyl)phenyl]-L-alaninamide (Synthon OX)

[1833] The title compound was prepared as described in Example 2.30, substituting Example 1.17.10 with Example 1.21.12. MS (ESI) m/e 1359.5 (M+H).sup.+ , 1357.5 (M-H).sup.-.

[1834] 2.45 Synthesis of N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[(({2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-6-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]phenyl}-L-alaninamide (Synthon OZ)

[1835] The title compound was prepared as described in Example 2.30, substituting Example 1.17.10 with Example 1.22.9. MS (ESI) m/e 1302.5 (M+H).sup.+ , 1300.5 (M-H).sup.-.

[1836] 2.46 Synthesis of 2-[(({2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)naphthalen-2-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](2-methoxyethyl)carbamoyl}oxy)methyl]-5-{2-[2-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid (Synthon PA)

[1837] 2.46.1 3-(1-((3-(2-(((4-(2-(2-aminoethoxy)ethoxy)-2-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(2-methoxyethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-

(8-(benzo[d]thiazol-2-ylcarbamoyl)naphthalen-2-yl)picolinic acid

[1838] The title compound was prepared as described in Example 2.43.1, substituting Example 2.11.6 with Example 2.26.7. MS (ESI) m/e 1228.1 (M-H).sup.-.

[1839] 2.46.2 3-(1-((3-(2-(((4-(2-(2-((R)-2-amino-3-sulfopropanamido)ethoxy)ethoxy)-2-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(2-methoxyethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)naphthalen-2-yl)picolinic acid

[1840] The title compound was prepared as described in Example 2.34.2, substituting Example 2.34.1 with Example 2.46.1. MS (ESI) m/e 1377.5 (M-H).sup.-.

[1841] 2.46.3 2-[(2-((3-((4-((6-((8-(1,3-benzothiazol-2-ylcarbamoyl)naphthalen-2-yl))-2-carboxypyridin-3-yl))-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)oxy)ethyl)(2-methoxyethyl)carbamoyl)oxy)methyl]-5-{2-[2-((N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl)amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid

[1842] The title compound was prepared as described in Example 2.34, substituting Example 2.34.2 with Example 2.46.2. .sup.1H NMR (501 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 13.08 (s, 1H), 9.00 (s, 1H), 8.36 (d, 1H), 8.25-8.12 (m, 3H), 8.05 (d, 1H), 8.00 (d, 1H), 7.92 (d, 1H), 7.85 (d, 1H), 7.78 (dd, 2H), 7.72-7.65 (m, 1H), 7.50-7.43 (m, 2H), 7.35 (t, 1H), 7.21-7.14 (m, 1H), 6.96 (s, 2H), 6.69 (d, 1H), 6.58 (d, 1H), 5.13-4.93 (m, 3H), 4.28 (q, 1H), 4.03 (dd, 2H), 3.94 (d, 1H), 3.86 (d, 2H), 3.67 (t, 2H), 3.31-3.08 (m, 8H), 2.83-2.64 (m, 2H), 2.21 (d, 3H), 2.04 (t, 2H), 1.53-1.30 (m, 5H), 1.30-0.89 (m, 11H), 0.89-0.75 (m, 6H). MS (ESI) m/e 1570.5 (M-H).sup.-.

[1843] 2.47 Synthesis of 2-[(2-((3-((4-((6-((5-(1,3-benzothiazol-2-ylcarbamoyl)quinolin-3-yl))-2-carboxypyridin-3-yl))-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)oxy)ethyl)(methyl)carbamoyl)oxy)methyl]-5-[2-(2-((6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl)amino)ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid (Synthon QL)

[1844] 2.47.1 3-(1-((3-(2-(((4-(2-(2-aminoethoxy)ethoxy)-2-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(5-(benzo[d]thiazol-2-ylcarbamoyl)quinolin-3-yl)picolinic acid

[1845] The title compound was prepared as described in Example 2.36.1, substituting Example 1.1.14 with Example 1.10.3.

[1846] 2.47.2 2-[(2-((3-((4-((6-((5-(1,3-benzothiazol-2-ylcarbamoyl)quinolin-3-yl))-2-carboxypyridin-3-yl))-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)oxy)ethyl)(methyl)carbamoyl)oxy)methyl]-5-[2-(2-((6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl)amino)ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid

[1847] The title compound was prepared as described in Example 2.36., substituting Example 2.36.1 with Example 2.47.1. .sup.1H NMR (501 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 13.17 (s, 1H), 9.70 (d, 1H), 9.39 (s, 1H), 8.31 (dd, 2H), 8.16 (d, 1H), 8.06 (dd, 1H), 8.01-7.90 (m, 2H), 7.83-7.71 (m, 2H), 7.52-7.43 (m, 2H), 7.39-7.31 (m, 1H), 7.18 (d, 1H), 6.96 (s, 2H), 6.65 (d, 1H), 6.58 (dd, 1H), 5.04 (s, 1H), 4.96 (s, 2H), 4.09 (dtd, 2H), 3.87 (s, 2H), 3.70 (t, 2H), 3.40-3.14 (m, 7H), 2.85 (d, 3H), 2.22 (s, 3H), 2.01 (t, 2H), 1.49-1.30 (m, 6H), 1.30-0.90 (m, 10H), 0.90-0.74 (m, 6H). MS (ESI) m/e 1400.4 (M+Na).sup.+.

[1848] 2.48 Synthesis of 4-[(2-((3-((4-((6-((5-(1,3-benzothiazol-2-ylcarbamoyl)quinolin-3-yl))-2-carboxypyridin-3-yl))-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)oxy)ethyl)(methyl)carbamoyl)oxy)methyl]-3-[2-(2-((6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl)amino)ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid (Synthon QM)

[1849] 2.48.1 3-(1-((3-(2-(((2-(2-(2-aminoethoxy)ethoxy)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(5-(benzo[d]thiazol-2-

ylcarbamoyl)quinolin-3-yl)picolinic acid

[1850] To a solution of Example 1.10.3 (208 mg) and Example 2.11.6 (267 mg) in N,N-dimethylformamide (2 mL) at 0° C. was added N,N-diisopropylethylamine (251 µL). The resulting mixture was stirred at room temperature overnight and concentrated. The residue was dissolved in methanol (3 mL) and tetrahydrofuran (5 mL). The solution was cooled in an ice water bath and 1M aqueous lithium hydroxide solution was added (2.87 mL). The mixture was stirred at 0° C. for 2 hours and was acidified with trifluoroacetic acid. The reaction mixture was concentrated under reduced pressure. The residue was diluted with dimethyl sulfoxide and purified by reverse-phase HPLC on a Gilson system (C18 column), eluting with 20-75% acetonitrile in water containing 0.1% trifluoroacetic acid, to provide the title compound. MS (ESI) m/e 1185.1 (M+H).sup.+.

[1851] 2.48.2 4-[(2-((3-((4-((6-((5-(1,3-benzothiazol-2-ylcarbamoyl)quinolin-3-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)oxy)ethyl)(methyl)carbamoyl)oxy)methyl]-3-[2-(2-((6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl)amino)ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid

[1852] The title compound was prepared as described in Example 2.36.2, substituting Example 2.36.1 with Example 2.48.1. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ 13.18 (s, 1H), 9.70 (d, 1H), 9.39 (s, 1H), 8.31 (dd, 2H), 8.16 (d, 1H), 8.06 (d, 1H), 8.01-7.90 (m, 2H), 7.80 (d, 2H), 7.52-7.43 (m, 2H), 7.39-7.32 (m, 1H), 7.18 (d, 1H), 6.96 (s, 2H), 6.67 (d, 1H), 6.58 (dd, 1H), 5.11-4.90 (m, 3H), 4.03 (d, 2H), 3.95-3.82 (m, 3H), 3.68 (t, 2H), 3.48-3.23 (m, 10H), 3.18 (t, 2H), 2.85 (d, 3H), 2.22 (s, 3H), 2.00 (t, 2H), 1.51-1.31 (m, 5H), 1.19 (dd, 10H), 0.83 (d, 6H). MS (ESI) m/e 1376.4 (M-H).sup.-.

[1853] 2.49 Synthesis of 6-[5-(1,3-benzothiazol-2-ylcarbamoyl)quinolin-3-yl]-3-(1-((3-(2-((6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl)(methyl)amino)ethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid (Synthon QN)

[1854] The title compound was prepared as described in Example 2.36.2, substituting Example 2.36.1 with Example 1.10.3. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ 13.21 (s, 1H), 9.70 (d, 1H), 9.40 (s, 1H), 8.42-8.27 (m, 2H), 8.16 (d, 1H), 8.06 (d, 1H), 8.04-7.90 (m, 2H), 7.80 (d, 1H), 7.56-7.44 (m, 2H), 7.42-7.31 (m, 1H), 6.95 (d, 2H), 3.87 (s, 2H), 3.55-3.18 (m, 5H), 2.95 (s, 1H), 2.76 (s, 2H), 2.28 (t, 1H), 2.22 (s, 4H), 1.53-1.29 (m, 6H), 1.28-0.91 (m, 10H), 0.84 (s, 6H). MS (ESI) m/e 949.1 (M+H).sup.+.

[1855] 2.50 Synthesis of 4-[(2-((3-((4-((6-((7-(1,3-benzothiazol-2-ylcarbamoyl)-1H-indol-2-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)oxy)ethyl)(methyl)carbamoyl)oxy)methyl]-2-((N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-beta-alanyl)amino)phenyl beta-D-glucopyranosiduronic acid (Synthon QT)

[1856] 2.50.1 3-(1-((3-(2-(((3-(3-aminopropanamido)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(7-(benzo[d]thiazol-2-ylcarbamoyl)-1H-indol-2-yl)picolinic acid

[1857] The title compound was prepared by substituting Example 1.27.4 for Example 2.32.24 in Example 2.32.25. MS (ESI) m/e:1156.6 (M+H).sup.+.

[1858] 2.50.2 4-[(2-((3-((4-((6-((7-(1,3-benzothiazol-2-ylcarbamoyl)-1H-indol-2-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)oxy)ethyl)(methyl)carbamoyl)oxy)methyl]-2-((N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-beta-alanyl)amino)phenyl beta-D-glucopyranosiduronic acid

[1859] The title compound was prepared by substituting Example 2.50.1 for Example 2.11.7 in Example 2.11.8. .sup.1H NMR (501 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 13.00 (s, 2H); 9.06 (s, 1H), 8.29 (dd, 1H), 8.22 (d, 1H), 8.18 (s, 1H), 8.04 (t, 2H), 7.97 (d, 1H), 7.90 (d, 1H), 7.79 (d, 1H), 7.50-7.43 (m, 3H), 7.35 (ddd, 1H), 7.25 (t, 1H), 7.06 (d, 1H), 7.01 (dd, 1H), 6.94 (s, 2H), 4.96 (s, 2H), 4.81 (s, 1H), 3.33-3.25 (m, 6H), 2.87 (d, 3H), 2.50 (d, 3H), 2.31 (dd, 2H), 2.21 (s, 3H),

1.38 (d, 2H), 1.30-0.77(m, 18H). MS (ESI) m/e 1305.2 (M-H).sup.-.

[1860] 2.51 Synthesis of 4-[(2-({3-[(4-{6-[7-(1,3-benzothiazol-2-ylcarbamoyl)-1H-indol-2-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl)(methyl)carbamoyl}oxy)methyl]-3-[2-(2-{[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid (Synthon RF)

[1861] 2.51.1 3-(1-((3-(2-(((2-(2-(2-aminoethoxy)ethoxy)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(7-(benzo[d]thiazol-2-ylcarbamoyl)-1H-indol-2-yl)picolinic acid

[1862] The title compound was prepared by substituting Example 1.27.4 for Example 1.12.10 in Example 2.11.7. MS (ESI) m/e:1172.9 (M+H).sup.+.

[1863] 2.51.2 4-[(2-({3-[(4-{6-[7-(1,3-benzothiazol-2-ylcarbamoyl)-1H-indol-2-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl)(methyl)carbamoyl}oxy)methyl]-3-[2-(2-{[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid

[1864] The title compound was prepared by substituting Example 2.51.1 for Example 2.11.7 in Example 2.11.8. .sup.1H NMR (501 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 11.16 (s, 2H), 8.27 (d, 1H), 8.19 (d, 1H), 8.06-7.94 (m, 3H), 7.88 (d, 1H), 7.77 (d, 1H), 7.50-7.39 (m, 3H), 7.33 (t, 1H), 7.26-7.13 (m, 2H), 6.93 (s, 2H), 6.63 (d, 1H), 6.57 (dd, 1H), 5.03 (d, 1H), 4.94 (s, 2H), 4.13-4.00 (m, 2H), 3.86 (d, 3H), 3.14 (q, 2H), 2.83 (d, 3H), 2.29 (t, 2H), 2.20 (s, 3H), 1.36 (d, 2H), 1.28-0.73 (m, 16H). MS (ESI) m/e 1322.4 (M-H).sup.-.

[1865] 2.52 Synthesis of 4-[(2-({3-[(4-{6-[7-(1,3-benzothiazol-2-ylcarbamoyl)-1H-indol-2-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl)(methyl)carbamoyl}oxy)methyl]-3-{2-[2-({N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-3-sulfo-L-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid (Synthon RG)

[1866] 2.52.1 3-(1-((3-(2-(((2-(2-(2-((R)-2-amino-3-sulfopropanamido)ethoxy)ethoxy)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(7-(benzo[d]thiazol-2-ylcarbamoyl)-1H-indol-2-yl)picolinic acid

[1867] The title compound was prepared by substituting Example 2.51.1 for Example 2.9.1 in Example 2.18.1. MS (ESI) m/e:1325.5 (M+H).sup.+.

[1868] 2.52.2 4-[(2-({3-[(4-{6-[7-(1,3-benzothiazol-2-ylcarbamoyl)-1H-indol-2-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl)(methyl)carbamoyl}oxy)methyl]-3-{2-[2-({N-[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]-3-sulfo-L-alanyl}amino)ethoxy]ethoxy}phenyl beta-D-glucopyranosiduronic acid

[1869] The title compound was prepared by substituting Example 2.52.1 for Example 2.11.7 in Example 2.11.8. .sup.1H NMR (501 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 11.17 (s, 2H), 8.27 (d, 1H), 8.20 (d, 1H), 8.03 (dd, 2H), 7.96 (d, 1H), 7.89 (d, 1H), 7.82-7.75 (m, 2H), 7.50 (s, 1H), 7.48-7.41 (m, 2H), 7.34 (t, 1H), 7.24 (t, 1H), 7.18 (d, 1H), 6.93 (s, 2H), 6.66 (d, 1H), 6.58 (dd, 1H), 5.04 (d, 1H), 4.95 (s, 2H), 3.70 (t, 2H), 3.58 (t, 2H), 3.48-3.14 (m, 11H), 2.89-2.79 (m, 4H), 2.73 (dd, 1H), 2.37 (m, 2H), 2.21 (s, 3H), 1.45-0.73 (m, 19H). MS (ESI) m/e 1473.3 (M-H).sup.-.

[1870] 2.53 Synthesis of 4-[(2-({3-[(4-{6-[7-(1,3-benzothiazol-2-ylcarbamoyl)-3-methyl-1H-indol-2-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl)(methyl)carbamoyl}oxy)methyl]-3-[2-(2-{[3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl]amino}ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid (Synthon SF)

[1871] 2.53.1 3-(1-((3-(2-(((2-(2-(2-aminoethoxy)ethoxy)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(7-(benzo[d]thiazol-2-

ylcarbamoyl)-3-methyl-1H-indol-2-yl)picolinic acid

[1872] The title compound was prepared by substituting Example 1.29.7 for Example 1.12.10 in Example 2.11.7. MS (ESI) m/e:1187.1 (M+H).sup.+.

[1873] 2.53.2 4-[(2-((3-((4-((6-((1,3-benzothiazol-2-ylcarbamoyl)-3-methyl-1H-indol-2-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl)(methyl)carbamoyl}oxy)methyl]-3-[2-(2-((3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)propanoyl)amino)ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid

[1874] The title compound was prepared by substituting Example 2.53.1 for Example 2.11.7 in Example 2.11.8. .sup.1H NMR (501 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 11.01 (s, 1H), 8.28 (d, 1H), 8.06-7.94 (m, 4H), 7.91 (d, 1H), 7.76 (d, 1H), 7.50-7.42 (m, 2H), 7.32 (td, 1H), 7.26-7.15 (m, 2H), 6.93 (s, 2H), 6.64 (d, 1H), 6.58 (dd, 1H), 5.03 (d, 1H), 4.95 (s, 2H), 4.11-3.99 (m, 2H), 3.87 (d, 3H), 3.68 (t, 2H), 3.56 (dd, 2H), 3.47-3.33 (m, 5H), 3.33-3.19 (m, 4H), 3.14 (q, 2H), 2.84 (d, 3H), 2.63 (s, 3H), 2.30 (dd, 2H), 2.21 (s, 3H), 1.42-0.72 (m, 21H). MS (ESI) m/e 1336.3 (M-H).sup.-.

[1875] 2.54 Synthesis of N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-[(2-((3-((4-((6-((1,3-benzothiazol-2-ylcarbamoyl)isoquinolin-6-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl)(methyl)carbamoyl}oxy)methyl]phenyl}-N5-carbamoyl-L-ornithinamide (Synthon SR)

[1876] The title compound was prepared as described in Example 2.2, substituting Example 1.3.2 with Example 1.26.10. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 13.28 (s, 2H), 9.96 (s, 1H), 9.59 (s, 1H), 9.03 (d, 2H), 8.53 (d, 1H), 8.42 (d, 1H), 8.25 (d, 1H), 8.05 (t, 2H), 7.97 (d, 1H), 7.78 (dd, 2H), 7.58 (d, 2H), 7.47 (d, 2H), 7.36 (t, 1H), 7.26 (d, 2H), 6.97 (s, 2H), 5.96 (s, 1H), 4.96 (s, 2H), 4.45-4.29 (m, 1H), 4.17 (t, 1H), 3.51-3.18 (m, 6H), 3.07-2.75 (m, 4H), 2.22 (s, 3H), 2.11 (dq, 1H), 2.02-1.82 (m, 1H), 1.76-0.88 (m, 18H), 0.81 (dd, 14H). MS (ESI) m/e 1352.4 (M-H).sup.-.

[1877] 2.55 Synthesis of 4-[(2-((3-((4-((6-((1,3-benzothiazol-2-ylcarbamoyl)-5,6-dihydroimidazo[1,5-a]pyrazin-7(8H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl)carbamoyl}oxy)methyl]-3-[2-(2-((2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl)amino)ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid (Synthon YZ)

[1878] 2.55.1 3-(1-((3-(2-(((2-(2-(2-aminoethoxy)ethoxy)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(1-(benzo[d]thiazol-2-ylcarbamoyl)-5,6-dihydroimidazo[1,5-a]pyrazin-7(8H)-yl)picolinic acid

[1879] The title compound was prepared by substituting Example 1.4.10 for Example 1.12.10 in Example 2.11.7. MS (ESI) m/e 1165 (M+H).sup.+ , 1163 (M-H).sup.-.

[1880] 2.55.2 4-[(2-((3-((4-((6-((1,3-benzothiazol-2-ylcarbamoyl)-5,6-dihydroimidazo[1,5-a]pyrazin-7(8H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl)carbamoyl}oxy)methyl]-3-[2-(2-((2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl)amino)ethoxy)ethoxy]phenyl beta-D-glucopyranosiduronic acid

[1881] The title compound was prepared by substituting Example 2.55.1 for Example 2.9.1 in Example 2.10. .sup.1H NMR (300 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 8.22 (t, 1H), 8.05 (s, 1H), 7.99 (d, 1H), 7.76 (d, 1H), 7.61 (d, 1H), 7.46 (t, 1H), 7.35-7.31 (m, 2H), 7.20 (d, 1H), 7.15 (d, 1H), 7.07 (s, 2H), 6.66 (d, 1H), 6.61 (dd, 1H), 5.12 (s, 2H), 5.08 (d, 1H), 4.94 (s, 2H), 4.28 (t, 2H), 4.09 (m, 4H), 4.03 (s, 2H), 3.91 (m, 3H), 3.84 (m, 4H), 3.73 (t, 2H), 3.49 (t, 2H), 3.40 (t, 2H), 3.34 (m, 2H), 3.30 (dd, 2H), 3.26 (m, 2H), 3.06 (q, 2H), 2.13 (s, 3H), 1.39 (bs, 2H), 1.26 (q, 4H), 1.13 (q, 4H), 1.02 (q, 2H), 0.85 (s, 6H). MS (ESI) m/e 1302 (M+H).sup.+.

[1882] 2.56 Synthesis of 2-[(2-((3-((4-((6-((5-(1,3-benzothiazol-2-ylcarbamoyl)quinolin-3-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl)carbamoyl}oxy)methyl]-4-[19-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-14-oxo-

4,7,10-trioxa-13-azanonadec-1-yl]phenyl beta-D-glucopyranosiduronic acid (Synthon QR)

[1883] 2.56.1 3-(1-(((3-(2-(((5-(3-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)propyl)-2-(((3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(5-(benzo[d]thiazol-2-ylcarbamoyl)quinolin-3-yl)picolinic acid

[1884] To a cold (0° C.) solution of (3R,4S,5S,6S)-2-(4-(1-(9H-fluoren-9-yl)-3-oxo-2,7,10,13-tetraoxa-4-azahexadecan-16-yl)-2-(((4-nitrophenoxy)carbonyl)oxy)methyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (56 mg) and Example 1.43.5 (47 mg) in N,N-dimethylformamide (2 mL) was added N,N-diisopropylethylamine (0.026 mL). The reaction was slowly warmed to room temperature and stirred overnight. To the reaction was added water (2 mL) and LiOH H.sub.2O (50 mg), and the mixture was stirred at room temperature for 3 hours. The mixture was acidified with trifluoroacetic acid, filtered and purified by reverse-phase HPLC on a Gilson system (C18 column), eluting with 20-80% acetonitrile in water containing 0.1% trifluoroacetic acid, to provide the title compound. MS (ESI) m/e 1255.4 (M-H).sup.-.

[1885] 2.56.2 2-[(2-({3-[(4-{6-[5-(1,3-benzothiazol-2-ylcarbamoyl)quinolin-3-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl]carbamoyl}oxy)methyl]-4-[19-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-14-oxo-4,7,10-trioxa-13-azanonadec-1-yl]phenyl beta-D-glucopyranosiduronic acid

[1886] To a solution of Example 2.56.1 (21 mg) in N,N-dimethylformamide (2 mL) was added 2,5-dioxopyrrolidin-1-yl 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoate (5.24 mg) and N,N-diisopropylethylamine (0.012 mL). The reaction mixture was stirred at room temperature overnight. The mixture was diluted with N,N-dimethylformamide (2 mL), filtered and purified by reverse-phase HPLC on a Gilson system (C18 column), eluting with 20-80% acetonitrile in water containing 0.1% trifluoroacetic acid, to provide the title compound. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 13.17 (s, 2H), 9.68 (d, 1H), 9.37 (s, 1H), 8.29 (dd, 2H), 8.14 (d, 1H), 8.04 (d, 1H), 8.01-7.88 (m, 2H), 7.82-7.69 (m, 2H), 7.51-7.40 (m, 2H), 7.38-7.29 (m, 1H), 7.17 (t, 1H), 7.13-7.01 (m, 2H), 6.95 (s, 3H), 5.02 (s, 2H), 4.94-4.86 (m, 1H), 3.91-3.79 (m, 4H), 3.33 (td, 9H), 3.29-3.22 (m, 2H), 3.12 (q, 2H), 3.04 (d, 2H), 2.20 (s, 3H), 1.98 (t, 2H), 1.70 (p, 2H), 1.42 (dt, 7H), 1.31-0.89 (m, 13H), 0.82 (s, 7H). MS (ESI) m/e 1448.3 (M-H).sup.-.

[1887] 2.57 Synthesis of 4-[(2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)naphthalen-2-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl](methyl)carbamoyl}oxy)methyl]-3-[4-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)butyl]phenyl beta-D-glucopyranosiduronic acid (Synthon SE)

[1888] 2.57.1 (2S,3R,4S,5S,6S)-2-(3-bromo-4-formylphenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

[1889] A mixture of (3R,4S,5S,6S)-2-bromo-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (2.67 g), 2-bromo-4-hydroxybenzaldehyde (0.90 g) and silver oxide (1.56 g) was stirred in acetonitrile (20 mL) at room temperature protected from light. After 3 hours, the reaction was diluted with dichloromethane (20 mL), filtered through diatomaceous earth, washed with additional dichloromethane (40 mL) and concentrated. The residue was purified by silica gel chromatography, eluting with a gradient of 5% to 50% hexanes/ethyl acetate over 30 minutes, to provide the title compound. MS (ESI) m/e 517.1 (M+H).sup.+.

[1890] 2.57.2 (9H-fluoren-9-yl)methyl but-3-yn-1-ylcarbamate

[1891] A solution of but-3-yn-1-amine hydrochloride (9 g) and N-ethyl-N-isopropylpropan-2-amine (44.7 mL) was stirred in dichloromethane (70 mL) and the mixture was cooled to 0° C. A solution of (9H-fluoren-9-yl)methyl carbonochloridate (22.06 g) in dichloromethane (35 mL) was added, and the reaction was stirred for 2 hours. The reaction mixture was concentrated. The crude material was deposited onto silica gel, loaded onto a silica gel column and eluted with petroleum diethyl ether/ethyl acetate (10%-25%) to provide the title compound. MS (ESI) m/e 314

(M+Na).sup.+.

[1892] 2.57.3 (2S,3R,4S,5S,6S)-2-(3-(4-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)but-1-yn-1-yl)-4-formylphenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

[1893] Example 2.57.1 (0.389 g), Example 2.57.2 (0.285 g), bis(triphenylphosphine)palladium(II) dichloride (0.053 g), and copper(I) iodide (0.014 g) were weighed into a vial and the vial was flushed with a stream of nitrogen. N,N-diisopropylethylamine (0.263 mL) and N,N-dimethylformamide (1.5 mL) were added, and the reaction was stirred at room temperature overnight. The reaction mixture was diluted with diethyl ether (50 mL) and washed with water (30 mL) and brine (30 mL). The organic layer was dried over magnesium sulfate, filtered, and concentrated. The residue was purified by silica gel chromatography, eluting with a gradient of 5% to 60% ethyl acetate/heptanes over 30 minutes, to provide the title compound. MS (ESI) m/e 728.4 (M+H).sup.+.

[1894] 2.57.4 (2S,3R,4S,5S,6S)-2-(3-(4-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)butyl)-4-formylphenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

[1895] Example 2.57.3 (262 mg) and tetrahydrofuran (10 mL) were added to 10% palladium/C (50 mg) in a 50 mL pressure bottle and the mixture was shaken for 2 hours at room temperature under 30 psi H.sub.2. The reaction mixture was filtered and concentrated to provide the title compound. MS (ESI) m/e 732.5 (M+H).sup.+.

[1896] 2.57.5 (2S,3R,4S,5S,6S)-2-(3-(4-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)butyl)-4-(hydroxymethyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

[1897] A solution of Example 2.57.4 (0.235 g) in tetrahydrofuran (1.0 mL) and methanol (1.0 mL) was cooled to 0° C., and sodium borohydride (6.07 mg) was added in one portion. The reaction was stirred for 15 minutes and was diluted with ethyl acetate (75 mL) and water (50 mL). The organic layer was separated, washed with brine (50 mL), dried over magnesium sulfate, filtered, and concentrated. The residue was purified by silica gel chromatography, eluting with a gradient of 10% to 70% ethyl acetate/heptanes, to provide the title compound. MS (ESI) m/e 734.5 (M+H).sup.+.

[1898] 2.57.6 (2S,3R,4S,5S,6S)-2-(3-(4-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)butyl)-4-(((4-nitrophenoxy)carbonyl)oxy)methyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

[1899] To an ambient solution of Example 2.57.5 (0.148 g) and bis(4-nitrophenyl) carbonate (0.123 g) in N,N-dimethylformamide (1.5 mL) was added N,N-diisopropylethylamine (0.053 mL). After 3 hours, the reaction mixture was concentrated. The residue was purified by silica gel chromatography, eluting with a gradient of 10% to 60% ethyl acetate/hexanes, to provide the title compound. MS (ESI) m/e 899.5 (M+H).sup.+.

[1900] 2.57.7 3-(1-((3-(2-(((2-(4-aminobutyl)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-yl)carbamoyl)naphthalen-2-yl)picolinic acid

[1901] To a solution of Example 1.6.3 (0.101 g) and Example 2.57.6 (0.095 g) in N,N-dimethylformamide (1.0 mL) was added N,N-diisopropylethylamine (0.055 mL), and the reaction was stirred at room temperature for 3 hours. The reaction was quenched with a mixture of 2,2,2-trifluoroacetic acid (0.204 mL), water (1 mL) and N,N-dimethylformamide (1 mL) and was purified by preparatory reverse-phase HPLC on a Gilson 2020 system using a gradient of 5% to 50% acetonitrile water over 30 minutes. The product-containing fractions were lyophilized to provide the title compound. MS (ESI) m/e 1152.7 (M+H).sup.+.

[1902] 2.57.8 3-(1-((3-(2-(((2-(4-((R)-2-amino-3-sulfopropanamido)butyl)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-yl)carbamoyl)naphthalen-2-yl)picolinic acid

[1903] To a stirred solution of (R)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-sulfopropanoic acid (0.058 g) and O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (0.054 g) in N,N-dimethylformamide (0.5 mL) was added N,N-diisopropylethylamine (0.051 mL). After stirring for 5 minutes, the mixture was added to a mixture of Example 2.57.7 (0.113 g) and N,N-diisopropylethylamine (0.051 mL) in N,N-dimethylformamide (0.5 mL). After stirring for 2 hours, diethylamine (0.102 mL) was added, and the reaction mixture was stirred for 30 minutes. The reaction mixture was diluted with a solution of 2,2,2-trifluoroacetic acid (0.189 mL) in water (1 mL) and was purified by preparatory reverse-phase HPLC on a Gilson 2020 system using a gradient of 5% to 85% acetonitrile water over 30 minutes. The product-containing fractions were lyophilized to provide the title compound. MS (ESI) m/e 1303.1 (M+H).sup.+.

[1904] 2.57.9 4-[(2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)naphthalen-2-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl)(methyl)carbamoyl}oxy)methyl]-3-[4-({N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-3-sulfo-L-alanyl}amino)butyl]phenyl beta-D-glucopyranosiduronic acid

[1905] To a solution of Example 2.57.8 (0.044 g) and 2,5-dioxopyrrolidin-1-yl 6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoate (0.012 g) in N,N-dimethylformamide (0.4 mL) was added N,N-diisopropylethylamine (0.027 mL), and the reaction mixture was stirred for 2 hours at room temperature. The reaction mixture was quenched with a mixture of 2,2,2-trifluoroacetic acid (0.060 mL), water (1 mL) and N,N-dimethylformamide (1 mL) and purified by preparatory reverse-phase HPLC on a Gilson 2020 system using a gradient of 5% to 50% acetonitrile water over 30 minutes. The product-containing fractions were lyophilized to provide the title compound. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) 6 13.10 (s, 1H), 9.02 (s, 1H), 8.38 (dd, 1H), 8.27-8.14 (m, 3H), 8.07 (d, 1H), 8.02 (d, 1H), 7.94 (d, 1H), 7.82 (dd, 2H), 7.79-7.66 (m, 2H), 7.53-7.44 (m, 1H), 7.48 (s, 1H), 7.37 (t, 1H), 7.23 (d, 1H), 6.98 (s, 2H), 6.88 (d, 1H), 6.82 (dd, 1H), 5.04 (d, 1H), 5.00 (s, 2H), 4.29 (q, 2H), 3.57 (s, 2H), 3.44 (s, 4H), 3.41 (d, 1H), 3.40-3.27 (m, 3H), 3.30-3.21 (m, 2H), 3.03 (t, 2H), 2.85 (s, 3H), 2.79 (dd, 1H), 2.70 (dd, 1H), 2.58 (s, 2H), 2.23 (s, 3H), 2.06 (t, 2H), 1.53-1.41 (m, 5H), 1.42 (s, 6H), 1.26 (s, 2H), 1.25-1.07 (m, 8H), 0.85 (s, 6H). MS (ESI) m/e 1494.1 (M-H).sup.-.

[1906] 2.58 Synthesis of 2-{6-[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl]-2-methyl-3,3-dioxido-7-oxo-8-oxa-3lambda6-thia-2,6-diazanonan-9-yl}-5-(4-[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino)butyl}phenyl beta-D-glucopyranosiduronic acid (Synthon UH)

[1907] 2.58.1 (9H-fluoren-9-yl)methyl but-3-yn-1-ylcarbamate

[1908] A solution of but-3-yn-1-amine hydrochloride (9 g) and N,N-diisopropylethylamine (44.7 mL) was stirred in dichloromethane (70 mL) and the mixture was cooled to 0° C. A solution of (9H-fluoren-9-yl)methyl carbonochloridate (22.06 g) in dichloromethane (35 mL) was added, and the reaction mixture was stirred for 2 hours. The reaction mixture was concentrated, and the residue was purified by silica gel chromatography, eluting with petroleum ether in ethyl acetate (10%-25%) to provide the title compound. MS (ESI) m/e 314 (M+Na).sup.+.

[1909] 2.58.2 (2S,3S,4S,5R,6S)-methyl 6-(5-(4-(((9H-fluoren-9-yl)methoxy)carbonylamino)but-1-ynyl)-2-formylphenoxy)-3,4,5-triacetoxy-tetrahydro-2H-pyran-2-carboxylate

[1910] Example 2.58.3 (2.7 g), Example 2.58.1 (2.091 g), bis(triphenylphosphine)palladium(II) chloride (0.336 g) and copper(I) iodide (0.091 g) were weighed into a vial and flushed with a stream of nitrogen. Triethylamine (2.001 mL) and tetrahydrofuran (45 mL) were added, and the reaction was stirred at room temperature. After stirring for 16 hours, the reaction mixture was diluted with ethyl acetate (200 mL) and washed with water (100 mL) and brine (100 mL). The organic layer was dried over magnesium sulfate, filtered, and concentrated. The residue was purified by silica gel chromatography, eluting with petroleum ether in ethyl acetate (10%-50%), to

provide the title compound. MS (ESI) m/e 750 (M+Na).sup.+.

[1911] 2.58.3 (2S,3S,4S,5R,6S)-methyl 6-(5-(4-(((9H-fluoren-9-yl)methoxy)carbonylamino)butyl)-2-formylphenoxy)-3,4,5-triacetoxy-tetrahydro-2H-pyran-2-carboxylate

[1912] Example 2.58.2 (1.5 g) and tetrahydrofuran (45 mL) were added to 10% Pd—C(0.483 g) in a 100 mL pressure bottle and the mixture was stirred for 16 hours under 1 atm H.sub.2 at room temperature. The reaction mixture was filtered and concentrated to provide the title compound. MS (ESI) m/e 754 (M+Na).sup.+.

[1913] 2.58.4 (2S,3S,4S,5R,6S)-methyl 6-(5-(4-(((9H-fluoren-9-yl)methoxy)carbonylamino)butyl)-2-(hydroxymethyl)phenoxy)-3,4,5-triacetoxy-tetrahydro-2H-pyran-2-carboxylate

[1914] A solution of Example 2.58.3 (2.0 g) in tetrahydrofuran (7.00 mL) and methanol (7 mL) was cooled to 0° C. and NaBH.sub.4 (0.052 g) was added in one portion. After 30 minutes, the reaction mixture was diluted with ethyl acetate (150 mL) and water (100 mL). The organic layer was separated, washed with brine (100 mL), dried over magnesium sulfate, filtered, and concentrated. The residue was purified by silica gel chromatography, eluting with petroleum ether in ethyl acetate (10%-40%), to provide the title compound. MS (ESI) m/e 756 (M+Na).sup.+.

[1915] 2.58.5 (2S,3S,4S,5R,6S)-methyl 6-(5-(4-(((9H-fluoren-9-yl)methoxy)carbonylamino)butyl)-2-(((4-nitrophenoxy)carbonyloxy)methyl)phenoxy)-3,4,5-triacetoxy-tetrahydro-2H-pyran-2-carboxylate

[1916] To a solution of Example 2.58.4 (3.0 g) and bis(4-nitrophenyl) carbonate (2.488 g) in dry acetonitrile (70 mL) at 0° C. was added N,N-diisopropylethylamine (1.07 mL). After stirring at room temperature for 16 hours, the reaction mixture was concentrated to give a residue, which was purified by silica gel chromatography, eluting with petroleum ether in ethyl acetate (10%-50%), to provide the title compound. MS (ESI) m/e 921 (M+Na).sup.+.

[1917] 2.58.6 3-(1-((3-(2-(((4-(4-aminobutyl)-2-(((2R,3S,4R,5R,6R)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(2-(N,N-dimethylsulfamoyl)ethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

[1918] To a cold (0° C.) solution of Example 2.58.5 (40.8 mg) and Example 1.36 (40 mg) in N,N-dimethylformamide (4 mL) was added N,N-diisopropylethylamine (0.026 mL). The reaction mixture was slowly warmed to room temperature and stirred overnight. To the reaction mixture was added water (2 mL) and LiOH H.sub.2O (50 mg), and the mixture was stirred at room temperature for 3 hours. The mixture was acidified with trifluoroacetic acid, filtered and purified by reverse-phase HPLC on a Gilson system (C18 column), eluting with 20-80% acetonitrile in water containing 0.1% trifluoroacetic acid, to provide the title compound. MS (ESI) m/e 1278.7 (M-H).sup.-.

[1919] 2.58.7 2-{6-[2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl]-2-methyl-3,3-dioxido-7-oxo-8-oxa-3λ⁶-thia-2,6-diazanonan-9-yl}-5-(4-[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}butyl)phenyl beta-D-glucopyranosiduronic acid

[1920] To a solution of Example 2.58.6 (35.1 mg) in N,N-dimethylformamide (4 mL) was added 2,5-dioxopyrrolidin-1-yl 2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetate (6.93 mg) and N,N-diisopropylethylamine (0.026 mL). The reaction mixture was stirred at room temperature overnight. The mixture was diluted with N,N-dimethylformamide (2 mL), filtered and purified by reverse-phase HPLC on a Gilson system (C18 column), eluting with 20-80% acetonitrile in water containing 0.1% trifluoroacetic acid, to provide the title compound. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 12.85 (s, 1H), 8.02 (dd, 2H), 7.76 (d, 1H), 7.58 (d, 1H), 7.53-7.37 (m, 3H), 7.32 (td, 2H), 7.24 (s, 1H), 7.16 (dd, 1H), 7.04 (s, 2H), 6.99-6.87 (m, 2H), 6.81 (d,

1H), 5.08 (d, 2H), 4.99 (d, 1H), 4.92 (s, 2H), 3.95 (s, 2H), 3.86 (q, 3H), 3.47-3.14 (m, 9H), 2.99 (dt, 4H), 2.72 (s, 3H), 2.60 (s, 3H), 2.06 (s, 3H), 1.49 (p, 2H), 1.41-1.27 (m, 4H), 1.29-0.86 (m, 10H), 0.80 (d, 7H). MS (ESI) m/e 1413.4 (M-H).sup.-.

[1921] 2.59 Synthesis of 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[3-(2-{([2-((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl]oxy)-4-(4-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}butyl)benzyl]oxy}carbonyl)[3-(dimethylamino)-3-oxopropyl]amino}ethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid (Synthon UI)

[1922] 2.59.1 3-(1-((3-(2-(((4-(4-aminobutyl)-2-(((2R,3S,4R,5R,6R)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(3-(dimethylamino)-3-oxopropyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

[1923] The title compound was prepared as described in Example 2.58.6, substituting Example 1.36 with Example 1.38. MS (ESI) m/e 1243.7 (M+H).sup.+.

[1924] 2.59.2 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[3-(2-{([2-((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl]oxy)-4-(4-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}butyl)benzyl]oxy}carbonyl)[3-(dimethylamino)-3-oxopropyl]amino}ethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl}-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid

[1925] The title compound was prepared as described in Example 2.58.7, substituting Example 2.58.6 with Example 2.59.1. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 8.02 (dd, 2H), 7.76 (d, 1H), 7.58 (d, 1H), 7.44 (ddd, 3H), 7.32 (td, 2H), 7.24 (s, 1H), 7.13 (dd, 1H), 7.04 (s, 2H), 6.99-6.86 (m, 2H), 6.81 (d, 1H), 5.06 (d, 2H), 4.98 (d, 1H), 4.92 (s, 2H), 3.95 (s, 2H), 3.85 (q, 3H), 3.77 (d, 2H), 3.39 (q, 5H), 3.27 (q, 4H), 2.99 (dt, 4H), 2.88 (s, 2H), 2.81-2.66 (m, 5H), 2.06 (d, 3H), 1.50 (p, 2H), 1.34 (dd, 4H), 1.27-0.85 (m, 9H), 0.79 (d, 6H). MS (ESI) m/e 1401.3 (M+H).sup.+.

[1926] 2.60 Synthesis of 2-[(2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl)(2-sulfamoyl)ethyl)carbamoyl}oxy)methyl]-5-(4-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}butyl)phenyl beta-D-glucopyranosiduronic acid (Synthon US)

[1927] 2.60.1 3-(1-((3-(2-(((4-(4-aminobutyl)-2-(((2R,3S,4R,5R,6R)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(2-sulfamoyl)ethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

[1928] The title compound was prepared as described in Example 2.58.6, substituting Example 1.36 with Example 1.18.20. MS (ESI) m/e 1251.2 (M+H).sup.+.

[1929] 2.60.2 2-[(2-({3-[(4-{6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl)(2-sulfamoyl)ethyl)carbamoyl}oxy)methyl]-5-(4-{[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}butyl)phenyl beta-D-glucopyranosiduronic acid

[1930] The title compound was prepared as described in Example 2.58.7, substituting Example 2.58.6 with Example 2.60.1. .sup.1H NMR (400 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 12.84 (s, 2H), 8.04 (dd, 2H), 7.77 (d, 1H), 7.60 (d, 1H), 7.53-7.38 (m, 3H), 7.38-7.30 (m, 2H), 7.26 (s, 1H), 7.16 (d, 1H), 7.05 (s, 2H), 6.96-6.77 (m, 5H), 5.09 (s, 2H), 5.00 (d, 1H), 4.94 (s, 2H), 3.97 (s, 2H), 3.87 (q, 3H), 3.48-3.16 (m, 5H), 3.09-2.94 (m, 4H), 2.07 (s, 3H), 1.50 (d, 2H), 1.36 (d, 3H), 1.29-0.88 (m, 9H), 0.81 (d, 7H). MS (ESI) m/e 1385.5 (M-H).sup.-.

[1931] 2.61 Synthesis of 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-{[3-(2-{([2-((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-

yl]oxy}-4-(4-(((2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}butyl)benzyl]oxy}carbonyl)[3-(methylamino)-3-oxopropyl]amino}ethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid (Synthon UY)

[1932] 2.61.1 3-(1-(((3-(2-(((4-(4-aminobutyl)-2-(((2R,3S,4R,5R,6R)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(3-(methylamino)-3-oxopropyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

[1933] The title compound was prepared as described in Example 2.58.6, substituting Example 1.36 with Example 1.39. MS (ESI) m/e 1228.8 (M+H).sup.+.

[1934] 2.61.2 6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]-3-(1-([3-(2-([2-([2-(2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl]oxy)-4-(4-(((2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}butyl)benzyl]oxy}carbonyl)[3-(methylamino)-3-oxopropyl]amino}ethoxy)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl)pyridine-2-carboxylic acid

[1935] The title compound was prepared as described in Example 2.58.7, substituting Example 2.58.6 with Example 2.61.1. .sup.1H NMR (501 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 12.83 (s, 1H), 8.06 (s, 1H), 8.01 (dd, 1H), 7.77 (d, 1H), 7.71 (d, 0H), 7.60 (d, 1H), 7.45 (tdd, 3H), 7.38-7.29 (m, 2H), 7.26 (s, 1H), 7.15 (d, 1H), 7.05 (d, 1H), 6.96-6.90 (m, 2H), 6.82 (d, 1H), 5.07 (s, 2H), 5.01 (t, 1H), 4.94 (s, 2H), 3.97 (s, 2H), 3.87 (q, 3H), 3.79 (d, 2H), 3.28 (p, 2H), 3.09-2.93 (m, 3H), 2.52 (d, 3H), 2.35-2.26 (m, 2H), 2.07 (d, 2H), 1.60-1.44 (m, 2H), 1.34 (d, 3H), 1.29-0.88 (m, 6H), 0.81 (d, 5H). MS (ESI) m/e 1363.5 (M-H).sup.-.

[1936] 2.62 Synthesis of 3-{1-[(3-{2-[(3-amino-3-oxopropyl)([2-[(2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl]oxy}-4-(4-[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}butyl)benzyl]oxy}carbonyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid (Synthon UX)

[1937] 2.62.1 3-(1-(((3-(2-((3-amino-3-oxopropyl)((4-(4-aminobutyl)-2-(((2R,3S,4R,5R,6R)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

[1938] The title compound was prepared as described in Example 2.58.6, substituting Example 1.36 with Example 1.32.2. MS (ESI) m/e 1214.6 (M+H).sup.+.

[1939] 2.62.2 3-{1-[(3-{2-[(3-amino-3-oxopropyl)([2-[(2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl]oxy}-4-(4-[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}butyl)benzyl]oxy}carbonyl)amino]ethoxy}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)methyl]-5-methyl-1H-pyrazol-4-yl}-6-[8-(1,3-benzothiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl]pyridine-2-carboxylic acid

[1940] The title compound was prepared as described in Example 2.58.7, substituting Example 2.58.6 with Example 2.62.1. .sup.1H NMR (501 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 12.83 (s, 2H), 8.06 (s, 1H), 8.01 (d, 1H), 7.77 (d, 1H), 7.60 (d, 1H), 7.53-7.38 (m, 3H), 7.34 (q, 2H), 7.26 (s, 1H), 7.15 (d, 1H), 7.05 (s, 2H), 6.93 (d, 2H), 6.87-6.73 (m, 2H), 5.07 (d, 2H), 5.04-4.97 (m, 1H), 4.94 (s, 2H), 3.97 (s, 2H), 3.87 (q, 3H), 3.79 (d, 2H), 3.29 (t, 3H), 3.10-2.95 (m, 4H), 2.32 (p, 2H), 2.07 (d, 3H), 1.51 (dd, 2H), 1.36 (dd, 5H), 1.30-0.86 (m, 8H), 0.81 (d, 6H). MS (ESI) m/e 1349.5 (M-H).sup.-.

[1941] 2.63 Synthesis of 2-[(2-[(3-[(4-{6-[3-(1,3-benzothiazol-2-ylcarbamoyl)-1H-indol-5-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl]oxy)ethyl](methyl)carbamoyl]oxy)methyl]-5-(4-[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]amino}butyl)phenyl beta-D-glucopyranosiduronic acid (Synthon WZ)

[1942] 2.63.1 3-(1-(((3-(2-(((4-(4-aminobutyl)-2-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(methyl)amino)ethoxy)-5,7-

dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(3-(benzo[d]thiazol-2-ylcarbamoyl)-1H-indol-5-yl)picolinic acid

[1943] The title compound was prepared by substituting Example 1.34.5 for Example 1.12.10 and Example 2.58.5 for Example 2.11.6 in Example 2.11.7.

[1944] 2.63.2 2-[(2-((3-((4-((6-((3-((1,3-benzothiazol-2-ylcarbamoyl)-1H-indol-5-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)oxy)ethyl)carbamoyl)oxy)methyl]-5-(4-((2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl)amino)butyl)phenyl beta-D-glucopyranosiduronic acid

[1945] The title compound was prepared by substituting Example 2.63.1 for Example 2.9.1 in Example 2.10. ¹H NMR (400 MHz, dimethyl sulfoxide-d₆) δ ppm 12.47 (bs, 1H), 12.16 (d, 1H), 9.01 (s, 1H), 8.69 (d, 1H), 8.11-8.04 (m, 4H), 7.99 (d, 1H), 7.76 (d, 1H), 7.64 (d, 1H), 7.48 (s, 1H), 7.45 (t, 1H), 7.31 (t, 1H), 7.19 (t, 1H), 7.07 (s, 1H), 6.94 (s, 1H), 6.86 (d, 1H), 5.10 (s, 2H), 5.03 (d, 1H), 3.99 (s, 2H), 3.90 (m, 3H), 3.48 (m, 3H), 3.28 (m, 2H), 3.05 (m, 4H), 2.93 (s, 2H), 2.88 (s, 2H), 2.54-2.53 (m, 2H), 2.24 (s, 3H), 1.54 (m, 2H), 1.40 (m, 4H), 1.30-1.22 (m, 6H), 1.20-1.14 (m, 6H), 1.11-0.96 (m, 2H), 0.87 (d, 6H). MS (ESI) m/e 1300 (M+Na)⁺, 1276 (M-H)⁻.

[1946] 2.64 Synthesis of 2-[(2-((3-((4-((6-((1-((1,3-benzothiazol-2-ylcarbamoyl)-5,6-dihydroimidazo[1,5-a]pyrazin-7(8H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)oxy)ethyl)carbamoyl)oxy)methyl]-5-(4-((2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl)amino)butyl)phenyl beta-D-glucopyranosiduronic acid (Synthon XO)

[1947] 2.64.1 3-(1-((3-((2-(((4-((4-aminobutyl)-2-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(1-(benzo[d]thiazol-2-ylcarbamoyl)-5,6-dihydroimidazo[1,5-a]pyrazin-7(8H)-yl)picolinic acid

[1948] The title compound was prepared by substituting Example 1.4.10 for Example 1.12.10 and Example 2.58.5 for Example 2.11.6 in Example 2.11.7. MS (ESI) m/e 1133 (M+H)⁺, 1131 (M-H)⁻.

[1949] 2.64.2 2-[(2-((3-((4-((6-((1-((1,3-benzothiazol-2-ylcarbamoyl)-5,6-dihydroimidazo[1,5-a]pyrazin-7(8H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)oxy)ethyl)carbamoyl)oxy)methyl]-5-(4-((2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl)amino)butyl)phenyl beta-D-glucopyranosiduronic acid

[1950] The title compound was prepared by substituting Example 2.64.1 for Example 2.9.1 in Example 2.10. ¹H NMR (400 MHz, dimethyl sulfoxide-d₆) δ ppm 8.08 (t, 1H), 8.01 (s, 1H), 7.99 (d, 1H), 7.76 (d, 1H), 7.61 (d, 1H), 7.46 (t, 1H), 7.34 (s, 1H), 7.33 (t, 1H), 7.17 (m, 3H), 7.08 (s, 2H), 6.92 (s, 1H), 6.84 (d, 1H), 5.12 (s, 2H), 5.05 (s, 2H), 5.02 (d, 1H), 4.27 (m, 2H), 4.10 (m, 2H), 3.99 (s, 2H), 3.91 (m, 2H), 3.84 (s, 2H), 3.70 (m, 2H), 3.42 (t, 2H), 3.35 (t, 2H), 3.30 (t, 2H), 3.06 (m, 5H), 2.53 (m, 2H), 2.14 (s, 3H), 1.53 (m, 2H), 1.43-1.35 (m, 4H), 1.27 (m, 4H), 1.14 (q, 4H), 1.03 (dd, 2H), 0.86 (s, 6H). MS (ESI) m/e 1270 (M+H)⁺, 1268 (M-H)⁻.

[1951] 2.65 Synthesis of (6S)-2,6-anhydro-6-(2-((2-((3-((4-((6-((1-((1,3-benzothiazol-2-ylcarbamoyl)-5,6-dihydroimidazo[1,5-a]pyrazin-7(8H)-yl)-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl)-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)oxy)ethyl)carbamoyl)oxy)methyl)-5-(N-((2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl)-L-valyl-L-alanyl)amino)phenyl)ethyl)-L-gulonic acid (Synthon XW)

[1952] 2.65.1 (3R,4S,5R,6R)-3,4,5-tris(benzyloxy)-6-(benzyloxymethyl)-tetrahydropyran-2-one

[1953] To a solution of (3R,4S,5R,6R)-3,4,5-tris(benzyloxy)-6-((benzyloxy)methyl)tetrahydro-2H-pyran-2-ol (75 g) in dimethyl sulfoxide (400 mL) at 0° C. was added acetic anhydride (225 mL). The mixture was stirred for 16 hours at room temperature before cooled to 0° C. A large volume of water was added, and the stirring was stopped and the reaction mixture was allowed to settle for 3 hours (the crude lactone was at the bottom of the flask). The supernatant was removed, and the

crude mixture was diluted with ethyl acetate, washed 3 times with water, neutralized with saturated aqueous solution of NaHCO₃, and washed again twice with water. The organic layer was then dried over magnesium sulfate, filtered and concentrated to provide the title compound. MS (ESI) m/e 561 (M+Na).sup.+.

[1954] 2.65.2 (3R,4S,5R,6R)-3,4,5-tris(benzyloxy)-6-(benzyloxymethyl)-2-ethynyl-tetrahydro-2H-pyran-2-ol

[1955] To a solution of ethynyltrimethylsilane (18.23 g) in tetrahydrofuran (400 mL) under nitrogen and chilled in a dry ice/acetone bath (internal temp -65°C .) was added 2.5M BuLi in hexane (55.7 mL) dropwise, keeping the temperature below -60°C . The mixture was stirred in a cold bath for 40 minutes, followed by an ice-water bath (internal temp rose to 0.4°C .) for 40 minutes, and finally cooled to -75°C . again. A solution of Example 2.55.1 (50 g) in tetrahydrofuran (50 mL) was added dropwise, keeping the internal temperature below -70°C . The mixture was stirred in a dry ice/acetone bath for an additional 3 hours. The reaction mixture was quenched with saturated aqueous NaHCO₃ solution (250 mL). The mixture was allowed to warm to room temperature, extracted with ethyl acetate (3 \times 300 mL), dried over MgSO₄, filtered, and concentrated in vacuo to provide the title compound. MS (ESI) m/e 659 (M+Na).sup.+.

[1956] 2.65.3 trimethyl(((3S,4R,5R,6R)-3,4,5-tris(benzyloxy)-6-(benzyloxymethyl)-tetrahydro-2H-pyran-2-yl)ethynyl)silane

[1957] To a mixture of Example 2.65.2 (60 g) in acetonitrile (450 mL) and dichloromethane (150 mL) at -15°C . in an ice-salt bath was added triethylsilane (81 mL) dropwise, followed by addition of boron trifluoride diethyl ether complex (40.6 mL) at such a rate that the internal temperature did not exceed -10°C . The mixture was stirred between -15°C . and -10°C . for 2 hours. The reaction mixture was quenched with saturated aqueous NaHCO₃ solution (275 mL) and stirred for 1 hour at room temperature. The mixture was extracted with ethyl acetate (3 \times 550 mL). The combined extracts were dried over MgSO₄, filtered, and concentrated. The residue was purified by flash chromatography eluting with a gradient of 0% to 7% ethyl acetate/petroleum ether to provide the title compound. MS (ESI) m/e 643 (M+Na).sup.+.

[1958] 2.65.4 (2R,3R,4R,5S)-3,4,5-tris(benzyloxy)-2-(benzyloxymethyl)-6-ethynyl-tetrahydro-2H-pyran

[1959] To a mixed solution of Example 2.65.3 (80 g) in dichloromethane (200 mL) and methanol (1000 mL) was added 1N aqueous NaOH solution (258 mL). The mixture was stirred at room temperature for 2 hours. The solvent was removed. The residue was then partitioned between water and dichloromethane. The extracts were washed with brine, dried over Na₂SO₄, filtered, and concentrated to provide the title compound. MS (ESI) m/e 571 (M+Na).sup.+.

[1960] 2.65.5 (2R,3R,4R,5S)-2-(acetoxymethyl)-6-ethynyl-tetrahydro-2H-pyran-3,4,5-triyl triacetate

[1961] To a solution of Example 2.65.4 (66 g) in acetic anhydride (500 mL) cooled by an ice/water bath was added boron trifluoride diethyl ether complex (152 mL) dropwise. The mixture was stirred at room temperature for 16 hours, cooled with an ice/water bath and neutralized with saturated aqueous NaHCO₃ solution. The mixture was extracted with ethyl acetate (3 \times 500 mL), dried over Na₂SO₄ and concentrated in vacuo. The residue was purified by flash chromatography eluting with a gradient of 0% to 30% ethyl acetate/petroleum ether to provide the title compound. MS (ESI) m/e 357 (M+H).sup.+.

[1962] 2.65.6 (3R,4R,5S,6R)-2-ethynyl-6-(hydroxymethyl)-tetrahydro-2H-pyran-3,4,5-triol

[1963] To a solution of Example 2.65.5 (25 g) in methanol (440 mL) was added sodium methanolate (2.1 g). The mixture was stirred at room temperature for 2 hours, then neutralized with 4M HCl in dioxane. The solvent was removed, and the residue was adsorbed onto silica gel and loaded onto a silica gel column. The column was eluted with a gradient of 0 to 100% ethyl acetate/petroleum ether then 0% to 12% methanol/ethyl acetate to provide the title compound. MS

(ESI) m/e 211 (M+Na).sup.+.

[1964] 2.65.7 (2S,3S,4R,5R)-6-ethynyl-3,4,5-trihydroxy-tetrahydro-2H-pyran-2-carboxylic acid

[1965] A three-necked round bottom flask was charged with Example 2.65.6 (6.00 g), KBr (0.30 g), tetrabutylammonium bromide (0.41 g) and 60 mL of saturated aqueous NaHCO₃ solution.

(2,2,6,6-Tetramethylpiperidin-1-yl)oxidanyl (0.15 g) in 60 mL dichloromethane was added. The mixture was stirred vigorously and cooled in an ice-salt bath to -2° C. internal temperature. A solution of brine (12 mL), aqueous NaHCO₃ solution (24 mL) and NaOCl (154 mL) was added dropwise such that the internal temperature was maintained below 2° C. The pH of the reaction mixture was maintained in the 8.2-8.4 range with the addition of solid Na₂CO₃. After a total of 6 hours the reaction was cooled to 3° C. internal temperature and ethanol (~20 mL) was added dropwise and was stirred for ~30 minutes. The mixture was transferred to a separatory funnel, and the dichloromethane layer was discarded. The pH of the aqueous layer was adjusted to 2-3 using 1 M aqueous HCl. The aqueous layer was then concentrated to dryness. Methanol (100 mL was) added to the dry solid, and the slurry was stirred for ~30 minutes. The mixture was filtered over a pad of diatomaceous earth, and the residue in the funnel was washed with ~100 mL of methanol. The filtrate was concentrated under reduced pressure to obtain the title compound.

[1966] 2.65.8 (2S,3S,4R,5R)-methyl 6-ethynyl-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylate

[1967] A 500 mL three-necked round bottom flask was charged with a suspension of Example 2.65.7 (6.45 g) in methanol (96 mL) and was cooled in an ice-salt-bath with internal temperature of -1° C. Neat thionyl chloride (2.79 mL) was carefully added. The internal temperature kept rising throughout the addition but did not exceed 10° C. The reaction was allowed to slowly warm up to 15-20° C. over 2.5 hours. After 2.5 hours, the reaction was concentrated to provide the title compound.

[1968] 2.65.9 (3S,4R,5S,6S)-2-ethynyl-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

[1969] Example 2.65.8 (6.9 g) as a solution in N,N-dimethylformamide (75 mL) was added 4-dimethylaminopyridine (0.17 g) and acetic anhydride (36.1 mL). The suspension was cooled in an ice-bath and pyridine (18.04 mL) was added via syringe over 15 minutes. The reaction was allowed to warm to room temperature overnight. Additional acetic anhydride (12 mL) and pyridine (6 mL) were added and stirring was continued for an additional 6 hours. The reaction was cooled in an ice-bath and 250 mL of saturated aqueous NaHCO₃ solution was added and stirred for 1 hour. Water (100 mL) was added, and the mixture was extracted with ethyl acetate. The organic extract was washed twice with saturated CuSO₄ solution, dried and concentrated. The residue was purified by flash chromatography, eluting with 50% ethyl acetate/petroleum ether to provide the title compound. .sup.1H NMR (500 MHz, methanol-d₄) δ ppm 5.29 (t, 1H), 5.08 (td, 2H), 4.48 (dd, 1H), 4.23 (d, 1H), 3.71 (s, 3H), 3.04 (d, 1H), 2.03 (s, 3H), 1.99 (s, 3H), 1.98 (s, 4H). MS (ESI) m/e 359.9 (M+NH₄).sup.+.

[1970] 2.65.10 2-iodo-4-nitrobenzoic acid

[1971] A 3 L fully jacketed flask equipped with a mechanical stirrer, temperature probe and an addition funnel under a nitrogen atmosphere, was charged with 2-amino-4-nitrobenzoic acid (69.1 g, Combi-Blocks) and sulfuric acid, 1.5 M aqueous (696 mL). The resulting suspension was cooled to 0° C. internal temperature, and a solution of sodium nitrite (28.8 g) in water (250 mL) was added dropwise over 43 minutes with the temperature kept below 1° C. The reaction mixture was stirred at ca. 0° C. for 1 hour. A solution of potassium iodide (107 g) in water (250 mL) was added dropwise over 44 minutes with the internal temperature kept below 1° C. (Initially addition was exothermic and there was gas evolution). The reaction mixture was stirred 1 hour at 0° C. The temperature was raised to 20° C. and then stirred at ambient temperature overnight. The reaction mixture became a suspension. The reaction mixture was filtered, and the collected solid was washed with water. The wet solid (~108 g) was stirred in 10% sodium sulfite (350 mL, with ~200 mL water used to wash in the solid) for 30 minutes. The suspension was acidified with

concentrated hydrochloric acid (35 mL), and the solid was collected by filtration and washed with water. The solid was slurried in water (1 L) and re-filtered, and the solid was left to dry in the funnel overnight. The solid was then dried in a vacuum oven for 2 hours at 60° C. The resulting solid was triturated with dichloromethane (500 mL), and the suspension was filtered and washed with additional dichloromethane. The solid was air-dried to provide the title compound. MS (ESI) m/e 291.8 (M-H).sup.-.

[1972] 2.65.11 (2-iodo-4-nitrophenyl)methanol

[1973] A flame-dried 3 L 3-necked flask was charged with Example 2.65.10 (51.9 g) and tetrahydrofuran (700 mL). The solution was cooled in an ice bath to 0.5° C., and borane-tetrahydrofuran complex (443 mL, 1M in THF) was added dropwise (gas evolution) over 50 minutes, reaching a final internal temperature of 1.3° C. The reaction mixture was stirred for 15 minutes, and the ice bath was removed. The reaction left to come to ambient temperature over 30 minutes. A heating mantle was installed, and the reaction was heated to an internal temperature of 65.5° C. for 3 hours, and then allowed to cool to room temperature while stirring overnight. The reaction mixture was cooled in an ice bath to 0° C. and quenched by dropwise addition of methanol (400 mL). After a brief incubation period, the temperature rose quickly to 2.5° C. with gas evolution. After the first 100 mL are added over ~30 minutes, the addition was no longer exothermic, and the gas evolution ceased. The ice bath was removed, and the mixture was stirred at ambient temperature under nitrogen overnight. The mixture was concentrated to a solid, dissolved in dichloromethane/methanol and adsorbed on to silica gel (~150 g). The residue was loaded on a plug of silica gel (3000 mL) and eluted with dichloromethane to provide the title compound. MS (DCI) m/e 296.8 (M+NH.sub.4).sup.+.

[1974] 2.65.12 (4-amino-2-iodophenyl)methanol

[1975] A 5 L flask equipped with a mechanical stirrer, heating mantle controlled by a JKEM temperature probe and condenser was charged with Example 2.65.11 (98.83 g) and ethanol (2 L). The reaction was stirred rapidly, and iron (99 g) was added, followed by a solution of ammonium chloride (20.84 g) in water (500 mL). The reaction was heated over the course of 20 minutes to an internal temperature of 80.3° C., when it began to reflux vigorously. The mantle was dropped until the reflux calmed. Thereafter, the mixture was heated to 80° C. for 1.5 hour. The reaction was filtered hot through a membrane filter, and the iron residue was washed with hot 50% ethyl acetate/methanol (800 mL). The eluent was passed through a diatomaceous earth pad, and the filtrate was concentrated. The residue was partitioned between 50% brine (1500 mL) and ethyl acetate (1500 mL). The layers were separated, and the aqueous layer was extracted with ethyl acetate (400 mL×3). The combined organic layers were dried over sodium sulfate, filtered and concentrated to provide the title compound, which was used without further purification. MS (DCI) m/e 266.9 (M+NH.sub.4).sup.+.

[1976] 2.65.13 4-(((tert-butyl)dimethylsilyl)oxy)methyl)-3-iodoaniline

[1977] A 5 L flask with a mechanical stirrer was charged with Example 2.65.12 (88 g) and dichloromethane (2 L). The suspension was cooled in an ice bath to an internal temperature of 2.5° C., and tert-butylchlorodimethylsilane (53.3 g) was added portion-wise over 8 minutes. After 10 minutes, 1H-imidazole (33.7 g) was added portionwise to the cold reaction. The reaction was stirred 90 minutes while the internal temperature rose to 15° C. The reaction mixture was diluted with water (3 L) and dichloromethane (1 L). The layers were separated, and the organic layer was dried over sodium sulfate, filtered, and concentrated to an oil. The residue was purified by silica gel chromatography (1600 g silica gel), eluting a gradient of 0-25% ethyl acetate in heptane, to provide the title compound.

[1978] 2.65.14 (S)-2-(((S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)propanoic acid

[1979] To a solution of (S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanoic acid (6.5 g) in dimethoxyethane (40 mL) was added (S)-2-aminopropanoic acid (1.393 g) and sodium

bicarbonate (1.314 g) in water (40 mL). Tetrahydrofuran (20 mL) was added to aid solubility. The resulting mixture was stirred at room temperature for 16 hours. Aqueous citric acid (15%, 75 mL) was added, and the mixture was extracted with 10% 2-propanol in ethyl acetate (2×100 mL). A precipitate formed in the organic layer. The combined organic layers were washed with water (2×150 mL). The organic layer was concentrated under reduced pressure and then triturated with diethyl ether (80 mL). After brief sonication, the title compound was collected by filtration. MS (ESI) m/e 411 (M+H).sup.+.

[1980] 2.65.15 (9H-fluoren-9-yl)methyl ((S)-1-(((S)-1-((4-(((tert-butyl)dimethylsilyl)oxy)methyl)-3-iodophenyl)amino)-1-oxopropan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate

[1981] A solution of Example 2.65.13 (5.44 g) and Example 2.65.14 (6.15 g) in a mixture of dichloromethane (70 mL) and methanol (35.0 mL) was added ethyl 2-ethoxyquinoline-1(2H)-carboxylate (4.08 g), and the reaction was stirred overnight. The reaction mixture was concentrated and the residue was loaded onto silica gel, eluting with a gradient of 10% to 95% heptane in ethyl acetate followed by 5% methanol in dichloromethane. The product-containing fractions were concentrated, dissolved in 0.2% methanol in dichloromethane (50 mL), loaded onto silica gel and eluted with a gradient of 0.2% to 2% methanol in dichloromethane. The product containing fractions were collected to provide the title compound. MS (ESI) m/e 756.0 (M+H).sup.+.

[1982] 2.65.16 (2S,3S,4R,5S,6S)-2-((5-((S)-2-((S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)propanamido)-2-(((tert-butyl)dimethylsilyl)oxy)methyl)phenyl)ethynyl)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

[1983] A solution of Example 2.65.9 (4.500 g), Example 2.65.15 (6.62 g), copper(I) iodide (0.083 g) and bis(triphenylphosphine)palladium(II) dichloride (0.308 g) were combined in vial and degassed. N,N-dimethylformamide (45 mL) and N-ethyl-N-isopropylpropan-2-amine (4.55 mL) were added, and the reaction vessel was flushed with nitrogen and stirred at room temperature overnight. The reaction was partitioned between water (100 mL) and ethyl acetate (250 mL). The layers were separated, and the organic layer was dried over magnesium sulfate, filtered, and concentrated. The residue was purified by silica gel chromatography, eluting with a gradient of 5% to 95% ethyl acetate in heptane. The product containing fractions were collected, concentrated and purified by silica gel chromatography, eluting with a gradient of 0.25% to 2.5% methanol in dichloromethane to provide the title compound. MS (ESI) m/e 970.4 (M+H).sup.+.

[1984] 2.65.17 (2S,3S,4R,5S,6S)-2-(5-((S)-2-((S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)propanamido)-2-(((tert-butyl)dimethylsilyl)oxy)methyl)phenethyl)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

[1985] Example 2.65.16 (4.7 g) and tetrahydrofuran (95 mL) were added to 5% Pt/C (2.42 g, wet) in a 50 mL pressure bottle and the reaction was shaken for 90 minutes at room temperature under 50 psi of hydrogen. The reaction mixture was filtered and concentrated to provide the title compound. MS (ESI) m/e 974.6 (M+H).sup.+.

[1986] 2.65.18 (2S,3S,4R,5S,6S)-2-(5-((S)-2-((S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)propanamido)-2-(hydroxymethyl)phenethyl)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

[1987] A solution of Example 2.65.17 (5.4 g) in tetrahydrofuran (7 mL), water (7 mL) and glacial acetic acid (21 mL) was stirred overnight at room temperature. The reaction mixture was diluted with ethyl acetate (200 mL) and was washed with water (100 mL), saturated aqueous NaHCO₃ solution (100 mL), and brine (100 mL), dried over magnesium sulfate, filtered, and concentrated. The residue was purified by silica gel chromatography, eluting with a gradient of 0.5% to 5% methanol in dichloromethane, to provide the title compound. MS (ESI) m/e 860.4 (M+H).sup.+.

[1988] 2.65.19 (2S,3S,4R,5S,6S)-2-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)propanamido)-2-(((4-nitrophenoxy)carbonyl)oxy)methyl)phenethyl)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate

[1989] To a solution of Example 2.65.18 (4.00 g) and bis(4-nitrophenyl) carbonate (2.83 g) in acetonitrile (80 mL) was added N-ethyl-N-isopropylpropan-2-amine (1.22 mL) at room temperature. After stirring overnight, the reaction mixture was concentrated, dissolved in dichloromethane (250 mL) and washed with saturated aqueous NaHCO₃ solution (4×150 mL). The organic layer was dried over magnesium sulfate, filtered, and concentrated. The resulting foam was purified by silica gel chromatography, eluting with a gradient of 5% to 75% ethyl acetate in hexanes to provide the title compound. MS (ESI) m/e 1025.5 (M+H)⁺.

[1990] 2.65.20 3-(1-((3-(2-(((4-((S)-2-((S)-2-amino-3-methylbutanamido)propanamido)-2-((2S,3R,4R,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)ethyl)benzyl)oxy)carbonyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(1-(benzo[d]thiazol-2-yl)carbamoyl)-5,6-dihydroimidazo[1,5-a]pyrazin-7(8H)-yl)picolinic acid

[1991] The title compound was prepared by substituting Example 1.4.10 for Example 1.12.10 and Example 2.65.19 for Example 2.11.6 in Example 2.11.7. MS (ESI) m/e 1257 (M-H)⁻.

[1992] 2.65.21 (6S)-2,6-anhydro-6-(2-{2-[(2-({3-[(4-{6-[1-(1,3-benzothiazol-2-yl)carbamoyl)-5,6-dihydroimidazo[1,5-a]pyrazin-7(8H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl]carbamoyl}oxy)methyl]-5-({N-[(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetyl]-L-valyl-L-alanyl}amino)phenyl}ethyl)-L-gulonic acid

[1993] The title compound was prepared by substituting Example 2.65.20 for Example 2.9.1 in Example 2.10. ¹H NMR (400 MHz, dimethyl sulfoxide-d₆) δ ppm 9.88 (s, 1H), 8.26 (t, 2H), 8.00 (m, 2H), 7.76 (d, 1H), 7.61 (d, 1H), 7.46 (m, 2H), 7.38-7.30 (m, 3H), 7.21 (d, 1H), 7.15 (d, 1H), 7.07 (s, 2H), 7.04 (t, 1H), 5.12 (s, 2H), 4.97 (s, 2H), 4.39 (m, 1H), 4.28 (m, 2H), 4.22 (m, 2H), 4.12 (s, 2H), 4.09 (m, 2H), 3.84 (s, 2H), 3.58 (m, 4H), 3.33 (m, 4H), 3.18-3.00 (m, 4H), 2.94 (t, 2H), 2.80-2.55 (m, 2H), 2.13 (s, 3H), 2.08-1.91 (m, 2H), 1.56 (m, 1H), 1.39 (s, 2H), 1.30-1.20 (m, 6H), 1.26-0.95 (m, 6H), 0.85 (m, 12H). MS (ESI) m/e 1395 (M-H)⁻.

[1994] 2.66 Synthesis of (6S)-2,6-anhydro-6-[2-(2-[(2-({3-[(4-16-[8-(1,3-benzothiazol-2-yl)carbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl}oxy)ethyl)(2-methoxyethyl)carbamoyl}oxy)methyl]-5-{{N-[(3S,5S)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-oxo-5-[(2-sulfoethoxy)methyl]pyrrolidin-1-yl}acetyl)-L-valyl-L-alanyl}amino}phenyl}ethyl]-L-gulonic acid (Synthon YG)

[1995] 2.66.1 (3R,7aS)-3-phenyltetrahydropyrrolo[1,2-c]oxazol-5(3H)-one

[1996] A solution of (S)-5-(hydroxymethyl)pyrrolidin-2-one (25 g), benzaldehyde (25.5 g) and para-toluenesulfonic acid monohydrate (0.50 g) in toluene (300 mL) was heated to reflux using a Dean-Stark trap under a drying tube for 16 hours. The reaction was cooled to room temperature, and the solvent was decanted from the insoluble materials. The organic layer was washed with saturated aqueous sodium bicarbonate solution (2×) and brine (1×). The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by flash chromatography on silica gel, eluting with 35/65 heptane/ethyl acetate, to provide the title compound. MS (DCI) m/e 204.0 (M+H)⁺.

[1997] 2.66.2 (3R,6R,7aS)-6-bromo-3-phenyltetrahydropyrrolo[1,2-c]oxazol-5(3H)-one

[1998] To a cold (-77° C.) solution of Example 2.66.1 (44.6 g) in tetrahydrofuran (670 mL) was added lithium bis(trimethylsilyl)amide (1.0M in hexanes) (250 mL) dropwise over 40 minutes, keeping T_{sub.rxn} < -73° C. The reaction mixture was stirred at -77° C. for 2 hours, and bromine (12.5 mL) was added dropwise over 20 minutes, keeping T_{sub.rxn} < -64° C. The reaction mixture

was stirred at -77°C . for 75 minutes and was quenched by the addition of 150 mL cold 10% aqueous sodium thiosulfate solution to the -77°C . reaction. The reaction mixture was warmed to room temperature and partitioned between half-saturated aqueous ammonium chloride solution and ethyl acetate. The layers were separated, and the organic was washed with water and brine, dried over sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluting with a gradient of 80/20, 75/25, and 70/30 heptane/ethyl acetate to provide the title compound. MS (DCI) m/e 299.0 and 301.0 ($M+\text{NH.sub.3}+\text{H}$).sup.+.

[1999] 2.66.3 (3R,6S,7aS)-6-bromo-3-phenyltetrahydropyrrolo[1,2-c]oxazol-5(3H)-one

[2000] The title compound was isolated as a by-product during the synthesis of Example 2.66.2.

MS (DCI) m/e 299.0 and 301.0 ($M+\text{NH.sub.3}+\text{H}$).sup.+.

[2001] 2.66.4 (3R,6S,7aS)-6-azido-3-phenyltetrahydropyrrolo[1,2-c]oxazol-5(3H)-one

[2002] To a solution of Example 2.66.2 (19.3 g) in N,N-dimethylformamide (100 mL) was added sodium azide (13.5 g). The reaction mixture was heated to 60°C . for 2.5 hours. The reaction mixture was cooled to room temperature and quenched by the addition of water (500 mL) and ethyl acetate (200 mL). The layers were separated, and the organic layer was washed brine. The combined aqueous layers were back-extracted with ethyl acetate (50 mL). The combined organic layers were dried with sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluting with 78/22 heptane/ethyl acetate, to provide the title compound. MS (DCI) m/e 262.0 ($M+\text{NH.sub.3}+\text{H}$).sup.+.

[2003] 2.66.5 (3R,6S,7aS)-6-amino-3-phenyltetrahydropyrrolo[1,2-c]oxazol-5(3H)-one

[2004] To a solution of Example 2.66.4 (13.5 g) in tetrahydrofuran (500 mL) and water (50 mL) was added polymer-supported triphenylphosphine (55 g). The reaction was mechanically stirred overnight at room temperature. The reaction mixture was filtered through diatomaceous earth, eluting with ethyl acetate and toluene. The solution was concentrated under reduced pressure, dissolved in dichloromethane (100 mL), dried with sodium sulfate, then filtered and concentrated to provide the title compound, which was used in the subsequent step without further purification. MS (DCI) m/e 219.0 ($M+\text{H}$).sup.+.

[2005] 2.66.6 (3R,6S,7aS)-6-(dibenzylamino)-3-phenyltetrahydropyrrolo[1,2-c]oxazol-5(3H)-one

[2006] To a solution of Example 2.66.5 (11.3 g) in N,N-dimethylformamide (100 mL) was added potassium carbonate (7.0 g), potassium iodide (4.2 g), and benzyl bromide (14.5 mL). The reaction was stirred at room temperature overnight and quenched by the addition of water and ethyl acetate. The layers were separated, and the organic layer was washed brine. The combined aqueous layers were back-extracted with ethyl acetate. The combined organic layers were dried with sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluting with a gradient of 10 to 15% ethyl acetate in heptane to give a solid that was triturated with heptane to provide the title compound. MS (DCI) m/e 399.1 ($M+\text{H}$).sup.+.

[2007] 2.66.7 (3S,5S)-3-(dibenzylamino)-5-(hydroxymethyl)pyrrolidin-2-one

[2008] To a solution of Example 2.66.6 (13 g) in tetrahydrofuran (130 mL) was added para-toluene sulfonic acid monohydrate (12.4 g) and water (50 mL), and the reaction was heated to 65°C . for 6 days. The reaction mixture was cooled to room temperature and was quenched by the addition of saturated aqueous sodium bicarbonate and ethyl acetate. The layers were separated, and the organic layer was washed with brine. The combined aqueous layers were back-extracted with ethyl acetate. The combined organic layers were dried with sodium sulfate, filtered and concentrated under reduced pressure. The waxy solids were triturated with heptane (150 mL) to provide the title compound. MS (DCI) m/e 311.1 ($M+\text{H}$).sup.+.

[2009] 2.66.8 (3S,5S)-5-(((tert-butyl)dimethylsilyl)oxy)methyl)-3-(dibenzylamino)pyrrolidin-2-one

[2010] To a solution of Example 2.66.7 (9.3 g) and 1H-imidazole (2.2 g) in N,N-dimethylformamide was added tert-butylchlorodimethylsilane (11.2 mL, 50 weight % in toluene), and the reaction was stirred overnight. The reaction mixture was quenched by the addition of water and diethyl ether. The layers were separated, and the organic layer was washed with brine. The

combined aqueous layers were back-extracted with diethyl ether. The combined organic layers were dried with sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluting with 35% ethyl acetate in heptane, to provide the title compound. MS (DCI) m/e 425.1 (M+H).sup.+.

[2011] 2.66.9 tert-butyl 2-((3S,5S)-5-(((tert-butyldimethylsilyl)oxy)methyl)-3-(dibenzylamino)-2-oxopyrrolidin-1-yl)acetate

[2012] To a cold (0° C.) solution of Example 2.66.8 (4.5 g) in tetrahydrofuran (45 mL) was added 95% sodium hydride (320 mg) in two portions. The cold solution was stirred for 40 minutes, and tert-butyl 2-bromoacetate (3.2 mL) was added. The reaction mixture was warmed to room temperature and stirred overnight. The reaction mixture was quenched by the addition of water and ethyl acetate. The layers were separated, and the organic layer was washed with brine. The combined aqueous layers were back-extracted with ethyl acetate. The combined organic layers were dried with sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluting with a gradient of 5-12% ethyl acetate in heptane, to provide the title compound. MS (DCI) m/e 539.2 (M+H).sup.+.

[2013] 2.66.10 tert-butyl 2-((3S,5S)-3-(dibenzylamino)-5-(hydroxymethyl)-2-oxopyrrolidin-1-yl)acetate

[2014] To a solution of Example 2.66.9 (5.3 g) in tetrahydrofuran (25 mL) was added tetrabutylammonium fluoride (11 mL, 1.0M in 95/5 tetrahydrofuran/water). The reaction mixture was stirred at room temperature for one hour and was quenched by the addition of saturated aqueous ammonium chloride solution, water and ethyl acetate. The layers were separated, and the organic layer was washed with brine. The combined aqueous layers were back-extracted with ethyl acetate. The combined organic layers were dried with sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluting with 35% ethyl acetate in heptane, to provide the title compound. MS (DCI) m/e 425.1 (M+H).sup.+.

[2015] 2.66.11 tert-butyl 2-((3S,5S)-5-((2-((4-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylbutoxy)sulfonyl)ethoxy)methyl)-3-(dibenzylamino)-2-oxopyrrolidin-1-yl)acetate

[2016] To a solution of Example 2.66.10 (4.7 g) in dimethyl sulfoxide (14 mL) was added a solution of 4-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylbutyl ethenesulfonate (14.5 g) in dimethyl sulfoxide (14 mL). Potassium carbonate (2.6 g) and water (28 µL) were added, and the reaction was heated at 60° C. under nitrogen for one day. The reaction was cooled to room temperature, and quenched by the addition of brine solution, water and diethyl ether. The layers were separated, and the organic layer was washed with brine. The combined aqueous layers were back-extracted with diethyl ether. The combined organic layers were dried with sodium sulfate, filtered and concentrated under reduced pressure. The residue was purified by silica gel chromatography, eluting with a gradient of 15-25% ethyl acetate in heptane, to provide the title compound. MS (ESI+) m/e 871.2 (M+H).sup.+.

[2017] 2.66.12 tert-butyl 2-((3S,5S)-3-amino-5-((2-((4-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylbutoxy)sulfonyl)ethoxy)methyl)-2-oxopyrrolidin-1-yl)acetate

[2018] Example 2.66.11 (873 mg) was dissolved in ethyl acetate (5 mL) and methanol (15 mL), and palladium hydroxide on carbon, 20% by wt (180 mg) was added. The reaction mixture was stirred under a hydrogen atmosphere (30 psi) at room temperature for 30 hours, then at 50° C. for one hour. The reaction was cooled to room temperature, filtered, and concentrated to give the desired product. MS (ESI+) m/e 691.0 (M+H).sup.+.

[2019] 2.66.13 4-(((3S,5S)-1-(2-(tert-butoxy)-2-oxoethyl)-5-((2-((4-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylbutoxy)sulfonyl)ethoxy)methyl)-2-oxopyrrolidin-3-yl)amino)-4-oxobut-2-enoic acid

[2020] Maleic anhydride (100 mg) was dissolved in dichloromethane (0.90 mL), and a solution of Example 2.66.12 (650 mg) in dichloromethane (0.90 mL) was added dropwise, and then heated at 40° C. for 2 hours. The reaction mixture was directly purified by silica gel chromatography, eluting

with a gradient of 1.0-2.5% methanol in dichloromethane containing 0.2% acetic acid. After concentrating the product-bearing fractions, toluene (10 mL) was added and the mixture was concentrated again to provide the title compound. MS (ESI-) m/e 787.3 (M-H).sup.-.

[2021] 2.66.14 tert-butyl 2-((3S,5S)-5-((2-((4-((tert-butyldiphenylsilyl)oxy)-2,2-dimethylbutoxy)sulfonyl)ethoxy)methyl)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-oxopyrrolidin-1-yl)acetate

[2022] Example 2.66.13 (560 mg) was slurried in toluene (7 mL), and triethylamine (220 µL) and sodium sulfate (525 mg) were added. The reaction mixture was heated at reflux under a nitrogen atmosphere for 6 hours, and the reaction mixture stirred at room temperature overnight. The reaction was filtered, and the solids rinsed with ethyl acetate. The eluent was concentrated under reduced pressure, and the residue was purified by silica gel chromatography, eluting with 45/55 heptane/ethyl acetate, ethyl acetate, and then 97.5/2.5/0.2 dichloromethane/methanol/acetic acid to provide the title compound.

[2023] 2.66.15 2-((3S,5S)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-oxo-5-((2-sulfoethoxy)methyl)pyrrolidin-1-yl)acetic acid

[2024] Example 2.66.14 (1.2 g) was dissolved in trifluoroacetic acid (15 mL) and heated to 65-70° C. under nitrogen overnight. The trifluoroacetic acid was removed under reduced pressure. The residue was dissolved in acetonitrile (2.5 mL) and purified by preparative reverse-phase liquid chromatography on a Luna C18(2) AXIA column (250×50 mm, 10µ particle size) using a gradient of 5-75% acetonitrile containing 0.1% trifluoroacetic acid in water over 30 minutes, to provide the title compound. MS (ESI-) m/e 375.2 (M-H).sup.-.

[2025] 2.66.16 3-(1-((3-(2-(((4-((S)-2-((S)-2-amino-3-methylbutanamido)propanamido)-2-(2-((2S,3R,4R,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)ethyl)benzyl)oxy)carbonyl)(2-methoxyethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-yl)carbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

[2026] Example 1.12.10 (75 mg) and Example 2.65.19 (100 mg) were dissolved in N,N-dimethylformamide (0.3 mL). 1-Hydroxybenzotriazole (13 mg) and N-ethyl-N-isopropylpropan-2-amine (50 µL) were added, and the reaction was stirred at room temperature for two hours. The reaction mixture was concentrated under reduced pressure. The residue was dissolved in tetrahydrofuran and methanol (0.3 mL each), and lithium hydroxide hydrate (55 mg) in water (0.6 mL) was added. The reaction mixture was stirred at room temperature for one hour and quenched by the addition of N,N-dimethylformamide/water 1/1 (1.5 mL) with trifluoroacetic acid (0.15 mL). The solution was washed with heptane (1 mL), then purified by reverse-phase chromatography (C18 column), eluting with 20-70% acetonitrile in 0.1% trifluoroacetic acid water, to provide the title compound as a trifluoroacetic acid salt. MS (ESI-) m/e 1355.6 (M-H).sup.-.

[2027] 2.66.17 (6S)-2,6-anhydro-6-[2-(2-[(2-((3-((4-((6-[8-(1,3-benzothiazol-2-yl)carbamoyl)-5-methoxy-3,4-dihydroisoquinolin-2(1H)-yl)]-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]dec-1-yl)oxy)ethyl](2-methoxyethyl)carbamoyl)oxy)methyl]-5-[[N-((3S,5S)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-oxo-5-[(2-sulfoethoxy)methyl]pyrrolidin-1-yl)acetyl)-L-valyl-L-alanyl]amino}phenyl]ethyl]-L-gulonic acid

[2028] To a solution of Example 2.66.15 (20 mg) in N,N-dimethylformamide (0.2 mL) was added 0-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (20 mg) and N,N-diisopropylethylamine (18 µL). The reaction mixture was stirred for 3 minutes at room temperature and was then added to a solution of Example 2.66.16 (57 mg) and N,N-diisopropylethylamine (30 µL) in N,N-dimethylformamide (0.7 mL). The reaction mixture was stirred at room temperature for 1 hour and diluted with N,N-dimethylformamide/water 1/1 (1.0 mL). The solution was purified by reverse-phase chromatography (C18 column), eluting with 20-70% acetonitrile in 0.1% trifluoroacetic acid water, to provide the title compound. .sup.1H NMR (400 MHz, dimethyl

sulfoxide-d.sub.6) δ ppm 9.84 (br d, 1H), 8.18 (br d, 1H), 8.04 (m, 1H), 8.01 (d, 1H), 7.77 (dd, 2H), 7.50 (d, 1H), 7.46 (m, 3H), 7.34 (t, 1H), 7.29 (s, 1H), 7.21 (br d, 1H), 7.07 (s, 2H), 7.01 (d, 1H), 6.99 (d, 1H), 5.00 (s, 4H), 4.64 (t, 1H), 4.37 (m, 1H), 4.18 (m, 2H), 4.01 (d, 1H), 3.88 (s, 3H), 3.87 (m, 2H), 3.81 (br d, 2H), 3.73 (br m, 1H), 3.63 (m, 2H), 3.55 (m, 2H), 3.49 (m, 2H), 3.36 (br m, 6H), 3.31 (m, 2H), 3.26 (br m, 2H), 3.19 (m, 2H), 3.14 (m, 1H), 3.10 (br m, 1H), 2.94 (t, 1H), 2.81 (m, 3H), 2.74 (m, 2H), 2.60 (br m, 1H), 2.36 (m, 1H), 2.09 (s, 3H), 2.00 (m, 2H), 1.85 (m, 1H), 1.55 (br m, 1H), 1.40-0.92 (m, 14H), 0.88, 0.86, 0.83, 0.79 (d,d, s, s, total 12H). MS (ESI⁻) m/e 1713.7 (M⁻1).

[2029] 2.67 Synthesis of 8-[2-({[(3-amino-3-oxopropyl){2-[(3-{[4-(6-{8-[(1,3-benzothiazol-2-yl)carbamoyl]-3,4-dihydroisoquinolin-2(1H)-yl}-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl]methyl}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]decan-1-yl)oxy]ethyl}carbamoyl]oxy}methyl)-5-{{[(2S)-2-({(2S)-2-[2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetamido]-3-methylbutanoyl}amino)propanoyl]amino}phenyl]-2,6-anhydro-7,8-dideoxy-L-glycero-L-gulo-octonic acid (Synthon ZT)

[2030] 2.67.1 3-(1-((3-(2-(((4-((S)-2-((S)-2-amino-3-methylbutanamido)propanamido)-2-(2-((3R,4R,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)ethyl)benzyl)oxy)carbonyl)(3-amino-3-oxopropyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

[2031] To a cold (0° C.) solution of Example 2.65.19 (66 mg) and Example 1.32.2 (60 mg) in N,N-dimethylformamide (6 mL) was added N,N-diisopropylethylamine (0.026 mL) and 1-hydroxybenzotriazole hydrate (16.23 mg). The reaction mixture was slowly warmed to room temperature and stirred overnight. To the reaction mixture was added water (1 mL) and LiOH H.sub.2O (20 mg). The mixture was stirred at room temperature for 3 hours. The mixture was acidified with trifluoroacetic acid, filtered and purified by reverse-phase HPLC on a Gilson system (C18 column), eluting with 20-80% acetonitrile in water containing 0.1% trifluoroacetic acid, to provide the title compound. MS (ESI) m/e 1338.5 (M⁻H).sup.-.

[2032] 2.67.2 8-[2-({[(3-amino-3-oxopropyl){2-[(3-{[4-(6-{8-[(1,3-benzothiazol-2-yl)carbamoyl]-3,4-dihydroisoquinolin-2(1H)-yl}-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl]methyl}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]decan-1-yl)oxy]ethyl}carbamoyl]oxy}methyl)-5-{{[(2S)-2-({(2S)-2-[2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetamido]-3-methylbutanoyl}amino)propanoyl]amino}phenyl]-2,6-anhydro-7,8-dideoxy-L-glycero-L-gulo-octonic acid

[2033] The title compound was prepared as described in Example 2.58.7, substituting Example 2.58.6 with Example 2.67.1. .sup.1H NMR (501 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 9.91 (d, 1H), 8.25 (dd, 2H), 8.03 (d, 1H), 7.79 (d, 1H), 7.61 (d, 6H), 7.55-7.30 (m, 7H), 7.28 (s, 1H), 7.22 (d, 1H), 7.07 (s, 2H), 6.94 (d, 1H), 6.89-6.74 (m, 1H), 5.01 (s, 3H), 4.96 (s, 2H), 4.38 (t, 1H), 4.27-4.17 (m, 1H), 4.12 (d, 2H), 3.88 (t, 2H), 3.79 (d, 1H), 3.41-3.30 (m, 3H), 3.24 (s, 2H), 3.12 (dt, 2H), 3.01 (t, 2H), 2.94 (t, 1H), 2.74 (d, 1H), 2.67-2.56 (m, 1H), 2.29 (t, 2H), 2.08 (d, 3H), 1.99 (d, 3H), 1.55 (d, 1H), 1.42-0.99 (m, 15H), 0.99-0.70 (m, 12H). MS (ESI) m/e 1477.2 (M⁺H).sup.+.

[2034] 2.68 Synthesis of 4-{{[(2-[(3-{[4-(6-{8-[(1,3-benzothiazol-2-yl)carbamoyl]-3,4-dihydroisoquinolin-2(1H)-yl}-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl]methyl}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]decan-1-yl)oxy]ethyl}{3-(methylamino)-3-oxopropyl}carbamoyl]oxy}methyl}-3-{3-[2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetamido]propoxy}phenyl beta-D-glucopyranosiduronic acid (Synthon AAN)

[2035] 2.68.1 3-(1-((3-(2-(((2-(3-aminopropoxy)-4-(((2S,3R,4S,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)oxy)benzyl)oxy)carbonyl)(3-(methylamino)-3-oxopropyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

[2036] To a cold (0° C.) solution of Example 2.28.3 (38.7 mg) and Example 1.39 (39.3 mg) in N,N-dimethylformamide (6 mL) was added N,N-diisopropylethylamine (0.026 mL) and 1-

hydroxybenzotriazole hydrate (6.58 mg). The reaction was slowly warmed to room temperature and stirred overnight. To the reaction was added water (2 mL) and LiOH H.sub.2O (50 mg), and the mixture was stirred at room temperature for 3 hours. The mixture was acidified with trifluoroacetic acid, filtered and purified by reverse-phase HPLC on a Gilson system (C18 column), eluting with 20-80% acetonitrile in water containing 0.1% trifluoroacetic acid, to provide the title compound. MS (ESI) m/e 1230.2 (M-H).sup.-.

[2037] 2.68.2 4-[[({2-[(3-{[4-(6-{8-[(1,3-benzothiazol-2-yl)carbamoyl]-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl]methyl}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]decan-1-yl)oxy]ethyl}[3-(methylamino)-3-oxopropyl]carbamoyl)oxy]methyl}-3-{3-[2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetamido]propoxy}phenyl beta-D-glucopyranosiduronic acid

[2038] The title compound was prepared as described in Example 2.58.7, substituting Example 2.58.6 with Example 2.68.1 .sup.1H NMR (501 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 12.88 (s, 2H), 9.93 (d, 1H), 8.36-8.22 (m, 2H), 8.04 (d, 1H), 7.80 (d, 2H), 7.76 (d, 0H), 7.62 (d, 1H), 7.56-7.42 (m, 5H), 7.41-7.33 (m, 3H), 7.28 (s, 1H), 7.22 (d, 1H), 7.08 (s, 2H), 6.95 (d, 1H), 5.01 (d, 3H), 4.96 (s, 2H), 4.39 (p, 1H), 4.22 (dd, 1H), 4.12 (d, 2H), 3.89 (t, 2H), 3.80 (d, 2H), 3.34 (t, 2H), 3.22 (d, 2H), 3.13 (dt, 2H), 3.02 (t, 2H), 2.94 (t, 1H), 2.86-2.71 (m, 1H), 2.60 (s, 2H), 2.54 (d, 4H), 2.29 (q, 2H), 2.09 (d, 3H), 2.07-1.90 (m, 3H), 1.60-1.48 (m, 1H), 1.39-1.00 (m, 17H), 0.97-0.74 (m, 15H). (ESI) m/e 1489.5 (M-H).sup.-.

[2039] 2.69 Synthesis of 2,6-anhydro-8-(2-[[({2-[(3-{[4-(6-{8-[(1,3-benzothiazol-2-yl)carbamoyl]-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl]methyl}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]decan-1-yl)oxy]ethyl}[3-(methylamino)-3-oxopropyl]carbamoyl)oxy]methyl}-5-[[{(2S)-2-((2S)-2-[2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetamido]-3-methylbutanoyl}amino)propanoyl]amino}phenyl)-7,8-dideoxy-L-glycero-L-gulo-octonic acid (Synthon AAO)

[2040] 2.69.1 3-(1-((3-(2-(((4-((S)-2-((S)-2-amino-3-methylbutanamido)propanamido)-2-(2-((2S,3R,4R,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)ethyl)benzyl)oxy)carbonyl)(3-(methylamino)-3-oxopropyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

[2041] The title compound was prepared as described in Example 2.67.1, substituting Example 1.32.2 with Example 1.39. MS (ESI) m/e 1352.6 (M-H).sup.-.

[2042] 2.69.2 2,6-anhydro-8-(2-[[({2-[(3-{[4-(6-{8-[(1,3-benzothiazol-2-yl)carbamoyl]-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl]methyl}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]decan-1-yl)oxy]ethyl}[3-(methylamino)-3-oxopropyl]carbamoyl)oxy]methyl}-5-[[{(2S)-2-((2S)-2-[2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetamido]-3-methylbutanoyl}amino)propanoyl]amino}phenyl)-7,8-dideoxy-L-glycero-L-gulo-octonic acid

[2043] The title compound was prepared as described in Example 2.58.7, substituting Example 2.58.6 with Example 2.67.1. .sup.1H NMR (501 MHz, dimethyl sulfoxide-d.sub.6) δ ppm 12.88 (s, 2H), 9.93 (d, 1H), 8.36-8.22 (m, 2H), 8.04 (d, 1H), 7.80 (d, 2H), 7.76 (d, 0H), 7.62 (d, 1H), 7.56-7.42 (m, 5H), 7.41-7.33 (m, 3H), 7.28 (s, 1H), 7.22 (d, 1H), 7.08 (s, 2H), 6.95 (d, 1H), 5.01 (d, 3H), 4.96 (s, 2H), 4.39 (p, 1H), 4.22 (dd, 1H), 4.12 (d, 2H), 3.89 (t, 2H), 3.80 (d, 2H), 3.34 (t, 2H), 3.22 (d, 2H), 3.13 (dt, 2H), 3.02 (t, 2H), 2.94 (t, 1H), 2.86-2.71 (m, 1H), 2.60 (s, 2H), 2.54 (d, 4H), 2.29 (q, 2H), 2.09 (d, 3H), 2.07-1.90 (m, 3H), 1.60-1.48 (m, 1H), 1.39-1.00 (m, 17H), 0.97-0.74 (m, 15H). MS (ESI) m/e 1489.5 (M-H).sup.-.

[2044] 2.70 Synthesis of 2,6-anhydro-8-(2-[[({2-[(3-{[4-(6-{8-[(1,3-benzothiazol-2-yl)carbamoyl]-3,4-dihydroisoquinolin-2(1H)-yl]-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl]methyl}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]decan-1-yl)oxy]ethyl}[3-(methylamino)-3-oxopropyl]carbamoyl)oxy]methyl}-5-[[{(2S)-2-[(2S)-2-(2-((3S,5S)-3-(2,5-dioxo-2,5-dihydro-1H-

pyrrol-1-yl)-2-oxo-5-[(2-sulfoethoxy)methyl]pyrrolidin-1-yl}acetamido)-3-methylbutanoyl]amino}propanoyl]amino}phenyl)-7,8-dideoxy-L-glycero-L-gulo-octonic acid (Synthon AAP)

[2045] To a solution of Example 2.66.15 (17 mg) in N,N-dimethylformamide (320 µL) was added 0-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (19 mg) and N,N-diisopropylethylamine (17 µL). The reaction mixture was stirred for 5 minutes and was added to a solution of Example 2.69.1 (39 mg) and N,N-diisopropylethylamine (36 µL) in N,N-dimethylformamide (320 µL). The reaction mixture was stirred for 2 hours and was diluted with N,N-dimethylformamide (2 mL). The solution was filtered and purified by reverse-phase HPLC on a Gilson system (C18 column), eluting with 20-80% acetonitrile in water containing 0.1% trifluoroacetic acid, to provide the title compound. ¹H NMR (501 MHz, dimethyl sulfoxide-d₆) δ ppm 9.82 (s, 1H), 8.15 (d, 1H), 8.00 (dd, 2H), 7.75 (d, 1H), 7.58 (d, 1H), 7.44 (ddd, 5H), 7.32 (td, 2H), 7.25 (s, 1H), 7.18 (d, 1H), 7.03 (s, 2H), 6.92 (d, 1H), 6.76 (s, 1H), 4.97 (s, 2H), 4.92 (s, 2H), 4.61 (t, 1H), 4.33 (p, 1H), 4.21-4.08 (m, 2H), 3.98 (d, 1H), 3.84 (t, 2H), 3.40-3.27 (m, 3H), 3.21 (s, 1H), 3.14-3.03 (m, 2H), 2.98 (t, 2H), 2.90 (t, 1H), 2.81-2.50 (m, 4H), 2.38-2.20 (m, 3H), 2.05 (s, 3H), 2.01-1.90 (m, 2H), 1.88-1.74 (m, 1H), 1.60-1.43 (m, 1H), 1.36-0.95 (m, 14H), 0.95-0.62 (m, 13H). MS (ESI) m/e 1710.5(M-H).sup.-.

[2046] 2.71 Synthesis of 6-{8-[(1,3-benzothiazol-2-yl)carbamoyl]-3,4-dihydroisoquinolin-2(1H)-yl}-3-[1-(3-[2-({[(4-[(2S)-5-(carbamoylamino)-2-[(2S)-2-{[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]amino}-3-methylbutanoyl]amino}pentanoyl]amino}phenyl)methoxy]carbonyl]amino)acetamido]-5,7-dimethyltricyclo[3.3.1.1^{3,7}]decan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl]pyridine-2-carboxylic acid (Synthon ABF)

[2047] The title compound was prepared as described in Example 2.2, substituting Example 1.3.2 with Example 1.40.11. ¹H NMR (501 MHz, dimethyl sulfoxide-d₆) δ ppm 9.96 (s, 1H), 8.03 (dd, 2H), 7.78 (d, 2H), 7.59 (dd, 3H), 7.53-7.39 (m, 3H), 7.35 (q, 2H), 7.30-7.23 (m, 3H), 7.20 (d, 1H), 6.98 (s, 2H), 6.94 (d, 1H), 4.94 (d, 4H), 4.38 (t, 1H), 4.17 (dd, 1H), 3.87 (t, 2H), 3.78 (s, 2H), 3.35 (t, 2H), 3.00 (t, 3H), 2.94 (s, OH), 2.16 (d, 1H), 2.09 (s, 3H), 1.95 (d, 1H), 1.74-1.27 (m, 10H), 1.13 (dq, 5H), 0.87-0.71 (m, 12H). MS (ESI) m/e 1355.5(M-H).sup.-.

[2048] 2.72 Synthesis of 8-[2-({[(3-amino-3-oxopropyl){2-[(3-{[4-(6-{8-[(1,3-benzothiazol-2-yl)carbamoyl]-3,4-dihydroisoquinolin-2(1H)-yl}-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-yl)methyl}-5,7-dimethyltricyclo[3.3.1.1^{3,7}]decan-1-yl]oxy}ethyl}carbamoyl]oxy}methyl)-5-[(2S)-2-[(2S)-2-(2-{(3S,5S)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-oxo-5-[(2-sulfoethoxy)methyl]pyrrolidin-1-yl}acetamido)-3-methylbutanoyl]amino}propanoyl]amino}phenyl]-2,6-anhydro-7,8-dideoxy-L-glycero-L-gulo-octonic acid (Synthon ZZ)

[2049] 2.72.1 3-(1-((3-(2-(((4-((S)-2-((S)-2-amino-3-methylbutanamido)propanamido)-2-(2-((3R,4R,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)ethyl)benzyl)oxy)carbonyl)(3-amino-3-oxopropyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)picolinic acid

[2050] To a cold (0° C.) solution of Example 2.65.19 (66 mg) and Example 1.32.2 (6 mL) were added N,N-diisopropylamine (0.026 mL) and 1-hydroxybenzotriazole hydrate (16.23 mg). The reaction mixture was slowly warmed to room temperature and stirred overnight. To the reaction mixture was added water (1 mL) and LiOH H₂O (20 mg), and the mixture was stirred at room temperature for 3 hours. The mixture was acidified with trifluoroacetic acid, filtered and was purified by reverse-phase HPLC on a Gilson system (C18 column), eluting with 20-80% acetonitrile in water containing 0.1% trifluoroacetic acid, to provide the title compound. MS (ESI) m/e 1338.5 (M-H).sup.-.

[2051] 2.72.2 8-[2-({[(3-amino-3-oxopropyl){2-[(3-{[4-(6-{8-[(1,3-benzothiazol-2-yl)carbamoyl]-3,4-dihydroisoquinolin-2(1H)-yl}-2-carboxypyridin-3-yl)-5-methyl-1H-pyrazol-1-

yl)methyl}-5,7-dimethyltricyclo[3.3.1.1.sup.3,7]decan-1-yl)oxy]ethyl}carbamoyl]oxy}methyl)-5-
{[(2S)-2-[(2S)-2-(2-[(3S,5S)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-oxo-5-[(2-
sulfoethoxy)methyl]pyrrolidin-1-yl)acetamido)-3-
methylbutanoyl]amino}propanoyl]amino}phenyl]-2,6-anhydro-7,8-dideoxy-L-glycero-L-gulo-
octonic acid

[2052] To a solution of 2-((3S,5S)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-oxo-5-((2-
sulfoethoxy)methyl)pyrrolidin-1-yl)acetic acid (17 mg) in N,N-dimethylformamide (320 μ L), was
added O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (19 mg) and
N-ethyl-N-isopropylpropan-2-amine (17 μ L). The reaction mixture was stirred for 5 minutes and
was added to a solution of Example 2.72.1 (50 mg) and N-ethyl-N-isopropylpropan-2-amine (36
 μ L) in N,N-dimethylformamide (320 μ L). The reaction mixture was stirred for 2 hours. The
reaction mixture was diluted with N,N-dimethylformamide/water (1/1, 1 mL) and purified by
reverse-phase HPLC on a Gilson system (C18 column), eluting with 20-80% acetonitrile in water
containing 0.1% trifluoroacetic acid, to provide the title compound. ¹H NMR (501 MHz,
dimethyl sulfoxide-d₆) δ ppm 9.82 (s, 1H), 8.15 (d, 1H), 8.00 (dd, 2H), 7.75 (d, 1H), 7.58 (d,
1H), 7.44 (ddd, 5H), 7.32 (td, 2H), 7.25 (s, 1H), 7.18 (d, 1H), 7.03 (s, 2H), 6.92 (d, 1H), 6.76 (s,
1H), 4.97 (s, 2H), 4.92 (s, 2H), 4.61 (t, 1H), 4.33 (p, 1H), 4.21-4.08 (m, 2H), 3.98 (d, 1H), 3.84 (t,
2H), 3.40-3.27 (m, 3H), 3.21 (s, 1H), 3.14-3.03 (m, 2H), 2.98 (t, 2H), 2.90 (t, 1H), 2.81-2.50 (m,
4H), 2.38-2.20 (m, 3H), 2.05 (s, 3H), 2.01-1.90 (m, 2H), 1.88-1.74 (m, 1H), 1.60-1.43 (m, 1H),
1.36-0.95 (m, 14H), 0.95-0.62 (m, 13H). MS (ESI) m/e 1697.5 (M-H).sup.-.

[2053] 2.73 Synthesis of N-[6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)hexanoyl]-L-valyl-N-{4-
[(2-[(3-[(4-{6-[8-(1,3-benzothiazol-2-yl)carbamoyl]-3,4-dihydroisoquinolin-2(1H)-yl]-2-
carboxypyridin-3-yl}-5-methyl-1H-pyrazol-1-yl)methyl]-5,7-dimethyltricyclo[3.3.1.1.3,7]dec-1-
yl)oxy]ethyl}(2-sulfoethyl)carbamoyl]oxy)methyl]phenyl}-N5-carbamoyl-L-ornithinamide
(Synthon CZ)

[2054] Example 1.44.2 (100 mg) and 4-((S)-2-((S)-2-(6-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-
yl)hexanamido)-3-methylbutanamido)-5-ureidopentanamido)benzyl (4-nitrophenyl) carbonate
(purchased from Synchem, 114 mg) in N,N-dimethylformamide (7 mL) was cooled in an water-ice
bath, and N,N-diisopropylethylamine (0.15 mL) was added. The mixture was stirred at 0° C. for 30
minutes and then at room temperature overnight. The reaction was purified by a reverse phase
HPLC using a Gilson system, eluting with 20-60% acetonitrile in water containing 0.1% v/v
trifluoroacetic acid, to provide the title compound. ¹H NMR (400 MHz, dimethylsulfoxide-
d₆) δ ppm 12.85 (s, 1H), 9.99 (s, 1H), 8.04 (t, 2H), 7.75-7.82 (m, 2H), 7.40-7.63 (m, 6H),
7.32-7.39 (m, 2H), 7.24-7.29 (m, 3H), 6.99 (s, 2H), 6.95 (d, 1H), 6.01 (s, 1H), 4.83-5.08 (m, 4H),
4.29-4.48 (m, 1H), 4.19 (t, 1H), 3.84-3.94 (m, 2H), 3.80 (d, 2H), 3.14-3.29 (m, 2H), 2.87-3.06 (m,
4H), 2.57-2.69 (m, 2H), 2.03-2.24 (m, 5H), 1.89-2.02 (m, 1H), 1.53-1.78 (m, 2H), 1.26-1.53 (m,
8H), 0.89-1.27 (m, 12H), 0.75-0.88 (m, 12H). MS (ESI) m/e 1452.2 (M+H).sup.+.

[2055] 2.74 Synthesis of 6-(8-(benzo[d]thiazol-2-yl)carbamoyl)-3,4-dihydroisoquinolin-2(1H)-
yl)-3-(1-((3-(2-(((2-(2-((2S,3R,4R,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-
yl)ethyl)-4-((S)-2-((S)-2-(2-((3S,5S)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-oxo-5-((2-
sulfoethoxy)methyl)pyrrolidin-1-yl)acetamido)-3-
methylbutanamido)propanamido)benzyl)oxy)carbonyl)(2-sulfoethyl)amino)ethoxy)-5,7-
dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid (Synthon TX)

[2056] 2.74.1 3-(1-(((1r,3s,5R,7S)-3-(2-(((4-((R)-2-((R)-2-amino-3-
methylbutanamido)propanamido)-2-(2-((2S,3R,4R,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-
2H-pyran-2-yl)ethyl)benzyl)oxy)carbonyl)(2-sulfoethyl)amino)ethoxy)-5,7-dimethyladamantan-1-
yl)methyl)-5-methyl-1H-pyrazol-4-yl)-6-(8-(benzo[d]thiazol-2-yl)carbamoyl)-3,4-
dihydroisoquinolin-2(1H)-yl)picolinic acid

[2057] To a cold (0° C.) solution of Example 2.65.19 (70 mg) and Example 1.44.2 (58.1 mg) in
N,N-dimethylformamide (4 mL) was added N-ethyl-N-isopropylpropan-2-amine (0.026 mL). The

reaction was slowly warmed to room temperature and stirred overnight. To the reaction mixture was added water (1 mL) and LiOH H.sub.2O (20 mg). The mixture was stirred at room temperature for 3 hours. The mixture was acidified with trifluoroacetic acid, filtered and purified by reverse-phase HPLC on a Gilson system (C18 column), eluting with 20-80% acetonitrile in water containing 0.1% trifluoroacetic acid, to give the title product. MS (ESI) m/e 1564.4 (M-H).sup.-.

[2058] 2.74.2 6-(8-(benzo[d]thiazol-2-ylcarbamoyl)-3,4-dihydroisoquinolin-2(1H)-yl)-3-(1-(((3-2-(((2-2-((2S,3R,4R,5S,6S)-6-carboxy-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)ethyl)-4-((S)-2-((S)-2-(2-((3S,5S)-3-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-oxo-5-((2-sulfoethoxy)methyl)pyrrolidin-1-yl)acetamido)-3-methylbutanamido)propanamido)benzyl)oxy)carbonyl)(2-sulfoethyl)amino)ethoxy)-5,7-dimethyladamantan-1-yl)methyl)-5-methyl-1H-pyrazol-4-yl)picolinic acid

[2059] The title compound was prepared by substituting Example 2.74.1 for Example 2.66.16 in Example 2.66.17. .sup.1H NMR (400 MHz, dimethylsulfoxide-d.sub.6) δ ppm 9.85 (s, 1H), 8.17 (br d, 1H), 8.01 (d, 2H), 7.77 (d, 1H), 7.59 (d, 1H), 7.53 (d, 1H), 7.43 (m, 4H), 7.34 (m, 3H), 7.19 (d, 1H), 7.06 (s, 2H), 6.96 (d, 1H), 4.99 (m, 2H), 4.95 (s, 2H), 4.63 (t, 1H), 4.36 (t, 1H), 4.19 (br m, 1H), 4.16 (d, 1H), 3.98 (d, 1H), 3.87 (br t, 2H), 3.81 (br d, 2H), 3.73 (br m, 1H), 3.63 (t, 2H), 3.53 (m, 2H), 3.44 (m, 4H), 3.31 (t, 2H), 3.21 (br m, 2H), 3.17 (m, 2H), 3.00 (m, 2H), 2.92 (br m, 1H), 2.75 (m, 3H), 2.65 (br m, 3H), 2.35 (br m, 1H), 2.07 (s, 3H), 1.98 (br m, 2H), 1.85 (m, 1H), 1.55 (br m, 1H), 1.34 (br m, 1H), 1.26 (br m, 6H), 1.09 (br m, 7H), 0.93 (br m, 1H), 0.87, 0.83, 0.79 (all d, total 12H). MS (ESI) m/e 1733.4 (M-H).sup.-.

[2060] Example 3. Synthesis of Exemplary Bcl-xL Inhibitory ADCs

[2061] Exemplary ADCs were synthesized using one of nine exemplary methods, described below. Table 6 correlates which method was used to synthesize each exemplary ADC.

[2062] Method A. A solution of Bond-Breaker™ tris(2-carboxyethyl)phosphine (TCEP) solution (10 mM, 0.017 mL) was added to a solution of antibody (10 mg/mL, 1 mL) preheated to 37° C. The reaction mixture was kept at 37° C. for 1 hour. The solution of reduced antibody was added to a solution of synthon (3.3 mM, 0.160 mL in dimethyl sulfoxide (DMSO)) and gently mixed for 30 minutes. The reaction solution was loaded onto a desalting column (PD10, washed with DPBS 3× before use), followed by Dulbecco's phosphate-buffered saline (DPBS) (1.6 mL) and eluted with additional DPBS (3 mL). The purified ADC solution was filtered through a 0.2 micron, low protein-binding 13 mm syringe-filter and stored at 4° C.

[2063] Method B. A solution of Bond-Breaker™ tris(2-carboxyethyl)phosphine (TCEP) solution (10 mM, 0.017 mL) was added to the solution of antibody (10 mg/mL, 1 mL) preheated to 37° C. The reaction mixture was kept at 37° C. for 1 hour. The solution of reduced antibody was adjusted to pH=8 by adding boric buffer (0.05 mL, 0.5 M, pH8), added to a solution of synthon (3.3 mM, 0.160 mL in DMSO) and gently mixed for 4 hours. The reaction solution was loaded onto a desalting column (PD10, washed with DPBS 3× before use), followed by DPBS (1.6 mL) and eluted with additional DPBS (3 mL). The purified ADC solution was filtered through a 0.2 micron, low protein-binding 13 mm syringe-filter and stored at 4° C.

[2064] Method C. Conjugations were performed using a PerkinElmer Janus (part AJL8M01) robotic liquid handling system equipped with an I235/96 tip ModuLar Dispense Technology (MDT), disposable head (part 70243540) containing a gripper arm (part 7400358), and an 8-tip Varispan pipetting arm (part 7002357) on an expanded deck. The PerkinElmer Janus system was controlled using the WinPREP version 4.8.3.315 Software.

[2065] A Pall Filter plate 5052 was prewet with 100 μ L 1× DPBS using the MDT. Vacuum was applied to the filter plate for 10 seconds and was followed by a 5 second vent to remove DPBS from filter plate. A 50% slurry of Protein A resin (GE MabSelect Sure) in DPBS was poured into an 8 well reservoir equipped with a magnetic ball, and the resin was mixed by passing a traveling magnet underneath the reservoir plate. The 8 tip Varispan arm, equipped with 1 mL conductive tips, was used to aspirate the resin (250 μ L) and transfer to a 96-well filter plate. A vacuum was applied

for 2 cycles to remove most of the buffer. Using the MDT, 150 μ L of 1 \times PBS was aspirated and dispensed to the 96-well filter plate holding the resin. A vacuum was applied, removing the buffer from the resin. The rinse/vacuum cycle was repeated 3 times. A 2 mL, 96-well collection plate was mounted on the Janus deck, and the MDT transferred 450 μ L of 5 \times DPBS to the collection plate for later use. Reduced antibody (2 mg) as a solution in (200 μ L) DPBS was prepared as described above for Conditions A and preloaded into a 96 well plate. The solutions of reduced antibody were transferred to the filter plate wells containing the resin, and the mixture was mixed with the MDT by repeated aspiration/dispensation of a 100 μ L volume within the well for 45 seconds per cycle. The aspiration/dispensation cycle was repeated for a total of 5 times over the course of 5 minutes. A vacuum was applied to the filter plate for 2 cycles, thereby removing excess antibody. The MDT tips were rinsed with water for 5 cycles (200 μ L, 1 mL total volume). The MDT aspirated and dispensed 150 μ L of DPBS to the filter plate wells containing resin-bound antibody, and a vacuum was applied for two cycles. The wash and vacuum sequence was repeated two more times. After the last vacuum cycle, 100 μ L of 1 \times DPBS was dispensed to the wells containing the resin-bound antibody. The MDT then collected 30 μ L each of 3.3 mM dimethyl sulfoxide solutions of synthons plated in a 96-well format and dispensed it to the filter plate containing resin-bound antibody in DPBS. The wells containing the conjugation mixture were mixed with the MDT by repeated aspiration/dispensation of a 100 μ L volume within the well for 45 seconds per cycle. The aspiration/dispensation sequence was repeated for a total of 5 times over the course of 5 minutes. A vacuum was applied for 2 cycles to remove excess synthon to waste. The MDT tips were rinsed with water for 5 cycles (200 μ L, 1 mL total volume). The MDT aspirated and dispensed DPBS (150 μ L) to the conjugation mixture, and a vacuum was applied for two cycles. The wash and vacuum sequence was repeated two more times. The MDT gripper then moved the filter plate and collar to a holding station. The MDT placed the 2 mL collection plate containing 450 μ L of 10 \times DPBS inside the vacuum manifold. The MDT reassembled the vacuum manifold by placement of the filter plate and collar. The MDT tips were rinsed with water for 5 cycles (200 μ L, 1 mL total volume). The MDT aspirated and dispensed 100 μ L of IgG Elution Buffer 3.75 (Pierce) to the conjugation mixture. After one minute, a vacuum was applied for 2 cycles, and the eluent was captured in the receiving plate containing 450 μ L of 5 \times DPBS. The aspiration/dispensation sequence was repeated 3 additional times to deliver ADC samples with concentrations in the range of 1.5-2.5 mg/mL at pH 7.4 in DPBS.

[2066] Method D. Conjugations were performed using a PerkinElmer Janus (part AJL8M01) robotic liquid handling system equipped with an I235/96 tip ModuLar Dispense Technology (MDT), disposable head (part 70243540) containing a gripper arm (part 7400358), and an 8-tip Varispan pipetting arm (part 7002357) on an expanded deck. The PerkinElmer Janus system was controlled using the WinPREP version 4.8.3.315 Software.

[2067] A Pall Filter plate 5052 was prewet with 100 μ L 1 \times DPBS using the MDT. Vacuum was applied to the filter plate for 10 seconds and was followed by a 5 second vent to remove DPBS from filter plate. A 50% slurry of Protein A resin (GE MabSelect Sure) in DPBS was poured into an 8-well reservoir equipped with a magnetic ball, and the resin was mixed by passing a traveling magnet underneath the reservoir plate. The 8 tip Varispan arm, equipped with 1 mL conductive tips, was used to aspirate the resin (250 μ L) and transfer to a 96-well filter plate. A vacuum was applied to the filter plate for 2 cycles to remove most of the buffer. The MDT aspirated and dispensed 150 μ L of DPBS to the filter plate wells containing the resin. The wash and vacuum sequence was repeated two more times. A 2 mL, 96-well collection plate was mounted on the Janus deck, and the MDT transferred 450 μ L of 5 \times DPBS to the collection plate for later use. Reduced antibody (2 mg) as a solution in (200 μ L) DPBS was prepared as described above for Method A and dispensed into the 96-well plate. The MDT then collected 30 μ L each of 3.3 mM dimethyl sulfoxide solutions of synthons plated in a 96-well format and dispensed it to the plate loaded with reduced antibody in DPBS. The mixture was mixed with the MDT by twice repeated aspiration/dispensation of a 100

μL volume within the well. After five minutes, the conjugation reaction mixture (230 μL) was transferred to the 96-well filter plate containing the resin. The wells containing the conjugation mixture and resin were mixed with the MDT by repeated aspiration/dispensation of a 100 μL volume within the well for 45 seconds per cycle. The aspiration/dispensation sequence was repeated for a total of 5 times over the course of 5 minutes. A vacuum was applied for 2 cycles to remove excess synthon and protein to waste. The MDT tips were rinsed with water for 5 cycles (200 μL, 1 mL total volume). The MDT aspirated and dispensed DPBS (150 μL) to the conjugation mixture, and a vacuum was applied for two cycles. The wash and vacuum sequence was repeated two more times. The MDT gripper then moved the filter plate and collar to a holding station. The MDT placed the 2 mL collection plate containing 450 L of 10× DPBS inside the vacuum manifold. The MDT reassembled the vacuum manifold by placement of the filter plate and collar. The MDT tips were rinsed with water for 5 cycles (200 μL, 1 mL total volume). The MDT aspirated and dispensed 100 μL of IgG Elution Buffer 3.75 (P) to the conjugation mixture. After one minute, a vacuum was applied for 2 cycles, and the eluent was captured in the receiving plate containing 450 μL of 5× DPBS. The aspiration/dispensation sequence was repeated 3 additional times to deliver ADC samples with concentrations in the range of 1.5-2.5 mg/mL at pH 7.4 in DPBS.

[2068] Method E. A solution of Bond-Breaker™ tris(2-carboxyethyl)phosphine (TCEP) solution (10 mM, 0.017 mL) was added to the solution of antibody (10 mg/mL, 1 mL) at room temperature. The reaction mixture was heated to 37° C. for 75 minutes. The solution of reduced antibody cooled to room temperature and was added to a solution of synthon (10 mM, 0.040 mL in DMSO) followed by addition of boric buffer (0.1 mL, 1M, pH 8). The reaction solution was let to stand for 3 days at room temperature, loaded onto a desalting column (PD10, washed with DPBS 3×5 mL before use), followed by DPBS (1.6 mL) and eluted with additional DPBS (3 mL). The purified ADC solution was filtered through a 0.2 micron, low protein-binding 13 mm syringe-filter and stored at 4 C.

[2069] Method F. Conjugations were performed using a Tecan Freedom Evo robotic liquid handling system. The solution of antibody (10 mg/mL) was preheated to 37° C. and aliquoted to a heated 96 deep-well plate in amounts of 3 mg per well (0.3 mL) and kept at 37° C. A solution of Bond-Breaker™ tris(2-carboxyethyl)phosphine (TCEP) solution (1 mM, 0.051 mL/well) was added to antibodies, and the reaction mixture was kept at 37° C. for 75 minutes. The solution of reduced antibody was transferred to an unheated 96 deep-well plate. Corresponding solutions of synthons (5 mM, 0.024 mL in DMSO) were added to the wells with reduced antibodies and treated for 15 minutes. The reaction solutions were loaded onto a platform (8×12) of desalting columns (NAPS, washed with DPBS 4× before use), followed by DPBS (0.3 mL) and eluted with additional DPBS (0.8 mL). The purified ADC solutions were further aliquoted for analytics and stored at 4° C.

[2070] Method G. Conjugations were performed using a Tecan Freedom Evo robotic liquid handling system. The solution of antibody (10 mg/mL) was preheated to 37° C. and aliquoted onto a heated 96 deep-well plate in amounts of 3 mg per well (0.3 mL) and kept at 37° C. A solution of Bond-Breaker™ tris(2-carboxyethyl)phosphine (TCEP) solution (1 mM, 0.051 mL/well) was added to antibodies, and the reaction mixture was kept at 37° C. for 75 minutes. The solutions of reduced antibody were transferred to an unheated 96 deep-well plate. Corresponding solutions of synthons (5 mM, 0.024 mL/well in DMSO) were added to the wells with reduced antibodies followed by addition of boric buffer (pH=8, 0.03 mL/well) and treated for 3 days. The reaction solutions were loaded onto a platform (8×12) of desalting columns (NAP5, washed with DPBS 4× before use), followed by DPBS (0.3 mL) and eluted with additional DPBS (0.8 mL). The purified ADC solutions were further aliquoted for analytics and stored at 4° C.

[2071] Method H. A solution of Bond-Breaker™ tris(2-carboxyethyl)phosphine (TCEP) solution (10 mM, 0.17 mL) was added to the solution of antibody (10 mg/mL, 10 mL) at room temperature. The reaction mixture was heated to 37° C. for 75 minutes. The solution of synthon (10 mM, 0.40

mL in DMSO) was added to a solution of reduced antibody cooled to room temperature. The reaction solution was let to stand for 30 minutes at room temperature. The solution of ADC was treated with saturated ammonium sulfate solution (~2-2.5 mL) until a slightly cloudy solution formed. This solution was loaded onto butyl sepharose column (5 mL of butyl sepharose) equilibrated with 30% phase B in phase A (phase A: 1.5 M ammonium sulfate, 25 mM phosphate; phase B: 25 mM phosphate, 25% isopropanol v/v). Individual fractions with DAR2 (also referred to as “E2”) and DAR4 (also referred to as “E4”) eluted upon applying gradient A/B up to 75% phase B. Each ADC solution was concentrated and buffer switched using centrifuge concentrators or TFF for larger scales. The purified ADC solutions were filtered through a 0.2 micron, low protein-binding 13 mm syringe-filter and stored at 4 C.

[2072] Method I. A solution of Bond-Breaker™ tris(2-carboxyethyl)phosphine (TCEP) solution (10 mM, 0.17 mL) was added to the solution of antibody (10 mg/mL, 10 mL) at room temperature. The reaction mixture was heated to 37° C. for 75 minutes. The solution of synthon (10 mM, 0.40 mL in DMSO) was added to a solution of reduced antibody cooled to room temperature. The reaction solution was let to stand for 30 minutes at room temperature. The solution of ADC was treated with saturated ammonium sulfate solution (~2-2.5 mL) until a slightly cloudy solution formed. This solution was loaded onto a butyl sepharose column (5 mL of butyl sepharose) equilibrated with 30% phase B in Phase A (phase A: 1.5 M ammonium sulfate, 25 mM phosphate; phase B: 25 mM phosphate, 25% isopropanol v/v). Individual fractions with DAR2 (also referred to as “E2”) and DAR 4 (also referred to as “E4”) eluted upon applying a gradient A/B up to 75% phase B. Each ADC solution was concentrated and buffer switched using centrifuge concentrators or TFF for larger scales. The ADC solutions were treated with boric buffer (0.1 mL, 1M, pH8). The reaction solution was let stand for 3 days at room temperature, then loaded onto a desalting column (PD10, washed with DPBS 3×5 mL before use), followed by DPBS (1.6 mL) and eluted with additional DPBS (3 mL). The purified ADC solution was filtered through a 0.2 micron, low protein-binding 13 mm syringe-filter and stored at 4 C.

[2073] Table 6 below indicates which exemplary ADCs were synthesized via which exemplary method. The AbB, AbG, AbK, AbA1 are affinity matured variants of Ab1 described in Table 2. Monoclonal antibody to CMV glycoprotein H (MSL109) is an isotype matched non-targeting control.

TABLE-US-00022 TABLE 6 Synthetic Methods Used to Synthesize Exemplary ADCs Ex. No. ADC Method 3.1 AbA-CZ G 3.2 AbA-TX G 3.3 AbA-TV G 3.4 AbA-YY G 3.5 AbA-AAA G 3.6 AbA-AAD G 3.7 AbB-CZ G 3.8 AbB-TX G 3.9 AbB-TV G 3.10 AbB-YY G 3.11 AbB-AAD G 3.12 AbG-CZ G 3.13 AbG-TX G 3.14 AbG-TV G 3.15 AbG-YY G 3.16 AbG-AAA G 3.17 AbG-AAD G 3.18 AbK-CZ G 3.19 AbK-TX G 3.20 AbK-TV G 3.21 AbK-YY G 3.22 AbK-AAA G 3.23 AbK-AAD G 3.24 MSL109-CZ G 3.25 MSL109-TX G 3.26 MSL109-TV G 3.27 MSL109-YY G 3.28 MSL109-AAA G 3.29 MSL109-AAD G 3.30 AbA-WD E 3.31 AbA-LB A 3.32 AbB-WD E 3.33 AbB-LB A 3.34 AbG-WD E 3.35 AbG-LB A 3.36 AbK-WD E 3.37 AbK-LB A 3.38 MSL109-WD E 3.39 MSL109-LB A 3.40 AbA-ZT G 3.41 AbA-ZZ G 3.42 AbA-XW G 3.43 AbA-SE A 3.44 AbA-SR A 3.45 AbA-YG E 3.46 AbA-KZ A 3.47 AbB-ZT G 3.48 AbB-ZZ G 3.49 AbB-XW G 3.50 AbB-SE A 3.51 AbB-SR A 3.52 AbB-YG E 3.53 AbB-KZ A 3.54 AbG-ZT G 3.55 AbG-ZZ G 3.56 AbG-XW G 3.57 AbG-SE A 3.58 AbG-SR A 3.59 AbG-YG E 3.60 AbG-KZ A 3.61 AbK-ZT G 3.62 AbK-ZZ G 3.63 AbK-XW G 3.64 AbK-SE A 3.65 AbK-SR A 3.66 AbK-YG E 3.67 AbK-KZ A 3.68 MSL109-ZT G 3.69 MSL109-ZZ G 3.70 MSL109-XW G 3.71 MSL109-SE A 3.72 MSL109-SR A 3.73 MSL109-YG E 3.74 MSL109-KZ A

[2074] Example 4. Drug to Antibody Ratio (DAR) and Aggregation of Exemplary ADCs

[2075] The DAR and percentage aggregation of exemplary ADCs synthesized as described in Example 3, above, were determined by LC-MS and size exclusion chromatography (SEC), respectively.

[2076] 4.1 LC-MS General Methodology

[2077] LC-MS analysis was performed using an Agilent 1100 HPLC system interfaced to an Agilent LC/MSD TOF 6220 ESI mass spectrometer. The ADC was reduced with 5 mM (final concentration) BOND BREAKER TCEP solution (Thermo Scientific, Rockford, IL), loaded onto a Protein Microtrap (Michrom Bioresources, Auburn, CA) desalting cartridge, and eluted with a gradient of 10% B to 75% B in 0.2 minutes at ambient temperature. Mobile phase A was H.sub.2O with 0.1% formic acid (FA), mobile phase B was acetonitrile with 0.1% FA, and the flow rate was 0.2 ml/min. Electrospray-ionization time-of-flight mass spectra of the co-eluting light and heavy chains were acquired using Agilent MassHunter™ acquisition software. The extracted intensity vs. m/z spectrum was deconvoluted using the Maximum Entropy feature of MassHunter software to determine the mass of each reduced antibody fragment. DAR was calculated from the deconvoluted spectrum by summing intensities of the naked and modified peaks for the light chain and heavy chain, normalized by multiplying intensity by the number of drugs attached. The summed, normalized intensities were divided by the sum of the intensities, and the summing results for two light chains and two heavy chains produced a final average DAR value for the full ADC.

[2078] Thiosuccinimide hydrolysis of a bioconjugate can be monitored by electrospray mass spectrometry, since the addition of water to the conjugate results in an increase of 18 Daltons to the observable molecular weight of the conjugate. When a conjugate is prepared by fully reducing the interchain disulfides of a human IgG1 antibody and conjugating the maleimide derivative to each of the resulting cysteines, each light chain of the antibody will contain a single maleimide modification and each heavy chain will contain three maleimide modifications, as described in FIG. 4. Upon complete hydrolysis of the resulting thiosuccinimides, the mass of the light chain will therefore increase by 18 Daltons, while the mass of each heavy chain will increase by 54 Daltons. This is illustrated in FIG. 5, with the conjugation and subsequent hydrolysis of an exemplary maleimide drug-linker (synthon TX, molecular weight 1736 Da) to the fully reduced AbA antibody. The presence of the single N-linked glycosylation site on the heavy chain results in the heterogeneity of mass observed in the unconjugated antibody.

[2079] FIG. 5 shows MS characterization of light chain and heavy chain of an exemplary antibody Aba 1) prior to conjugation, 2) after conjugation to a maleimide derivative to give a thiosuccinimide intermediate and 3) post pH8-mediated hydrolysis of the thiosuccinimide ring.

[2080] 4.2 Size Exclusion Chromatography General Methodology

[2081] Size exclusion chromatography (SEC) was performed using a Shodex KW802.5 column in 0.2M potassium phosphate pH 6.2 with 0.25 mM potassium chloride and 15% isopropyl alcohol at a flow rate of 0.75 ml/min. The peak area absorbance at 280 nm was determined for each of the high molecular weight and monomeric eluents by integration of the area under the curve. The % aggregate fraction of the conjugate sample was determined by dividing the peak area absorbance at 280 nm for the high molecular weight eluent by the sum of the peak area absorbances at 280 nm of the high molecular weight and monomeric eluents multiplied by 100%.

[2082] 4.3. Results

[2083] The average DAR values were determined using the above LC-MS method. The % aggregate fraction for the ADCs was also determined using the SEC method described in Example 4.2. The DAR and % aggregation are both reported below in Table 7.

TABLE-US-00023 TABLE 7 ADC Analytical Characterization

ADC	DAR (by MS)	% Agg (by SEC)	Ex. No.
3.1 AbA-CZ	3.2	4.7	3.2
3.2 AbA-TX	2.8	0.7	3.3
3.3 AbA-TV	3.7	2.4	3.4
3.4 AbA-YY	2.2	18.8	3.5
3.5 AbA-AAA	2	19	3.6
3.6 AbA-AAD	3.3	3.6	3.7
3.7 AbB-CZ	3.5	0	3.8
3.8 AbB-TX	2.2	0	3.9
3.9 AbB-TV	2.3	0.7	3.10
3.10 AbB-YY	2.2	0	3.11
3.11 AbB-AAD	2.7	0	3.12
3.12 AbG-CZ	3.4	4	3.13
3.13 AbG-TX	3.3	1.6	3.14
3.14 AbG-TV	3.7	1.4	3.15
3.15 AbG-YY	2.2	16.5	3.16
3.16 AbG-AAA	1.9	17.5	3.17
3.17 AbG-AAD	3.4	2.5	3.18
3.18 AbK-CZ	3.4	3.3	3.19
3.19 AbK-TX	2.2	1.6	3.20
3.20 AbK-TV	2.4	2.6	3.21
3.21 AbK-YY	1.7	20	3.22
3.22 AbK-AAA	1.6	20.4	3.23
3.23 AbK-AAD	2.8	3.6	3.24
3.24 MSL109-CZ	3.4	4.1	3.25
3.25 MSL109-TX	3.5	0.7	3.26
3.26 MSL109-TV	4.2	0.7	3.27
3.27 MSL109-YY	2.3	17.5	3.28
3.28 MSL109-AAA	2.2	17.7	3.29
3.29 MSL109-AAD	3.6	2.9	3.30
3.30 AbA-WD	1.8	0	3.31
3.31 AbA-LB	2.4	14.5	3.32
3.32 AbB-WD	1.6	0	3.33
3.33 AbB-LB	1.8	0	3.34

AbG-WD 3.4 3.2 3.35 AbG-LB 2.5 15.3 3.36 AbK-WD 1.7 4.9 3.37 AbK-LB 1.8 13.6 3.38
 MSL109-WD 2.9 0 3.39 MSL109-LB 1.8 0 3.40 AbA-ZT 2 17.1 3.41 AbA-ZZ 1.3 19.2 3.42 AbA-
 XW 3.7 6.6 3.43 AbA-SE 2.8 0 3.44 AbA-SR 2.3 37.1 3.45 AbA-YG 1.9 0 3.46 AbA-KZ 2 4.4
 3.47 AbB-ZT 1.4 0 3.48 AbB-ZZ 1.1 0 3.49 AbB-XW 3.2 0 3.50 AbB-SE 2.2 0 3.51 AbB-SR 2.1 0
 3.52 AbB-YG 1.1 0 3.53 AbB-KZ 1.9 0 3.54 AbG-ZT 1.6 12.4 3.55 AbG-ZZ 1.4 16.8 3.56 AbG-
 XW 3.7 5.9 3.57 AbG-SE 3.8 2.1 3.58 AbG-SR 2.8 36.7 3.59 AbG-YG 3.7 2.4 3.60 AbG-KZ 2.7
 11.6 3.61 AbK-ZT 1.3 13.4 3.62 AbK-ZZ 1.9 4.5 3.63 AbK-XW 2.8 6.2 3.64 AbK-SE 2.7 2.5 3.65
 AbK-SR 2.3 30.1 3.66 AbK-YG 0.9 0 3.67 AbK-KZ 2.3 10.2 3.68 MSL109-ZT 2.3 7.5 3.69
 MSL109-ZZ 1.4 15 3.70 MSL109-XW 3.3 3.7 3.71 MSL109-SE 3.6 33.4 3.72 MSL109-SR 1.8 2.3
 3.73 MSL109-YG 3.1 13.2 3.74 MSL109-KZ 2.5 18

[2084] Example 5. EGFR-Targeted ADCs Inhibit the Growth of Cancer Cells In Vitro

[2085] The cytotoxicity of the anti-EGFR antibodies AbB, AbG, AbK, and AbL as Bcl-xL ADCs against the EGFR positive non-small cell lung cancer cells (NCI-H1650) was compared to non-targeting MSL109 isotype matched exemplary ADCs. To further evaluate the in vitro efficacy of these exemplary EGFR-targeted Bcl-xL-ADCs, human EGFR was over-expressed in mcl-1.sup.-/- mouse embryonic fibroblasts (MEFs). Mcl-1 refers to the gene myeloid cell leukemia 1. Mcl-1.sup.-/- MEFs are dependent upon Bcl-xL for survival (Lessene et al., 2013, *Nature Chemical Biology* 9:390-397).

[2086] 5.1 Methods

[2087] Retroviral supernatants were produced through transfection of the GP2-293 packaging cell line (Clontech) with the retroviral construct pLVC-IRES-Hygro (Clontech) containing huEGFR sequence or the empty vector utilizing FuGENE 6 transfection reagent (Roche Molecular Biochemicals, Mannheim, Germany). After 48 hours of culture, virus-containing supernatant was harvested and applied to mcl-1.sup.-/- MEFs in 75 cm.sup.2 culture flasks (0.5×10⁶ per flask) for a further 48 hours in the presence of polybrene (8 µg/ml; Sigma). Mcl-1.sup.-/- MEFs were washed and selected after 3 days with 250 g/ml hygromycin B (Invitrogen) in the full complement of media. The expression of huEGFR was confirmed by flow cytometry and compared to the parental cell line or those transfected with the empty vector.

[2088] Mcl-1.sup.-/- MEFs expressing huEGFR or the pLVX empty vector (Vct Ctrl) were treated with AB033-targeted Bcl-xL-ADCs, AB033 alone or MSL109-targeted Bcl-xL-ADCs for 96 hours in DMEM containing 10% FBS. For the assay, the cells were plated at 250 cells per well in 384-well tissue culture plates (Corning, Corning, NY) in a total volume of 25 µL of assay media (DMEM and 10% HI FBS). The plated cells were treated with a 4-fold serial dilution of the Antibody Drug Conjugates of interest from either 1 µM or 0.5 µM to either 1 50 µM or 25 µM, respectively 1 µM to 1 µM dispensed by an Echo 550 Acoustic Liquid Handler (Labcyte). Each concentration was tested in at least three replicates for the Mcl-1.sup.-/- MEF huEGFR cell line and for the Mcl-1.sup.-/- MEF vector cell line. The fraction of viable cells following 96 hours of Antibody Drug Conjugate treatment at 37° C. and 5% CO₂ was determined using the CellTiter-Glo Luminescent Cell Viability Assay according to the manufacturer's recommendations (Promega Corp., Madison, WI). The plates were read in a Perkin Elmer Envision using a Luminescence protocol with 0.1 sec integration time. The replicate values for each dilution point were averaged and the EC₅₀ values for the Antibody Drug Conjugates were generated by fitting the data with GraphPad Prism 5 (GraphPad Software, Inc.) to a sigmoidal curve model using linear regression, $Y = ((\text{Bottom} - \text{Top}) / (1 + ((x/K)^n))) + \text{Top}$, where Y is the measured response, x is the compound concentration, n is the Hill Slope and K is the EC₅₀ and Bottom and Top are the lower and higher asymptotes respectively. Visual inspection of curves was used to verify curve fit results. Mcl-1.sup.-/- MEFs were obtained from David C. S. Huang of the Walter and Eliza Hall Institute of Medical Research.

[2089] NCI-H1650 cells stably overexpressing eGFP were maintained in RPMI media (Invitrogen cat #22400) containing 10% Fetal Bovine Serum (Invitrogen cat #10082). The cells were removed

from plates with Trypsin and plated at 300 cells/well in 25 μ L of the same media in Corning 384 well spheroid plates (cat #3830). The plates were centrifuged at 500 \times g for 5 minutes and placed in an Essen INCUCYTE Zoom Live Cell Analysis System in a 37 $^{\circ}$ C. with 5% CO₂ and 95% humidity. The cells were allowed to form spheroids for 3 days before dosing with an equal volume of the antibody drug conjugates at 2 \times the indicated concentration. The spheroids were incubated for an additional 6 days while monitoring growth and GFP Fluorescence in the Incucyte Zoom prior to addition of 40 μ L of Promega CELLTITER-GLO 3D (cat #G968B) and subsequent luminescent reading. IC₅₀s were determined from both the final GFP fluorescence monitored by the Incucyte Zoom (referred to as “H1650 GFP Fluorescence EC₅₀ (μ g/mL)” in Table 8) and the chemiluminescent readings from the CellTiter-Glo reagent (referred to as “H1650 CTG-3D EC₅₀ (μ g/mL)” in Table 8).

[2090] 5.2 Results

[2091] Cell viability assay results (EC₅₀ in nanomolar or g/mL) for representative ADCs are provided below in Table 8.

TABLE-US-00024 TABLE 8 In Vitro Cell Viability Efficacy of Exemplary EGFR-Targeted ADCs

huEGFR.sup.+ mcl - 1.sup.-/- MEF	H1650 GFP	H1650 ADC	mcl - 1.sup.-/- MEF	Vector
Control	Flourescence	CTG-3D	Code.	DAR
EC ₅₀ (μ M)	EC ₅₀ (μ M)	EC ₅₀ (μ g/mL)	EC ₅₀ (μ g/mL)	AbA-CZ
3.2	0.0003	>0.5	1.24	0.88
AbA-TX	2.8	0.016	>0.5	13.88
>40	AbA-TV	3.7	0.003	0.364
0.69	0.34	AbA-YY	2.2	0.46
>0.5	7.85	>40	AbA-AAA	2
0.22	0.306	0.98	0.65	AbA-AAD
3.3	0.065	>0.5	0.36	0.79
AbB-CZ	3.5	0.0059	0.104	0.88
1.27	AbB-TX	2.2	0.011	0.491
3.72	2.39	AbB-TV	2.3	0.0024
0.31	0.74	0.86	AbB-YY	2.2
0.051	0.4	7.73	8.6	AbB-AAD
2.7	0.0046	>10	0.59	0.16
AbG-CZ	3.4	0.0034	0.194	0.25
0.07	AbG-TX	3.3	0.0053	0.368
0.51	0.15	AbG-TV	3.7	0.0026
0.196	0.04	0.03	AbG-YY	2.2
>0.5	>0.5	1.1	0.4	AbG-AAA
1.9	0.22	>0.5	0.17	0.12
AbG-AAD	3.4	0.108	>0.5	0.03
0.02	AbK-CZ	3.4	0.0001	>0.5
0.21	0.08	AbK-TX	2.2	0.00079
>1.0	1.47	1.07	AbK-TV	2.4
0.00015	0.455	0.16	0.05	AbK-YY
1.7	0.047	>1.0	1.09	0.49
AbK-AAA	1.6	0.0034	>1.0	0.16
0.11	AbK-AAD	2.8	0.0004	>0.5
0.06	0.01	MSL109-CZ	3.4	0.087
0.154	>40	>40	MSL109-TX	3.5
0.191	>0.5	24	22	MSL109-TV
4.2	0.143	0.411	>40	>40
MSL109-YY	2.3	>0.5	>0.5	>40
>40	>40	MSL109-AAA	2.2	>0.5
>0.5	>40	>40	MSL109-AAD	3.6
>0.5	>40	>40	AbA-WD	1.8
0.304	NT	7.62	23.59	AbA-LB
2.4	0.027	NT	2.48	4.05
AbB-WD	1.6	0.023	NT	1.01
2.69	AbB-LB	1.8	0.031	NT
2.38	1.07	AbG-WD	3.4	0.0058
NT	0.36	2.03	AbG-LB	2.5
0.024	NT	1.62	1.68	AbK-WD
1.7	0.035	NT	2.57	4.36
AbK-LB	1.8	0.017	NT	1.33
1.23	MSL109-WD	2.9	0.22	NT
1.791	7.668	MSL109-LB	1.8	0.025
NT	0.5744	0.6426	AbA-ZT	2
0.104	>0.5	1.13	0.35	AbA-ZZ
1.3	0.186	>0.5	1.18	1.86
AbA-XW	3.7	0.032	0.229	>40
>40	AbA-SE	2.8	0.105	NT
4.23	4.00	AbA-SR	2.3	0.0059
NT	2.22	2.12	AbA-YG	1.9
0.0064	NT	2.84	3.80	AbA-KZ
2	0.152	NT	29.31	>40
AbB-ZT	1.4	0.0089	0.404	0.64
0.26	AbB-ZZ	1.1	0.0074	0.311
0.61	0.52	AbB-XW	3.2	0.00065
>0.5	12.14	6.92	AbB-SE	2.2
0.039	NT	2.18	0.59	AbB-SR
2.1	0.007	NT	2.10	0.62
AbB-YG	1.1	0.0033	NT	1.55
3.28	AbB-KZ	1.9	0.055	NT
37.73	16.47	AbG-ZT	1.6	0.033
>0.5	0.08	0.1	AbG-ZZ	1.4
0.068	>0.5	0.44	0.47	AbG-XW
3.7	0.019	0.246	>40	>40
AbG-SE	3.8	0.024	NT	0.99
0.96	AbG-SR	2.8	0.007	NT
1.38	1.31	AbG-YG	3.7	0.001
NT	0.63	1.24	AbG-KZ	2.7
0.096	NT	38.19	38.34	AbK-ZT
1.3	0.0002	>0.5	0.12	0.05
AbK-ZZ	1.9	0.045	>0.5	0.16
0.03	AbK-XW	2.8	0.0006	>0.5
8.53	>40	AbK-SE	2.7	0.161
NT	1.68	2.67	AbK-SR	2.3
0.0089	NT	1.36	1.74	AbK-YG
0.9	0.037	NT	10.40	12.32
AbK-KZ	2.3	0.224	NT	>40
>40	MSL109-ZT	2.3	>0.5	>0.5
>40	MSL109-ZZ	1.4	>0.5	>0.5
>40	MSL109-XW	3.3	0.297	0.494
>40	MSL109-SE	3.6	>0.5	NT
3.692	6.079	MSL109-SR	1.8	0.142
NT	2.046	9.138	MSL109-YG	3.1
0.057	NT	3.895	5.852	MSL109-KZ
2.5	0.255	NT	>40	>40

[2092] As described above in Table 8, anti-EGFR ADCs comprising an anti-EGFR antibody and a Bcl-xL inhibitor, were effective at reducing cell viability of the human EGFR expressing mcl1.sup.-/- fibroblasts with a range of potencies. The anti-EGFR Bcl-xL ADCs also inhibited the growth of NSCLC spheroids (H1650 GFP) as measured by remaining cell fluorescence and reduced viability. In contrast many of the non-targeting (MSL109) control Bcl-xL ADCs displayed reduced potency.

These results support targeted delivery of the Bcl-xL warhead by the EGFR antibody, a conclusion supported by low activity of the EGFR Bcl-xL ADCs against the isotype matched mcl1-/- MEFs vector control line lacking human EGFR expression (Table 8).

[2093] Example 6. In Vivo Efficacy of Anti-EGFR-BCL-X.sub.L ADCs

[2094] The in vivo anti-tumor activity of the anti-EGFR antibodies AbB, AbG, AbK, and AbA as Bcl-xL inhibiting ADCs was evaluated using a murine xenograft non-small cell lung cancer (NSCLC) model. Specifically, EGFR positive NSCLC NCI-H1650 cells (ATCC deposit no. CRL-5883) were grown as a flank xenograft in mice. The activity of ADCs was compared to non-targeting IgG isotype matched antibody (AB095) (a human IgG1 antibody recognizing tetanus toxoid; see Larrick et al., 1992, *Immunological Reviews* 69-85) was used as a negative control. The results are presented in Tables 9, 10 and 11 below.

[2095] 6.1 Evaluation of Efficacy in Xenograft Models Methods

[2096] The cell line NCI-H1650 was obtained from the American Type Culture Collection (ATCC Deposit No. CRL-5883, Manassas, VA). The cells were cultured as monolayers in RPMI-1640 that was supplemented with 10% Fetal Bovine Serum (FBS, Hyclone, Logan, UT). To generate xenografts, 5×10^6 viable cells were inoculated subcutaneously into the right flank of immune deficient female SCID/bg mice (Charles River Laboratories, Wilmington, MA) respectively. The injection volume was 0.2 ml and composed of a 1:1 mixture of S MEM and Matrigel (BD, Franklin Lakes, NJ). Tumors were size matched at approximately 200 mm³.

[2097] The control antibody and ADCs were formulated in 0.9% sodium chloride for injection and injected intraperitoneally. Injection volume did not exceed 200 μ l. Therapy began within 24 hours after size matching of the tumors. Mice weighed approximately 22 g at the onset of therapy. Anti-EGFR ADCs and AB095 were administered intraperitoneally (IP) for a single dose (QD \times 1) or weekly for a total of six doses (Q7D \times 6).

[2098] Tumor volume was estimated two to three times weekly. Measurements of the length (L) and width (W) of the tumor were taken via electronic caliper and the volume was calculated according to the following equation: $V = L \times W^2 / 2$. Eight mice were housed per cage. Food and water were available ad libitum. Mice were acclimated to the animal facilities for a period of at least one week prior to commencement of experiments. Animals were tested in the light phase of a 12-hour light: 12-hour dark schedule (lights on at 06:00 hours). Mice were euthanized when tumor volume reached 3,000 mm³ or skin ulcerations occurred.

[2099] To refer to efficacy of therapeutic agents, parameters of amplitude (TGI.sub.max), durability (TGD) of therapeutic response were used. TGI.sub.max is the maximum tumor growth inhibition during the experiment. Tumor growth inhibition was calculated by $100 \times (1 - T_{\text{sub.v}} / C_{\text{sub.v}})$ where $T_{\text{sub.v}}$ and $C_{\text{sub.v}}$ are the mean tumor volumes of the treated and control groups, respectively. TGD or tumor growth delay is the extended time of a treated tumor needed to reach a volume of 1 cm³ relative to the control group (AB095). TGD is calculated by $100 \times (T_{\text{sub.t}} / C_{\text{sub.t}} - 1)$ where $T_{\text{sub.t}}$ and $C_{\text{sub.t}}$ are the median time periods to reach 1 cm³ of the treated and control groups, respectively.

[2100] Certain anti-Bcl-xL inhibiting synthons were conjugated to EGFR targeting antibodies AbA, AbB, AbG and AbK according to the synthetic methods noted in Tables 9, 10 and 11 (and described in the above Examples).

TABLE-US-00025 TABLE 9 In vivo efficacy of anti-EGFR-Bcl-xL ADCs in NCI-H1650 model of NSCLC Synthetic Dose TGI.sub.max TGD Drug Method DAR (mg/kg/day) Regimen/Route N (%) (%) AB095** — — 10 QD \times 1/IP 8 0 0 AbA-CZ A 3.7 3 QD \times 1/IP 8 81 100 AbA-CZ A 3.7 10 QD \times 1/IP 8 99 144 **IgG1 mAb

TABLE-US-00026 TABLE 10 In vivo efficacy of anti-EGFR-Bcl-xL ADCs in NCI-H1650 model of NSCLC Conjugation Dose TGI.sub.max Drug Method DAR (mg/kg/day) Regimen/Route N (%) AB095** — — 10 QD \times 1/IP 8 0 AbG-TX E 3.4 10 QD \times 1/IP 8 88 AbG-AAA E 3.6 10 QD \times 1/IP 8 76 AbG-XW E 4.2 10 QD \times 1/IP 8 76 AbK-CZ A 3.5 10 QD \times 1/IP 8 77 AbK-AAA E 3.1 10 QD \times 1/IP

8 84 AbB-CZ A 3.5 10 QDx1/IP 8 82 **IgG1 mAb

[2101] The results provided in Tables 9 and 10 indicate that the anti-EGFR antibodies AbB, AbG, AbK, AbA as Bcl-xL inhibitor ADCs were similarly effective at tumor growth inhibition of the H1650 xenograft non-small cell lung cancer (NSCLC) model.

[2102] The in vivo anti-tumor activity of anti-EGFR antibodies AbA and AbG were compared as DAR2 (E2) and DAR4 (E4) Bcl-xL inhibitor conjugates against the EGFR positive non-small cell lung cancer model NCI-H1650 grown as a flank xenograft in mice. The activity of these ADCs was compared to non-targeting IgG isotype matched antibody (AB095) as control. The results are presented in Table 11. The results shown in Table 11 indicate that the anti-EGFR antibodies AbA and AbG as Bcl-xL ADCs were effective as either purified DAR2 or DAR4 conjugates against the H1650 xenograft model, with TGI and TGD proportional to the total amount of Bcl-xL warhead dosed. Moreover, a comparison of the efficacy of the conjugates listed in Table 11 revealed that the growth inhibition was proportional to the amount of Bcl-xL ADC administered.

TABLE-US-00027 TABLE 11 In vivo efficacy of anti-EGFR-BCL-XL ADCs in NCI-H1650 model of NSCLC Conjugation Dose TGI.sub.max TGD Drug Method DAR (mg/kg/day) Regimen/Route N (%) (%) AB095** — — 8 Q7Dx6/IP 8 0 0 AbA-CZ H 2 2 Q7Dx6/IP 8 67 93 E2 AbA-CZ H 2 4 Q7Dx6/IP 8 67 93 E2 AbA-CZ H 2 8 Q7Dx6/IP 8 83 193 E2 AbA-CZ H 4 1 Q7Dx6/IP 8 62 75 E4 AbA-CZ H 4 2 Q7Dx6/IP 8 73 100 E4 AbA-CZ H 4 4 Q7Dx6/IP 8 77 114 E4 AbG-CZ H 2 2 Q7Dx6/IP 8 64 93 E2 AbG-CZ H 2 4 Q7Dx6/IP 8 80 143 E2 AbG-CZ H 2 8 Q7Dx6/IP 8 75 143 E2 AbG-CZ H 4 1 Q7Dx6/IP 8 61 64 E4 AbG-CZ H 4 2 Q7Dx6/IP 8 80 114 E4 AbG-CZ H 4 4 Q7Dx6/IP 8 74 114 E4 **IgG1 mAb

[2103] As a control, the in vivo anti-tumor activity of an ADC comprising the non-targeting antibody MSL109 (MSL109 is a monoclonal antibody to CMV glycoprotein H) conjugated to Bcl-xL inhibitors was evaluated against the EGFR positive non-small cell lung cancer model N-H1650 grown as a flank xenograft in mice. The activity of these ADCs was compared to non-targeting IgG isotype matched antibody (AB095) as control showing very modest tumor growth inhibition and low or no tumor growth delay. The results are presented in Table 12, and show only modest tumor growth inhibition and low or no tumor growth delay caused by of Bcl-xL ADCs that use a non-targeting antibody as a carrier. This low anti-tumor activity is contrasted with much greater TGI and TGD observed with the EGFR-targeting Bcl-xL ADCs (Tables 9 and 10), and reflected the antigen dependent delivery of these ADCs in EGFR expressing models.

TABLE-US-00028 TABLE 12 In vivo efficacy of non-targeting (MSL109) Bcl-xL inhibiting ADCs in NCI-H1650 model of NSCLC Growth Dose.sup.[a]/ Inhibition route/ TGI.sub.max Treatment regimen (%) TGD (%) MSL109.sup.†-H 3/IP/Q4Dx6 18* 0 MSL109.sup.†-H 10/IP/Q4Dx6 43* 20* MSL109.sup.†-H 10/IP/Q4Dx6 8 0 MSL109.sup.†-CZ 3/IP/Q4Dx6 29* 0 MSL109.sup.†-CZ 3/IP/Q7Dx6 18* 0 MSL109.sup.†-CZ 10/IP/Q4Dx6 32* 16 MSL109.sup.†-CZ 3/IP/Q4Dx6 32* 12 .sup.†Non-targeting antibody .sup.[a]dose is given in mg/kg/day *p < 0.05 as compared to control treatment (AB095) Q4Dx6 indicates one dose every 4 days for a total of 6 doses

[2104] While various specific embodiments have been illustrated and described, it will be appreciated that various changes can be made without departing from the spirit and scope of the disclosure.

TABLE-US-00029 SEQUENCE TABLE SEQ ID NO: Description 1 Ab1 VH amino acid sequence 2 Ab1, AbC, AbD, and AbE VH CDR1 amino acid sequence 3 Ab1, AbC, AbD, AbE, AbF, AbJ, and AbN VH CDR2 amino acid sequence 4 Ab1, AbC, AbD, and AbE VH CDR3 amino acid sequence 5 Ab1 and AbA VL amino acid sequence 6 Ab1, AbA, AbB, AbC, and AbF VL CDR1 amino acid sequence 7 Ab1, AbA, AbB, and AbC, and AbF VL CDR2 amino acid sequence 8 Ab1, AbA, AbB, and AbF VL CDR3 amino acid sequence 9 AbA VH FIG. 1 10 AbA, AbF, and AbK VH CDR1 amino acid sequence 11 AbA, AbH, AbK, AbL, AbM, AbO, and AbQ VH CDR2 amino acid sequence 12 AbA, AbF, AbM, AbN, and AbO VH CDR3 amino acid sequence 13 Ab1 and AbA light chain amino acid sequence 14 Ab1 heavy chain amino acid sequence 15 AbA heavy chain

amino acid sequence 16 AbB and AbG VH CDR1 amino acid sequence 17 AbB and AbG VH CDR2 amino acid sequence 18 AbG, AbH, AbJ, and AbL VH CDR3 amino acid sequence 19 AbB and AbK VH CDR3 amino acid sequence 20 AbM and AbN VH CDR1 amino acid sequence 21 AbP VH CDR1 amino acid sequence 22 AbP and AbQ VH CDR3 amino acid sequence 23 AbG, AbH, and AbJ VL CDR1 amino acid sequence 24 AbG, AbH, and AbJ VL CDR2 amino acid sequence 25 AbG, AbH, and AbJ VL CDR3 amino acid sequence 26 AbK, AbL, AbM, AbN, and AbO VL CDR1 amino acid sequence 27 AbE, AbK, AbL, AbM, AbN, and AbO VL CDR2 amino acid sequence 28 AbK, AbL, AbM, AbN, and AbO VL CDR3 amino acid sequence 29 AbP and AbQ VL CDR1 amino acid sequence 30 AbP and AbQ VL CDR2 amino acid sequence 31 AbD, AbP, and AbQ VL CDR3 amino acid sequence 32 Human EGFR amino acid sequence (with signal sequence) 33 Human Epidermal Growth Factor Receptor variant III (hEGFRvIII) amino acid 34 Human EGFR extracellular domain (ECD) amino acid sequence 35 VH CDR1 consensus sequence of AbA, AbG, AbK, AbM, and AbP 36 VH CDR2 consensus sequence of AbA, AbG, AbK, AbM, and AbP 37 VH CDR3 consensus sequence of AbA, AbG, AbK, AbM, and AbP 38 VL CDR1 consensus sequence of AbA, AbG, AbK, AbM, and AbP 39 VL CDR2 consensus sequence of AbA, AbG, AbK, AbM, and AbP 40 VL CDR3 consensus sequence of AbA, AbG, AbK, AbM, and AbP 41 Ig gamma-1 constant region 42 Ig gamma-1 constant region mutant 43 Ig kappa constant region 44 Ig lambda constant region 45 Epitope of EGFR 46 ECD of EGFRvIII amino acid sequence 47 EGFR 1-525 amino acid sequence 48 Heavy chain amino acid sequence Ab2 49 Light chain amino acid sequence Ab2 50 VH amino acid sequence AbE 51 VL amino acid sequence AbE 52 VH amino acid sequence AbF 53 VL amino acid sequence AbF 54 VH amino acid sequence AbH 55 VL amino acid sequence AbH 56 VH amino acid sequence AbJ 57 VL amino acid sequence AbJ 58 VH amino acid sequence AbL 59 VL amino acid sequence AbL 60 VH amino acid sequence AbN 61 VL amino acid sequence AbN 62 VH amino acid sequence AbO 63 VL amino acid sequence AbO 64 VH amino acid sequence AbB 65 VL amino acid sequence AbB 66 VH amino acid sequence AbC 67 VL amino acid sequence AbC 68 VH amino acid sequence AbD 69 VL amino acid sequence AbD 70 VH amino acid sequence AbQ 71 VL amino acid sequence AbQ 72 VH amino acid sequence AbG 73 VL amino acid sequence AbG 74 VH amino acid sequence AbK 75 VL amino acid sequence AbK 76 VH amino acid sequence AbM 77 VL amino acid sequence AbM 78 VH amino acid sequence AbP 79 VL amino acid sequence AbP 80 AbH, AbJ, AbL, and AbO VH CDR1 amino acid sequence 81 AbQ VH CDR1 amino acid sequence 82 AbD and AbE VL CDR1 amino acid sequence 83 AbD VL CDR2 amino acid sequence 84 AbC VL CDR3 amino acid sequence 85 AbE VL CDR3 amino acid sequence 86 AbA heavy chain nucleic acid sequence 87 AbA light chain nucleic acid sequence 88 Heavy chain amino acid leader sequence 89 Light chain amino acid leader sequence 90 AbB heavy chain amino acid sequence 91 AbB heavy chain amino acid sequence, LALA mutation 92 AbB light chain amino acid sequence 93 AbG heavy chain amino acid sequence 94 AbG heavy chain amino acid sequence, LALA mutation 95 AbG light chain amino acid sequence 96 AbK heavy chain amino acid sequence 97 AbK heavy chain amino acid sequence, LALA mutation 98 AbK light chain amino acid sequence

Claims

1. An anti-human Epidermal Growth Factor Receptor (hEGFR) antibody drug conjugate (ADC) comprising a drug linked to an anti-human Epidermal Growth Factor (hEGFR) antibody via a linker, wherein the drug is a Bcl-xL inhibitor according to structural formula (IIa) or (IIb):
 ##STR00286## wherein: Ar.sup.1 is selected from ##STR00287## and is optionally substituted with one or more substituents independently selected from halo, hydroxy, nitro, lower alkyl, lower heteroalkyl, C.sub.1-4alkoxy, amino, cyano and halomethyl; Ar.sup.2 is selected from ##STR00288## ##STR00289## and is optionally substituted with one or more substituents independently selected from halo, hydroxy, nitro, lower alkyl, lower heteroalkyl, C.sub.1-4alkoxy,

amino, cyano and halomethyl, wherein the #—N(R.sup.4)—R.sup.13—Z.sup.2b— substituent of formula (IIb) is attached to Ar.sup.2 at any Ar.sup.2 atom capable of being substituted; Z.sup.1 is selected from N, CH, C-halo and C—CN; Z.sup.2a, Z.sup.2b, and Z.sup.2c are each, independent from one another, selected from a bond, NR.sup.6, CR.sup.6aR.sup.6b, O, S, S(O), SO.sub.2, NR.sup.6C(O), NR.sup.6aC(O)NR.sup.6b, and NR.sup.6C(O)O; R.sup.1 is selected from hydrogen, methyl, halo, halomethyl, ethyl and cyano; R.sup.2 is selected from hydrogen, methyl, halo, halomethyl and cyano; R.sup.3 is selected from hydrogen, lower alkyl and lower heteroalkyl; R.sup.4 is selected from hydrogen, lower alkyl, monocyclic cycloalkyl, monocyclic heterocyclyl, and lower heteroalkyl or is taken together with an atom of R.sup.13 to form a cycloalkyl or heterocyclyl ring having between 3 and 7 ring atoms, wherein the lower alkyl, monocyclic cycloalkyl, monocyclic heterocyclyl, and lower heteroalkyl are optionally substituted with one or more halo, cyano, hydroxy, C.sub.1-4alkoxy, monocyclic cycloalkyl, monocyclic heterocyclyl, C(O)NR.sup.6aR.sup.6b, S(O).sub.2NR.sup.6aR.sup.6b, NHC(O)CHR.sup.6aR.sup.6b, NHS(O)CHR.sup.6aR.sup.6b, NHS(O).sub.2CHR.sup.6aR.sup.6b, S(O).sub.2CHR.sup.6aR.sup.6b or S(O).sub.2NH.sub.2 groups; R.sup.6, R.sup.6a and R.sup.6b are each, independent from one another, selected from hydrogen, lower alkyl, lower heteroalkyl, optionally substituted monocyclic cycloalkyl and monocyclic heterocyclyl, or are taken together with an atom from R.sup.13 to form a cycloalkyl or heterocyclyl ring having between 3 and 7 ring atoms; R.sup.10 is selected from cyano, OR.sup.14, SR.sup.14, SOR.sup.14, SO.sub.2R.sup.14, SO.sub.2NR.sup.14aR.sup.14b, NR.sup.14aR.sup.14b, NHC(O)R.sup.14 and NHSO.sub.2R.sup.14; R.sup.11a and R.sup.11b are each, independently of one another, selected from hydrogen, halo, methyl, ethyl, halomethyl, hydroxyl, methoxy, CN, and SCH.sub.3; R.sup.12 is selected from hydrogen, halo, cyano, lower alkyl, lower heteroalkyl, cycloalkyl, and heterocyclyl, wherein the alkyl, heteroalkyl, cycloalkyl, and heterocyclyl are optionally substituted with one or more halo, cyano, C.sub.1-4alkoxy, monocyclic cycloalkyl, monocyclic heterocyclyl, NHC(O)CHR.sup.6aR.sup.6b, NHS(O)CHR.sup.6aR.sup.6b, NHS(O).sub.2CHR.sup.6aR.sup.6b or S(O).sub.2CHR.sup.6aR.sup.6b groups; R.sup.13 is selected from a bond, optionally substituted lower alkylene, optionally substituted lower heteroalkylene, optionally substituted cycloalkyl or optionally substituted heterocyclyl; R.sup.14 is selected from hydrogen, optionally substituted lower alkyl and optionally substituted lower heteroalkyl; R.sup.14a and R.sup.14b are each, independently of one another, selected from hydrogen, optionally substituted lower alkyl, and optionally substituted lower heteroalkyl, or are taken together with the nitrogen atom to which they are bonded to form an optionally substituted monocyclic cycloalkyl or monocyclic heterocyclyl ring; R.sup.15 is selected from hydrogen, halo, C.sub.1-6 alkanyl, C.sub.2-4 alkenyl, C.sub.2-4 alkynyl, and C.sub.1-4 haloalkyl and C.sub.1-4 hydroxyalkyl, with the proviso that when R.sup.15 is present, R.sup.4 is not C.sub.1-4 alkyl, C.sub.2-4 alkenyl, C.sub.2-4 alkynyl, C.sub.1-4 haloalkyl or C.sub.1-4 hydroxyalkyl, wherein the R.sup.4 C.sub.1-6 alkanyl, C.sub.2-4 alkenyl, C.sub.2-4 alkynyl, C.sub.1-4 haloalkyl and C.sub.1-4 hydroxyalkyl are optionally substituted with one or more substituents independently selected from OCH.sub.3, OCH.sub.2CH.sub.2OCH.sub.3, and OCH.sub.2CH.sub.2NHCH.sub.3; and #represents a point of attachment to a linker; and wherein the anti-hEGFR antibody has the following characteristics: binds to an epitope within the amino acid sequence CGADSYEMEEDGVRKC (SEQ ID NO: 45) or competes with a second anti-hEGFR antibody for binding to epidermal growth factor receptor variant III (EGFRvIII) (SEQ ID NO: 33) in a competitive binding assay, wherein the second anti-EGFR antibody comprises a heavy chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 1 and a light chain variable domain comprising the amino acid sequence set forth in SEQ ID NO: 5; and binds to EGFR(1-525) (SEQ ID NO: 47) with a dissociation constant (K.sub.d) of about 1×10⁻⁶ M or less, as determined by surface plasmon resonance.

2. The ADC of claim 1, which is a compound according to structural formula (I): ##STR00290## wherein: D is the Bcl-xL inhibitor drug of formula (IIa) or (IIb); L is the linker; Ab is the anti-

hEGFR antibody; LK represents a covalent linkage linking the linker (L) to the anti-hEGFR antibody (Ab); and m is an integer ranging from 1 to 20.

3. (canceled)

4. The ADC of claim 2, selected from the group consisting of AbA-ZT, AbA-ZZ, AbA-XW, AbA-SE, AbA-SR, AbA-YG, AbA-KZ, AbB-ZT, AbB-ZZ, AbB-XW, AbB-SE, AbB-SR, AbB-YG, AbB-KZ, AbG-ZT, AbG-ZZ, AbG-XW, AbG-SE, AbG-SR, AbG-YG, AbG-KZ, AbK-ZT, AbK-ZZ, AbK-XW, AbK-SE, AbK-SR, AbK-YG, and AbK-KZ, wherein KZ, SR, SE, XW, YG, ZT and ZZ are synthons disclosed in Table 5, and wherein the synthons are either in open or closed form.

5. The ADC of claim 2, selected from the group consisting of formulae i-xiv: ##STR00291## ##STR00292## ##STR00293## ##STR00294## ##STR00295## ##STR00296## wherein m is an integer from 1 to 6.

6. The ADC of claim 1, wherein the anti-hEGFR antibody comprises a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 12, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 11, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 10; a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 8, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 7, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 6; or wherein the antibody comprises a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 40, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 39, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 38; and a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 37, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 36, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 35; or wherein the antibody comprises a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 8, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 7, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 6; and a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 19, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 17, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 16; or wherein the antibody comprises a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 25, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 24, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 23; and a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 18, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 17, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 16; or wherein the antibody comprises a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 28, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 27, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 26; and a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 19, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 11, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 10.

7. The ADC of claim 1, wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 9, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 5.

8. The ADC of claim 1, wherein the antibody comprises a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 15, and a light chain comprising the amino acid sequence set forth in SEQ ID NO: 13.

9. The ADC of claim 1, wherein the antibody comprises a heavy chain variable region comprising

an amino acid sequence selected from the group consisting of 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, and 78; and a light chain variable region comprising an amino acid sequence selected from the group consisting of 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, and 79.

10. The ADC of claim 1, wherein the antibody comprises a heavy chain CDR set (CDR1, CDR2, and CDR3) selected from the group consisting of SEQ ID NOs: 10, 11, and 12; SEQ ID NOs: 16, 17, and 18; SEQ ID NOs: 10, 11, and 19; SEQ ID NOs: 20, 11, and 12; SEQ ID NOs: 21, 3, and 22; SEQ ID NOs: 16, 17, and 19; SEQ ID NOs: 2, 3, and 4; SEQ ID NOs: 10, 3, and 12; SEQ ID NOs: 80, 11, and 18; SEQ ID NOs: 80, 3, and 18; SEQ ID NOs: 20, 3, and 12; SEQ ID NOs: 80, 11, and 12; and SEQ ID NOs: 81, 11, and 22; and a light chain CDR set (CDR1, CDR2, and CDR3) selected from the group consisting of SEQ ID NOs: 6, 7, and 8; SEQ ID NOs: 23, 24, and 25; SEQ ID NOs: 26, 27, and 28; SEQ ID NOs: 29, 30, and 31; SEQ ID NOs: 6, 7, and 84; SEQ ID NOs: 82, 83, and 31; and SEQ ID NOs: 82, 27, and 85, wherein the antibody does not comprise both the heavy chain CDR set of SEQ ID NOs: 2, 3, and 4, and the light chain CDR set of SEQ ID NOs: 6, 7, and 8.

11. The ADC of claim 1, wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 64, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 65.

12. The ADC of claim 1, wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 72, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 73.

13. The ADC of claim 1, wherein the antibody comprises a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 74, and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 75.

14. The ADC of claim 1, wherein the antibody is a monoclonal IgG antibody.

15. A pharmaceutical composition comprising an effective amount of an ADC according to claim 1, and a pharmaceutically acceptable carrier.

16. A pharmaceutical composition comprising an ADC mixture comprising a plurality of the ADC of claim 1, and a pharmaceutically acceptable carrier.

17. A method for treating cancer, comprising administering a therapeutically effective amount of the ADC of claim 1 to a subject in need thereof.

18. A method for inhibiting or decreasing solid tumor growth in a subject having a solid tumor, said method comprising administering an effective amount of the ADC of claim 1 to the subject having the solid tumor, such that the solid tumor growth is inhibited or decreased.

19. The method of claim 18, wherein the ADC is administered in combination with an additional agent or an additional therapy.

20. A process for the preparation of an ADC according to structural formula (I): ##STR00297## wherein: D is the Bcl-xL inhibitor drug of formula (IIa) or (IIb); L is the linker; Ab is an hEGFR antibody, wherein the hEGFR antibody comprises the heavy and light chain CDRs of AbA; AbB; AbG; and AbK; LK represents a covalent linkage linking linker L to antibody Ab; and m is an integer ranging from 1 to 20; the process comprising: treating an antibody in an aqueous solution with an effective amount of a disulfide reducing agent at 30-40° C. for at least 15 minutes, and then cooling the antibody solution to 20-27° C.; adding to the reduced antibody solution a solution of water/dimethyl sulfoxide comprising a synthon selected from the group of 2.1 to 2.31 and 2.34 to 2.72 (Table 5); adjusting the pH of the solution to a pH of 7.5 to 8.5; allowing the reaction to run for 48 to 80 hours to form the ADC; wherein the mass is shifted by 18±2 amu for each hydrolysis of a succinimide to a succinamide as measured by electron spray mass spectrometry; and wherein the ADC is optionally purified by hydrophobic interaction chromatography.

21. (canceled)

22. An anti-human Epidermal Growth Factor Receptor (hEGFR) antibody drug conjugate (ADC) selected from the group consisting of formulae (i) or (ii): ##STR00298## wherein m is an integer

from 1 to 6, optionally from 2 to 6; and wherein Ab is either an anti-hEGFR antibody comprising a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 12, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 11, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 10; a comprising light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 8, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 7, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 6; or an anti-hEGFR antibody comprising a light chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 25, a light chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 24, and a light chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 23; and a heavy chain CDR3 domain comprising the amino acid sequence set forth in SEQ ID NO: 18, a heavy chain CDR2 domain comprising the amino acid sequence set forth in SEQ ID NO: 17, and a heavy chain CDR1 domain comprising the amino acid sequence set forth in SEQ ID NO: 16.
