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UNITED STATES DISTRICT COURT
DISTRICT OF NEW JERSEY

JANSSEN PRODUCTS, L.P. and PHARMA MAR, S.A., Plaintiffs, v. MEDCHEMEXPRESS LLC, Defendant.	Case No. 2:25-cv-01867 (MCA)(JSA) (Filed Electronically)

AMENDED COMPLAINT FOR PATENT INFRINGEMENT

Plaintiffs Janssen Products, L.P., ("Janssen") and Pharma Mar, S.A. ("Pharma Mar") (collectively, "Plaintiffs"), for their Complaint against Medchemexpress LLC ("Medchemexpress" or "Defendant"), allege as follows:[1]

NATURE OF THE ACTION

1. This is a civil action for infringement by Defendant of U.S. Patent No. 8,895,557 (the "'557 Patent") and U.S. Patent No. 7,420,051 (the "'051 Patent") arising under the patent laws of the United States, 35 U.S.C. §§ 1 et seq., and for a declaratory judgment of infringement of the '557 Patent and under 35 U.S.C. §§ 1 et seq., 28 U.S.C. §§ 2201 and 2202.

2. This action arises out of, among other things, Defendant Medchemexpress' submission, in concert with EVER Valinject GmbH's ("EVER Valinject") and other parties described below, of New Drug Application No. 219617 ("NDA 219617") under § 505(b)(2) of the Federal Food, Drug and Cosmetic Act ("FDCA"), and submission of Drug Master File ("DMF") Nos. 36724 (referenced in the NDA) and 36899, to the United States Food and Drug Administration ("FDA") seeking approval to sell a version of Plaintiffs' highly successful Yondelis® (trabectedin) 1 mg/vial ("NDA Product") prior to the expiration of the '557 Patent and the '051 Patent (together, the "Patents-in-Suit").

3. Yondelis® was the first drug in decades to show consistent and meaningful clinical benefit as a second-line treatment option for patients with unresectable and metastatic liposarcomas or leiomyosarcomas after the failure of conventional therapy. Trabectedin, the active ingredient in Yondelis®, is manufactured by the processes claimed in the '051 Patent, which was the first and still only synthetic process that allows manufacture of this active pharmaceutical ingredient ("API") at a commercial scale. The '557 Patent includes claims to the novel formulation

[1] Plaintiffs file this Amended Complaint with Defendant's consent pursuant to Fed. R. Civ. P. 15(a)(2).

for Yondelis®, which is formulated to help reduce the formation of impurities during lyophilization of trabectedin and during storage. Plaintiffs' manufacture of trabectedin using the manufacturing process of the '051 Patent and use of the '557 Patent formulation helps ensure that commercial quantities of Yondelis® with sufficient storage stability are available to meet the needs of the vulnerable population of cancer patients for which it is indicated.

4. Plaintiffs originally filed suit against EVER Valinject, Nexus Pharmaceuticals LLC ("Nexus"), Shanghai Haoyuan Chemexpress Co., Ltd. ("Haoyuan Chemexpress"), Ruyuan HEC Pharm Co., Ltd. ("HEC"), and Medchemexpress (collectively, the "EVER Defendants") in the Northern District of Illinois. *See Janssen Prods. L.P., et al v. EVER Valinject GmbH, et al*, No. 1-24-cv-07319, Dkt. 1, 73 (N.D. Ill. Aug. 15, 2024). Defendant Medchemexpress moved to dismiss, *inter alia*, under Fed. R. Civ. P. 12(b)(3) for improper venue. *Id.* at Dkt. 86, 87. The court denied Medchemexpress' motion to dismiss on that basis, instead severing Medchemexpress and transferring the case against it to the District of New Jersey. *Id.* at Dkt. 113. The court did not address other aspects of Medchemexpress' motion to dismiss.

THE PARTIES

5. Plaintiff Janssen Products, L.P., is a partnership organized under the laws of the State of New Jersey, having its headquarters and principal place of business at 800/850 Ridgeview Drive, Horsham, PA 19044.

6. Plaintiff Pharma Mar, S.A. is a Spanish corporation having a principal place of business at Avda. de los Reyes, 1 Pol. Ind. La Mina, 28770, Colmenar Viejo, Madrid, Spain.

7. On information and belief, Defendant Medchemexpress is a limited liability company organized and existing under the laws of the State of New Jersey, having a principal place of business at 1 Deerpark Dr. Ste. Q, Monmouth Junction, New Jersey 08852. On

information and belief, Medchemexpress is in the business of, among other things, sales of chemical and biochemicals for the development of pharmaceutical products, including trabectedin. On information and belief, Medchemexpress is a subsidiary of Haoyuan Chemexpress and, among other things, the authorized U.S. agent for DMF Nos. 36724 and 36899. On information and belief, Medchemexpress operates in concert with and/or under the direction of Haoyuan Chemexpress, EVER Valinject and Nexus in, *inter alia*, seeking approval for NDA 219617, supported by DMF Nos. 36724 and 36899, and the development and manufacture of the trabectedin API and proposed NDA Product.

MEDCHEMEXPRESS' COLLABORATORS

8. On information and belief, Defendant Medchemexpress is acting in concert with defendants in the related Northern District of Illinois Action to infringe the '557 and '051 Patents.

9. On information and belief, EVER Valinject is in the business of, among other things, marketing and selling generic copies of branded pharmaceutical products for the U.S. market. EVER Valinject is the holder of NDA 219617.

10. On information and belief, Nexus is in the business of, among other things, marketing and selling generic copies of branded pharmaceutical products for the U.S. market. On information and belief, Nexus is the registered U.S. agent for the NDA 219617 submission and marketing partner for the proposed NDA Product.

11. On information and belief, Haoyuan Chemexpress is in the business of, among other things, manufacturing generic copies of branded pharmaceutical products for the U.S. market and/or manufacturing APIs and intermediates used to make APIs for generic copies of branded pharmaceutical products for the U.S. market. Haoyuan Chemexpress is the holder of DMF No. 36724 for trabectedin, and DMF No. 36899 which, on information and belief, is directed to an

intermediate and/or starting material for the manufacture of trabectedin, including for purposes of manufacturing the proposed NDA Product.

12. On information and belief, HEC is in the business of, among other things, developing, manufacturing, selling and distributing API for generic pharmaceutical products. On information and belief, HEC will manufacture the trabectedin API for the proposed NDA Product. On information and belief, HEC operates in concert with and under the direction of Haoyuan Chemexpress, EVER Valinject, Nexus and Medchemexpress in, *inter alia*, the development and manufacture of the trabectedin API for the proposed NDA Product.

13. On information and belief, Medchemexpress and the remaining EVER Defendants collaborated in the research, development, preparation and submission of NDA 219617, supporting DMF Nos. 36724 and 36899, and proposed NDA Product and continue to actively collaborate in seeking approval from the FDA for NDA 219617, supporting DMFs, and the NDA Product for marketing in the U.S., including in the State of New Jersey.

14. On information and belief, Medchemexpress and the remaining EVER Defendants actively collaborate with respect to the development, regulatory approval, commercial manufacture, marketing, sale, offer for sale, and/or distribution of the proposed NDA Product for the U.S. market, including in the State of New Jersey.

15. On information and belief, Medchemexpress and the remaining EVER Defendants rely on material assistance from one another to market, distribute, offer for sale, and/or sell the proposed NDA Product in the U.S. market, including in the State of New Jersey. On information and belief, Medchemexpress intends to act collaboratively with the remaining EVER Defendants to commercially manufacture, market, distribute, offer for sale and/or sell the proposed NDA Product, in the event FDA approves NDA 219617. On information and belief, Medchemexpress

will financially benefit in the event the FDA approves NDA 219617 and from the use, marketing and/or sale of the proposed NDA Product in the U.S., including in the State of New Jersey.

JURISDICTION AND VENUE

16. This Court has jurisdiction over the subject matter of this action pursuant to 28 U.S.C. §§ 1331, 1338(a), 2201, and 2202.

17. On information and belief, Medchemexpress is a limited liability company with a principal place of business at 1 Deerpark Dr. Ste. Q, Monmouth Junction, New Jersey 08852. By virtue of its principal place of business in New Jersey, this Court has personal jurisdiction over Medchemexpress.

18. On information and belief, this court has personal jurisdiction over Medchemexpress because, *inter alia*, Medchemexpress has purposely availed itself of the benefits and protections of New Jersey laws such that it should reasonably anticipate being haled into court here.

19. On information and belief, Medchemexpress has had persistent and continuous contacts with this Judicial District, including developing and marketing pharmaceutical products that are sold in this Judicial District, and selling pharmaceutical products in this Judicial District.

20. This Court also has personal jurisdiction over Medchemexpress because, *inter alia* and on information and belief, Medchemexpress has been and is engaging in activities directed toward infringement of the Patents-in-Suit by, among other things, preparing and submitting DMF Nos. 36724 and 36899 in support of NDA 219617, and acting in concert with the remaining EVER Defendants in the preparation and submission of NDA 219617 seeking FDA approval to market the proposed NDA Product throughout the U.S., including in New Jersey, before expiration of the Patents-in-Suit. On information and belief, Medchemexpress has purposely availed itself of the

benefits and protections of New Jersey laws such that it should reasonably anticipate being haled into court here.

21. On information and belief, Medchemexpress has been and is engaging in activities directed toward infringement of the Patents-in-Suit, including by acting in concert with the remaining EVER Defendants with respect to the development, regulatory approval, commercial manufacture, marketing, sale, offer for sale and/or distribution of the proposed NDA Product before expiration of the Patents-in-Suit. On information and belief, Medchemexpress acted in concert with the remaining EVER Defendants in the development, preparation, and submission of NDA 219617, and the development, preparation, and submission of supporting DMF Nos. 36724 and 36899. On information and belief, Medchemexpress continues to act in concert with the remaining EVER Defendants in seeking approval for NDA 219617 and in taking all steps to gain marketing approval from the FDA for the NDA Product for sale in the U.S., including in New Jersey. On information and belief, Medchemexpress intends to act in concert with the remaining EVER Defendants in importing, marketing, selling, distributing and/or using the proposed NDA Product before expiration of the Patents-in-Suit throughout the U.S., including in New Jersey. The conduct of Medchemexpress will therefore cause injury to Plaintiffs in New Jersey.

22. On information and belief, Medchemexpress derives substantial revenue from selling APIs and/or generic pharmaceutical products sold throughout the U.S., including in New Jersey.

23. Venue is proper in this district for Medchemexpress pursuant to 28 U.S.C. § 1400(b) because, *inter alia*, Medchemexpress has committed and will commit acts of infringement in this Judicial District and has a regular and established place of business at its headquarters in Monmouth Junction, New Jersey, located within this Judicial District.

THE ASSERTED PATENTS

24. On November 25, 2014, the U.S. Patent and Trademark Office ("PTO") issued the '557 Patent, entitled "Pharmaceutical Formulations of Ecteinascidin Compounds." A true and correct copy of the '557 Patent is attached hereto as Exhibit A.

25. Pharma Mar holds title to the '557 Patent.

26. Janssen holds an exclusive license to the '557 Patent.

27. The '557 Patent expires on January 7, 2028.

28. The FDA has awarded 6 months of pediatric exclusivity for Yondelis® (trabectedin). The period of pediatric exclusivity applicable to the '557 Patent does not expire until July 7, 2028.

29. Janssen is the holder of approved New Drug Application ("NDA") No. 207953 for Yondelis®.

30. Janssen sells Yondelis® in the U.S.

31. Yondelis® is included in the FDA's list of "Approved Drug Products With Therapeutic Equivalence Evaluations," also known as the "Orange Book." The FDA's "Orange Book" also lists patents associated with approved drugs. The '557 Patent is listed in the "Orange Book" in association with Yondelis®. The claims of the '557 Patent cover Yondelis®.

32. On September 2, 2008, the PTO issued the '051 Patent, entitled "Synthetic Process for the Manufacture of an Ecteinascidin Compound." A true and correct copy of the '051 Patent is attached hereto as Exhibit B.

33. Pharma Mar holds title to the '051 Patent.

34. Janssen holds an exclusive license to the '051 Patent for the commercialization of Yondelis®.

35. The claims of the ’051 Patent protect Yondelis®. Yondelis® is commercially manufactured by the processes claimed in the ’051 Patent.

36. The FDA has awarded a patent term extension for the ’051 Patent.

37. The patent term extension for the ’051 Patent expires on January 21, 2026.

38. On information and belief, Medchemexpress has, in concert with the EVER Defendants, made and will continue to make substantial and meaningful preparations to import into the U.S. and/or offer to sell, sell and/or use within the U.S. products containing trabectedin (also known as “ecteinascidin 743” or “ET-743”), which are made by processes patented by the ’051 Patent prior to its expiration.

39. On information and belief, Medchemexpress’ and the other EVER Defendants’ preparations include, but are not limited to, the development of the API and proposed NDA Product, and the submission of NDA 219617 to FDA with a Paragraph IV certification for the ’557 Patent.

40. On information and belief, Medchemexpress intends to financially benefit from NDA 219617 and DMF Nos. 36724 and 36899 by the sale and distribution of the proposed NDA Product upon approval.

PRE-SUIT COMMUNICATIONS AND DISCOVERY

41. The EVER Defendants’ purported July 3, 2024 Paragraph IV Letter stated that NDA No. 219617 was submitted to the FDA under § 505(b)(2) of the FDCA, 21 U.S.C. § 355(b), seeking approval to engage in the commercial manufacture, use, importation, offer for sale and/or sale of the proposed NDA Product prior to the expiration of the ’557 Patent. The Paragraph IV

Letter also stated that the EVER Defendants intended to engage in the commercial use, importation, offer for sale and/or sale of the NDA Product prior to the expiration of the ’051 Patent.

42. After receiving the purported July 3, 2024 letter, Plaintiffs contacted the EVER Defendants and asked for information documenting the process that has been and will be used to manufacture trabectedin for the proposed NDA Product and the formulation of the NDA Product so that Plaintiffs could evaluate infringement of the ’557 Patent and the ’051 Patent. Plaintiffs requested NDA 219617, associated DMFs, executed batch records, representative samples of the NDA Product and API, a manufacturing facility inspection and all agreements relating to the marketing of the proposed NDA Product. Despite repeated requests, Plaintiffs did not receive the requested information.

43. Beginning with correspondence on July 12, 2024, outside counsel for Plaintiffs negotiated in good faith with counsel identified in the Paragraph IV Letter in an attempt to reach agreement on reasonable terms of confidential access to the NDA, associated DMFs, batch records for the drug product and API, representative samples of finished product and API for exhibit batches provided to FDA, manufacturing facility inspection and all agreements relating to the marketing of the NDA Product. Plaintiffs repeated their request on July 16, 19, 22 and 23. Plaintiffs repeatedly stressed that it was in the best interests of all parties for Plaintiffs to receive information relevant to infringement of both the Orange Book patent and process patent (*i.e.*, NDA 219617, associated DMFs, batch records for the drug product and API, representative samples of finished product and API and manufacturing facility inspection).

44. On July 26, 2024, the EVER Defendants provided Plaintiffs with certain selected portions of NDA 219617 and selections of DMF No. 36724. The EVER Defendants, however, refused to produce the remaining materials, including the full NDA and DMFs, batch records

showing the process used to manufacture the trabectedin API, any raw analytical data, samples or other information requested by Plaintiffs.

45. On August 5, 2024, Plaintiffs repeated their request that the EVER Defendants produce the complete DMFs, raw analytical data and batch records concerning the manufacture of trabectedin. The EVER Defendants again refused to produce these documents, despite the full DMF(s) having already been provided to FDA in support of NDA 219617.

46. On August 6, 2024, Plaintiffs' counsel again wrote to counsel for EVER Valinject and Nexus, stating that the assertions in the July 3, 2024 letter were not supported by the documents produced and are scientifically implausible.

47. On August 15, 2024, Plaintiffs commenced a lawsuit against the EVER Defendants in the Northern District of Illinois: Civil Action No. 1:24-cv-07319. That lawsuit was commenced within 45 days of the date Plaintiffs received the purported Paragraph IV Letter dated July 3, 2024.

48. As of August 15, 2024, the EVER Defendants had not produced the requested materials, including the complete NDA, DMFs, raw analytical data and batch records concerning the manufacture of trabectedin.

49. On August 26, 2024, Plaintiffs received a second purported Paragraph IV Letter (dated August 23, 2024) ("PIV Letter") stating that NDA No. 219617 was submitted to the FDA under § 505(b)(2) of the FDCA, 21 U.S.C. § 355(b), seeking approval to engage in the commercial manufacture, use, importation, offer for sale and/or sale of the proposed NDA Product prior to the expiration of the '557 Patent. The PIV Letter also stated that the EVER Defendants intended to engage in the commercial use, importation, offer for sale and/or sale of the NDA Product prior to the expiration of the '051 Patent.

50. After receiving the PIV Letter, and pursuant to its Offer of Confidential Access ("OCA"), Plaintiffs again contacted the EVER Defendants and requested the full NDA 219617 and associated DMFs, all batch records for each of the exhibit batches of API, representative samples of the NDA Product and API, manufacturing facility inspection records and all agreements relating to the marketing of the proposed NDA Product. On September 5, 2024, Counsel for EVER Valinject and Nexus declined Plaintiffs' requests, inaccurately characterizing those requests as premature Rule 26 discovery. The EVER Defendants' withholding of this information is contrary to the EVER Defendants' purported OCA and has impeded Plaintiffs' ability to fully evaluate infringement of the '051 Patent.

51. The information provided by the documents that have been produced to date, and Medchemexpress' and the other EVER Defendants' failure to produce the requested API batch records, raw analytical data and complete DMFs, supports Plaintiffs' belief that the process used by Medchemexpress and the other EVER Defendants to manufacture trabectedin for the proposed NDA Product is the same as the commercial process invented by Pharma Mar and protected by the '051 Patent. Not only have Medchemexpress and the other EVER Defendants failed to produce the requested API batch records, but the documents produced to date reflect that the method they purport to use to manufacture trabectedin is scientifically implausible for commercial-scale manufacture and unlikely to be used to prepare the API for the proposed NDA Product. For example, *inter alia*, the process Medchemexpress and the other EVER Defendants allege to use (1) would not yield trabectedin API that is sufficiently pure and (2) would give rise to multiple known impurities that, on information and belief, Medchemexpress and the other EVER Defendants have failed to identify, test, control, or to demonstrate removal of. Medchemexpress and the EVER Defendants also have failed to produce any other documents—including documents that on information and belief are in

the possession of Medchemexpress in New Jersey—prepared in the ordinary course of business reflecting the preparation of trabectedin.

52. Further, as set forth above, the processes claimed in the ’051 Patent are important for the commercial manufacture of trabectedin. Prior art processes are not usable on a commercial scale for the manufacture of trabectedin API. On information and belief, Medchemexpress and the other EVER Defendants use the claimed processes of the ’051 Patent to manufacture trabectedin for the proposed NDA Product.

53. Plaintiffs have a good faith belief that the prior art process for manufacture of trabectedin (that Medchemexpress and the other EVER Defendants allege they are using) is not commercially feasible. This has been demonstrated by Plaintiffs’ own efforts to develop the prior art process for commercial scale manufacture—Plaintiffs were unable to do so and instead developed and patented the methods claimed in the ’051 Patent. Indeed, the chemist who developed the prior art process, Nobel Laureate E.J. Corey of Harvard, advised Plaintiff Pharma Mar that the process would not work on a commercial scale and that they should pursue alternative APIs.

54. Moreover, other groups of generic manufacturers who sought to copy Yondelis® initially engaged in attempts to manufacture trabectedin through the prior art process and/or initially alleged they used the prior art process for the manufacture of trabectedin. Each has stipulated to infringement of the ’051 Patent or is enjoined from commercial manufacture of trabectedin until patent expiration. *See, e.g.*, *Janssen Prods., L.P., et al. v. eVenus Pharms. Laby’s. Inc., et al.*, 1-20-cv-09369 (D.N.J.), Dkt. 445; *see also* Dkt. 491 at 24 (Ret. U.S.M.J. Joel Schneider as Special Master finding that “plaintiffs [Janssen and Pharma Mar] had a well-grounded good faith belief that defendants [eVenus-Fresenius-Hengrui] had no intention of using” the prior art process). Natco

Pharma Limited, Sun Pharmaceutical Industries Ltd. and Sun Pharmaceutical Industries Inc. stipulated to infringement of the ’051 Patent. *Id.* at Dkt. 104.

55. These facts, coupled with Medchemexpress’ and the other EVER Defendants’ failure to produce the full DMFs, manufacturing batch records for the trabectedin API in their proposed NDA Product, and the requested manufacturing inspection, strongly support the conclusion that the commercial process that Medchemexpress and the other EVER Defendants will actually use to manufacture trabectedin for the NDA Product is the process that was invented by Pharma Mar and covered by the ’051 Patent. Indeed, the facts, as outlined herein, give rise to a presumption under 35 U.S.C. § 295 that the trabectedin API in the proposed NDA Product is made using the processes claimed in the ’051 Patent.

56. On information and belief, the Medchemexpress and the other EVER Defendants also have knowledge of the validity of the ’051 Patent and that the patent was not found invalid in litigation. Their PIV Letter provides no reason that the claims of the ’051 Patent are not valid. Similarly, MSN stipulated to the validity of the claims of the ’051 Patent at the outset of litigation. Stipulated Order Regarding Infringement and Validity of U.S. Patent No. 8,895,557 and Validity of U.S. Patent No. 7,420,051 (Dkt. 14), *Janssen Prods., L.P., et al. v. MSN Pharms. Inc., et al.*, 3-21-cv-14622 (D.N.J. Dec. 17, 2021).

57. On information and belief, Medchemexpress and the other EVER Defendants had actual and constructive notice of the ’051 Patent prior to the submission of NDA 219617 seeking approval of the NDA Product.

58. On information and belief, Medchemexpress and the other EVER Defendants have made, and continue to make substantial preparations in the United States to offer to sell, sell, use and/or import the NDA Product prior to the expiration of the ’051 Patent.

59. On information and belief, Medchemexpress' and the other EVER Defendants' actions include, but are not limited to, the development of the NDA Product and the filing of NDA 219617 with a Paragraph IV certification.

60. On information and belief, the EVER Defendants continue to seek approval of NDA 219617 from the FDA and intend to collaborate in the manufacture, marketing, and sale of the NDA Product (including the commercial marketing and sale of such product in the State of New Jersey) in the event that the FDA approves NDA 219617.

61. On October 3, 2024, Plaintiffs commenced a lawsuit against the EVER Defendants in the Northern District of Illinois: Civil Action No. 1:24-cv-09441. That lawsuit was commenced within 45 days of the date they received EVER's notice dated August 23, 2024 of NDA No. 219617 containing a Paragraph IV certification.

62. Plaintiffs thereafter filed a consolidated complaint as ordered by the court. The consolidated complaint contains the allegations made in the original complaints of cases 1:24-cv-07319 and 1:24-cv-09941. Filing the consolidated complaint did not impact the 30-month regulatory stay triggered by Plaintiffs' commencement of those actions.

63. Medchemexpress moved to dismiss the consolidated complaint under, *inter alia*, Fed. R. Civ. P. 12(b)(3) for improper venue. No. 1:24-cv-07319, Dkt. 86, 87. The court denied Medchemexpress' motion to dismiss with respect to improper venue, instead severing Medchemexpress and transferring the case against it to the District of New Jersey. *Id.* at Dkt. 113.

64. Medchemexpress also moved to the dismiss the consolidated complaint under Fed. R. Civ. P. 12(b)(1), (2), and (6). No. 1:24-cv-07319, Dkt. 86, 87. The court "declined to reach the merits" of those motions after transferring the case to this District. *Id.* at Dkt. 113, p. 21, n. 10.

65. This Court has previously exercised declaratory judgment jurisdiction relating to claims of infringement of the ’051 Patent over entities seeking to make a generic version of Yondelis. *See, e.g.*, *Janssen Prods., L.P., et al. v. eVenus Pharms. Laby’s. Inc., et al.*, 1-20-cv-09369 (D.N.J.), Dkt. 32.

66. On information and belief, Medchemexpress is in possession of discovery relevant to infringement of the ’557 and ’051 Patents. On information and belief, discovery relating to the actions of Medchemexpress and its parent company, Haoyuan Chemexpress, is in the possession of Medchemexpress and is located in New Jersey. On information and belief, discovery of such information from Medchemexpress is critical in proving Plaintiffs’ claims.

67. Prior to initiating suit in the Northern District of Illinois, Plaintiffs requested basic information necessary to determine the roles of the parties in infringement of Plaintiffs’ patents, including for example, license and collaboration agreements between the EVER Defendants, the complete NDA, batch records, samples of the trabectedin API and proposed NDA Product, and the full DMFs Nos. 36724 and 36899, which are used to make the trabectedin API in the proposed NDA Product. To date, the requested information still has not been produced in the Northern District of Illinois Action. On information and belief, much of this discovery is in the possession or control Medchemexpress and located in New Jersey.

MEDCHEMEXPRESS’ INFRINGEMENT OF THE ’051 PATENT

68. On information and belief, Medchemexpress and the other EVER Defendants intend to use the processes claimed in the ’051 Patent to prepare the API, trabectedin, contained in the proposed NDA Product.

69. The processes claimed in the ’051 Patent are important for the commercial-scale manufacture of trabectedin. Prior art processes for manufacturing trabectedin are not commercially

feasible. The inventive processes claimed in the ’051 Patent were crucial to being able to make trabectedin available to vulnerable cancer patents. No generic drug manufacturer has been found not to infringe the ’051 Patent or to use a prior art process or any other process, despite claims to the contrary. *See* Stipulation and Order Regarding Infringement of U.S. Patent No 7,420,051 (Dkt. 104), *Janssen Prods., L.P., et al. v. eVenus Pharms. Laby’s Inc., et al.*, 1-20-cv-09369 (D.N.J. June 2, 2021); Stipulation and Order (Dkt. 445), *Janssen Prods., L.P., et al. v. eVenus Pharms. Laby’s Inc., et al.*, 1-20-cv-09369 (D.N.J. Dec. 22, 2022).

70. On information and belief, trabectedin is present in the proposed NDA Product without material change from trabectedin made by use of Plaintiffs’ patented processes.

71. On information and belief, trabectedin resulting from Plaintiffs’ patented processes is the API of the proposed NDA Product and, therefore, essential to the proposed NDA Product.

72. On information and belief, Medchemexpress submitted DMF Nos. 36724 and 36899 knowing that a process of making trabectedin that infringed the ’051 was and would actually be used to make trabectedin and that such trabectedin would be used, sold, offered for sale in the United States and/or imported into the United States, including for use as the API in the NDA Product.

73. Further, on information and belief, Medchemexpress presently sells and/or offers for sale in the United States trabectedin that is made by Plaintiffs’ patented processes. *See, e.g.*, https://www.medchemexpress.com/trabectedin-d3.html.

MEDCHEMEXPRESS’ INFRINGEMENT OF THE ’557 PATENT

74. In the PIV Letter, the EVER Defendants alleged non-infringement of the claimed commercial formulation of the ’557 Patent. In particular, the EVER Defendants alleged that their

proposed NDA Product uses L-arginine instead of a disaccharide as claimed in the ’557 Patent. Plaintiffs’ Yondelis® uses sucrose, a disaccharide.

75. Claim 1 of the ’557 Patent claims, “[a] lyophilised anti-tumor composition comprising a single active anti-tumor compound and a disaccharide selected from sucrose, lactose and a combination thereof, wherein the anti-tumor compound is ET-743 and wherein the disaccharide is present in a sufficient amount to inhibit conversion of the ET-743 into ET-701, such that the ET-743 composition comprises less than 2% ET-701 after storage of the ET-743 composition at 5° C for 3 months.” Medchemexpress and the other EVER Defendants do not dispute that the proposed NDA Product meets every claimed limitation other than the disaccharide limitation. Medchemexpress and the other EVER Defendants do not contest that their proposed product is “[a] lyophilized anti-tumor composition comprising a single active anti-tumor compound … wherein the anti-tumor compound is ET-743, trabectedin.” They also do not contest that their “ET-743 composition comprises less than 2% ET-701 after storage of the ET-743 composition at 5° C for 3 months.” They merely argue that they use L-arginine as opposed to sucrose “to inhibit conversion of the ET-743 into ET-701, such that the ET-743 composition comprises less than 2% ET-701 after storage of the ET-743 composition at 5° C for 3 months.” On information and belief, the ingredients in the proposed NDA Product perform substantially the same function, in substantially the same way, with substantially the same results as in the claims of the ’557 Patent.

76. On information and belief, the proposed NDA Product merely substitutes L-arginine for the specified disaccharide of, e.g., claims 1-8, 11-20 and 22-26 of the ’557 Patent. For example, both L-arginine and sucrose act to stabilize the trabectedin formulation by limiting conversion of ET-743 to ET-701 such that the composition has less than 2% ET-701 after storage

at 5° C or 25° C for 3 months. Medchemexpress and the other EVER Defendants do not dispute that the proposed NDA Product literally meets each of the other limitations of claims 1-8, 11-20 and 22-26 of the ’557 Patent.

77. On information and belief, Medchemexpress and the other EVER Defendants have not informed FDA that there are substantial differences between L-arginine and the disaccharide of claims 1-8, 11-20 and 22-26 of the ’557 Patent as relevant to the claims. Instead, Medchemexpress and the other EVER Defendants rely on Yondelis® as the reference product for approval of the NDA Product. On information and belief, NDA 219617 and the communications with FDA will support that ingredients in the proposed NDA Product perform substantially the same function, in substantially the same way, with substantially the same result as in the claims of the ’557 Patent.

78. On information and belief, Medchemexpress and the other EVER Defendants, had actual and constructive notice of the ’557 Patent prior to the submission of NDA 219617 seeking approval of the NDA Product. Indeed, their PIV Letter cites to and reflects knowledge of the prior Hatch-Waxman litigations involving the ’557 Patent and ’051 Patent.

79. On information and belief, as with the ’051 Patent, Medchemexpress and the other EVER Defendants had knowledge of the validity of the claims of the ’557 Patent. For example, publicly available documents show that eVenus and Hengrui are enjoined from infringing the ’557 Patent and have withdrawn their ANDA and Fresenius will never market their infringing product and cannot make use of the ANDA or supporting DMFs. Similarly, public records show that MSN stipulated to the validity of the claims of the ’557 Patent. *See* Stipulation and Order (Dkt. 445), *Janssen Prods., L.P., et al. v. eVenus Pharms. Laby’s Inc., et al.*, 1-20-cv-09369 (D.N.J. Dec. 22, 2022); Stipulated Order Regarding Infringement and Validity of U.S. Patent No. 8,895,557 and

Validity of U.S. Patent No. 7,420,051 (Dkt. 14), *Janssen Prods., L.P., et al. v. MSN Pharms. Inc., et al.*, 3-21-cv-14622 (D.N.J. Dec. 17, 2021).

80. On information and belief, Medchemexpress submitted DMF Nos. 36724 and 36899 with the knowledge and intent that the trabectedin is made by a process that infringes the ’051 Patent, that trabectedin will be imported into the United States as the API of the NDA Product, and with knowledge that such NDA Product infringes the ’557 Patent.

81. On information and belief, Medchemexpress continues to seek approval of NDA 219617 and take acts in furtherance of such approval, including through further acts, including submission of DMF Nos. 36724 and 36899 and NDA 219617, and communications with the FDA in furtherance of approval of DMF Nos. 36724 and 36899 and NDA 219617.

COUNT I

Infringement Of The ’557 Patent Under 35.U.S.C. § 271(b) and/or (e)(2)(A)

82. Plaintiffs repeat and reallege each and every allegation above as if fully set forth herein.

83. Medchemexpress has itself infringed the ’557 Patent under 35 U.S.C. §271(e)(2)(A), at least under the doctrine of equivalents, by in concert with the remaining EVER Defendants submitting NDA No. 219617 with a Paragraph IV certification and seeking FDA approval of NDA No. 219617 to market the proposed NDA Product prior to the expiration of the ’557 Patent.

84. Further, under 35 U.S.C. § 271(b), Medchemexpress has induced infringement of the ’557 Patent under § 271(e)(2)(A) by actively inducing the remaining EVER Defendants to submit NDA No. 219617 with a Paragraph IV certification and seeking FDA approval of NDA No. 219617 to market the proposed NDA Product prior to the expiration of the ’557 Patent,

knowing that the proposed NDA Product infringes claims 1-8, 11-20 and 22-26 of the ’557 Patent under the doctrine of equivalents and with the intent to cause such infringement.

85. On information and belief, Medchemexpress’ and the other EVER Defendants’ commercial manufacture, importation, use, sale and/or offer for sale of the proposed NDA Product prior to the expiration of the ’557 Patent would infringe, contribute to the infringement of, and/or induce the infringement of at least claims 1-8, 11-20 and 22-26 of the ’557 Patent under the doctrine of equivalents.

86. On information and belief, Medchemexpress has and will actively induce, encourage, aid, and abet that conduct with knowledge and specific intent that the conduct infringes the ’557 Patent.

87. Through at least the foregoing actions, Medchemexpress has and will actively induce the infringement of at least one claim of the ’557 Patent under 35 U.S.C. § 271(b).

88. Medchemexpress had actual and constructive notice of the ’557 Patent prior to filing NDA No. 219617 seeking approval for the proposed NDA Product, or at the very least, as of August 15, 2024 – the date of the first complaint in the Northern District of Illinois.

89. Plaintiffs have no adequate remedy at law to redress the infringement by Medchemexpress.

90. Plaintiffs will be irreparably harmed if Medchemexpress is not enjoined from infringing or actively inducing or contributing to infringement of the ’557 Patent.

COUNT II

Declaratory Judgment Of Infringement Of The ’557 Patent Under 35.U.S.C. § 271(a), (b) and/or (c)

91. Plaintiffs repeat and reallege each and every allegation contained above as if fully set forth herein.

92. A definite and concrete, real and substantial, justiciable controversy of sufficient immediacy and reality exists between Plaintiffs and Medchemexpress regarding infringement of the ’557 Patent.

93. On information and belief, Medchemexpress and the remaining EVER Defendants have made and will continue to make substantial and meaningful preparations to commercially manufacture, import into the U.S. and/or to offer to sell, sell, and/or use within the U.S. the proposed NDA Product prior to the expiration of the ’557 Patent.

94. The PIV Letter admits that the EVER Defendants will engage in the commercial manufacture, use and/or sale of the proposed NDA Product if approved by the FDA.

95. Medchemexpress’ and the other EVER Defendants’ actions, including, but not limited to, the submission of NDA 219617 with a Paragraph IV certification, submission of DMF Nos. 36724 and 36899 in support of NDA 219617 and in advance of its submission, and their systematic attempts to meet the applicable regulatory requirements for approval of NDA 219617, along with the supporting DMFs, indicate a refusal to change their course of action and intent to launch imminently.

96. FDA issued a tentative approval of NDA 219617 on or around April 9, 2025.

97. Medchemexpress and the EVER Defendants have indicated their intent to launch the NDA Product as soon as possible.

98. Medchemexpress’ and the EVER Defendants’ commercial manufacture, importation, use, sale and/or offer for sale of the proposed NDA Product prior to the expiration of the ’557 Patent would infringe, contribute to the infringement of, and/or induce the infringement of at least claims 1-8, 11-20 and 22-26 of the ’557 Patent under §§ 271(a), (b) and/or (c) under the doctrine of equivalents.

99. On information and belief, Medchemexpress will actively induce, encourage, aid, and abet that conduct with knowledge and specific intent that the conduct infringes the ’557 Patent.

100. Through at least the foregoing actions, Medchemexpress will actively induce the infringement of at least one claim of the ’557 Patent under 35 U.S.C. § 271(b).

101. Plaintiffs should be granted a judicial declaration that the commercial manufacture, importation, use, offer for sale, and/or sale in the U.S. of the proposed NDA Product will constitute infringement of the claims of the ’557 Patent under §§ 271(a), (b) and/or (c) under the doctrine of equivalents.

102. Plaintiffs have no adequate remedy at law to redress infringement by Medchemexpress.

103. Plaintiffs will be irreparably harmed if Medchemexpress is not enjoined from infringing or actively inducing or contributing to infringement of the ’557 Patent.

COUNT III

Infringement Of The ’051 Patent Under 35.U.S.C. § 271(a) and/or (g)

104. Plaintiffs repeat and reallege each and every allegation above as if fully set forth herein.

105. On information and belief, Medchemexpress presently makes, sells, and/or offers to sell within the United States, or imports into the United States, the API trabectedin that is prepared using the processes claimed in the ’051 Patent.

106. Medchemexpress’ manufacture, sale, offer for sale, and/or use within the United States, and/or importation into the United States of trabectedin before the expiration of the ’051

Patent has infringed and will infringe one or more claims of the ’051 Patent under 35 U.S.C. § 271(a) and/or (g).

107. Plaintiffs are entitled to injunctive relief prohibiting Medchemexpress from making, selling, offering to sell, and using within the United States, and importing into the United States, trabectedin, including as an API contained in the proposed NDA Product, before the expiration of the ’051 Patent.

108. Plaintiffs do not have an adequate remedy at law.

109. Medchemexpress’ manufacture sale, offer for sale, and/or use within the United States, and/or importation into the United States, of the API, trabectedin, including that contained in the proposed NDA Product, before the expiration of the ’051 Patent has and will cause Plaintiffs injury, entitling Plaintiffs to damages under 35 U.S.C. § 284.

COUNT IV

Declaratory Judgment Of Infringement Of The ’051 Patent Under 35.U.S.C. § 271(g)

110. Plaintiffs repeat and reallege each and every allegation above as if fully set forth herein.

111. A definite and concrete, real and substantial, justiciable controversy of sufficient immediacy and reality exists between Plaintiffs and Medchemexpress regarding infringement of the ’051 Patent.

112. On information and belief, Medchemexpress, alone and in collaboration with the remaining EVER Defendants, has made and will continue to make substantial and meaningful preparations to import into the U.S. or offer to sell, sell, and/or use within the U.S. the NDA Product – a product which is made by a process patented by the ’051 Patent prior to its expiration.

113. The PIV Letter admits that Medchemexpress and the other EVER Defendants will engage in the commercial manufacture, use and/or sale of the proposed NDA Product if approved by the FDA.

114. Claims relating to the ’051 Patent were dismissed without prejudice from the case proceeding in the Northern District of Illinois. There, the remaining EVER Defendants indicated their intent to market the NDA Product immediately upon FDA approval.

115. On information and belief, the FDA assesses and acts on NDAs within ten to twelve months of submission. On information and belief, there is immediacy and imminent infringement of the ’051 Patent, because FDA issued tentative approval of NDA 219617 on or around April 9, 2025.

116. On information and belief, Medchemexpress and the other EVER Defendants have or will begin importing API and/or the NDA Product to stockpile in anticipation of FDA final approval. In doing so, Medchemexpress and the other EVER Defendants will import trabectedin made using the process of Plaintiffs’ ’051 Patent, and will therefore infringe the ’051 Patent. Medchemexpress’ infringement of the ’051 Patent is therefore imminent.

117. Medchemexpress’ and the other EVER Defendants’ actions, including, but not limited to, the submission of NDA No. 219617 with a Paragraph IV certification, submission of DMF Nos. 36724 and 36899 in support of NDA 219617 and in advance of its submission, and the EVER Defendants’ systematic attempts to meet the applicable regulatory requirements for approval of NDA No. 219617, including approval of the supporting DMFs, indicate a refusal to change their course of action and intent to launch imminently.

118. On information and belief (including Medchemexpress’ and the other EVER Defendants’ failure to produce requested manufacturing information and the fact that they have

not provided any scientifically plausible basis to contest infringement of the claims of the ’051 Patent, including for at least claims 12-14), Medchemexpress’ and the other EVER Defendant’s importation, use, sale, and/or offer for sale of the proposed NDA Product prior to the expiration of the ’051 Patent would infringe, contribute to the infringement of, and/or induce the infringement of one or more claims of the ’051 Patent under 35 U.S.C. § 271(g), including at least claims 12-14.

119. On information and belief, Medchemexpress had actual and constructive notice of the ’051 Patent prior to the filing of NDA No. 219617 seeking approval of the proposed NDA Product, or at the very least as of August 15, 2024 – the filing date of the first complaint in the Northern District of Illinois.

120. On information and belief, Medchemexpress’ infringement of the ’051 Patent would be willful.

121. Plaintiffs should be granted a judicial declaration that the importation into the U.S. and/or use, offer for sale, and/or sale in the U.S. of trabectedin in the proposed NDA Product will constitute infringement of the claims of the ’051 Patent under 35 U.S.C. § 271(g).

122. Plaintiffs have no adequate remedy at law to redress infringement by Medchemexpress.

123. Plaintiffs will be irreparably harmed if Medchemexpress is not enjoined from infringing or actively inducing or contributing to infringement of the ’051 Patent.

PRAYER FOR RELIEF

WHEREFORE, Plaintiffs respectfully request that the Court grant the following relief:

(a) a judgment that Medchemexpress has infringed the ’557 Patent under 35 U.S.C. § 271(b) and/or (e)(2)(A);

(b) a judgment, pursuant to 35 U.S.C. § 271(e)(4)(A), that the effective date of any FDA approval of NDA 219617 under § 505(b)(2) of the Federal Food, Drug, and Cosmetic Act (21 U.S.C. § 355(b)) is not earlier than the day after the expiration of the ’557 Patent, including any additional exclusivity period applicable to the patent;

(c) a judgment declaring that the making, using, selling, offering to sell, or importing of the trabectedin 1 mg/vial described in NDA 219617 would constitute infringement of the ’557 Patent, and/or inducement and/or contribution to such conduct, by Medchemexpress pursuant to 35 U.S.C. § 271(a), (b) and/or (c);

(d) a judgment permanently enjoining Medchemexpress and each of its officers, agents, servants and employees, and those persons in active concert or participation with them, from commercially manufacturing, selling or offering for sale, using, or importing the trabectedin 1 mg/vial described in NDA 219617, or any colorable variations thereof, until the day after the expiration of the ’557 Patent, including any additional exclusivity period applicable to the ’557 Patent, and from otherwise infringing one or more claims of the ’557 Patent;

(e) a judgment declaring that making, importing, selling, offering to sell, or using trabectedin constitutes infringement of the ’051 Patent, and/or inducement or contribution to such conduct, by Medchemexpress pursuant to 35 U.S.C. § 271(a) and (g);

(f) a judgment declaring that importing, selling, offering to sell, or using the trabectedin 1 mg/vial described in NDA 219617 would constitute infringement of the ’051 Patent, and/or inducement and/or contribution to such conduct, by Medchemexpress pursuant to 35 U.S.C. § 271(g);

(g) a declaration that Medchemexpress would willfully infringe the ’051 Patent;

(h) a judgment permanently enjoining Medchemexpress and each of their officers, agents, servants and employees, and those persons in active concert or participation with them, from commercially importing, selling, offering for sale, or using the trabectedin 1 mg/vial described in NDA No. 219617, or any trabectedin product that is made by any colorable variation of the processes used to make the proposed NDA Product, until after the expiration of the ’051 Patent, including any additional exclusivity period applicable to the ’051 Patent, and from otherwise infringing one or more claims of the ’051 Patent;

(i) a judgment permanently enjoining Medchemexpress and each of their officers, agents, servants and employees, and those persons in active concert or participation with them, from commercially importing, selling, offering for sale, or using trabectedin or any trabectedin product that is made by any colorable variation of the processes claimed in the ’051 Patent, until after the expiration of the ’051 Patent, including any additional exclusivity period applicable to the ’051 Patent, and from otherwise infringing one or more claims of the ’051 Patent;

(j) an award of damages under 35 U.S.C. § 284 for Medchemexpress’ infringement of the ’051 Patent;

(k) a declaration that this case is exceptional;

(l) an award of Plaintiffs’ costs, expenses, reasonable attorneys’ fees, and such other relief as the Court deems just and proper pursuant to 35 U.S.C. § 271(e)(4) and 35 U.S.C. § 285; and

(m) such other and further relief as the Court may deem just and proper.

Dated: April 21, 2025

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EXHIBIT A

US008895557B2

(12) **United States Patent**
Beijnen et al.

(10) **Patent No.: US 8,895,557 B2**
(45) **Date of Patent: Nov. 25, 2014**

(54) **PHARMACEUTICAL FORMULATIONS OF ECTEINASCIDIN COMPOUNDS**

(75) Inventors: **Jacob Hendrik Beijnen**, Amsterdam (NL); **Bastiaan Nuijen**, Amsterdam (NL); **Pilar Calvo Salve**, Madrid (ES); **Maria Tobio Barreira**, Madrid (ES)

(73) Assignee: **Pharma Mar, S.A., Sociedad Unipersonal**, Madrid (ES)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 801 days.

(21) Appl. No.: **11/261,876**

(22) Filed: **Oct. 28, 2005**

(65) **Prior Publication Data**

US 2006/0094687 A1 May 4, 2006

Related U.S. Application Data

(60) Provisional application No. 60/623,813, filed on Oct. 29, 2004.

(51) **Int. Cl.**

A61K 31/50	(2006.01)
A61K 31/495	(2006.01)
A61K 31/715	(2006.01)
A61K 47/26	(2006.01)
A61K 47/18	(2006.01)
A61K 47/32	(2006.01)
A61K 47/02	(2006.01)
A61K 9/19	(2006.01)
A61K 31/498	(2006.01)
A61K 31/4995	(2006.01)
A61K 9/00	(2006.01)

(52) **U.S. Cl.**

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USPC **514/249**; 514/250; 514/53

(58) **Field of Classification Search**

USPC 514/249, 250

See application file for complete search history.

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(57) **ABSTRACT**

Ecteinascidin formulations, methods of preparing the same, articles of manufacture and kits with such formulations, and methods of treating proliferative diseases with the same formulations are provided.

26 Claims, 5 Drawing Sheets

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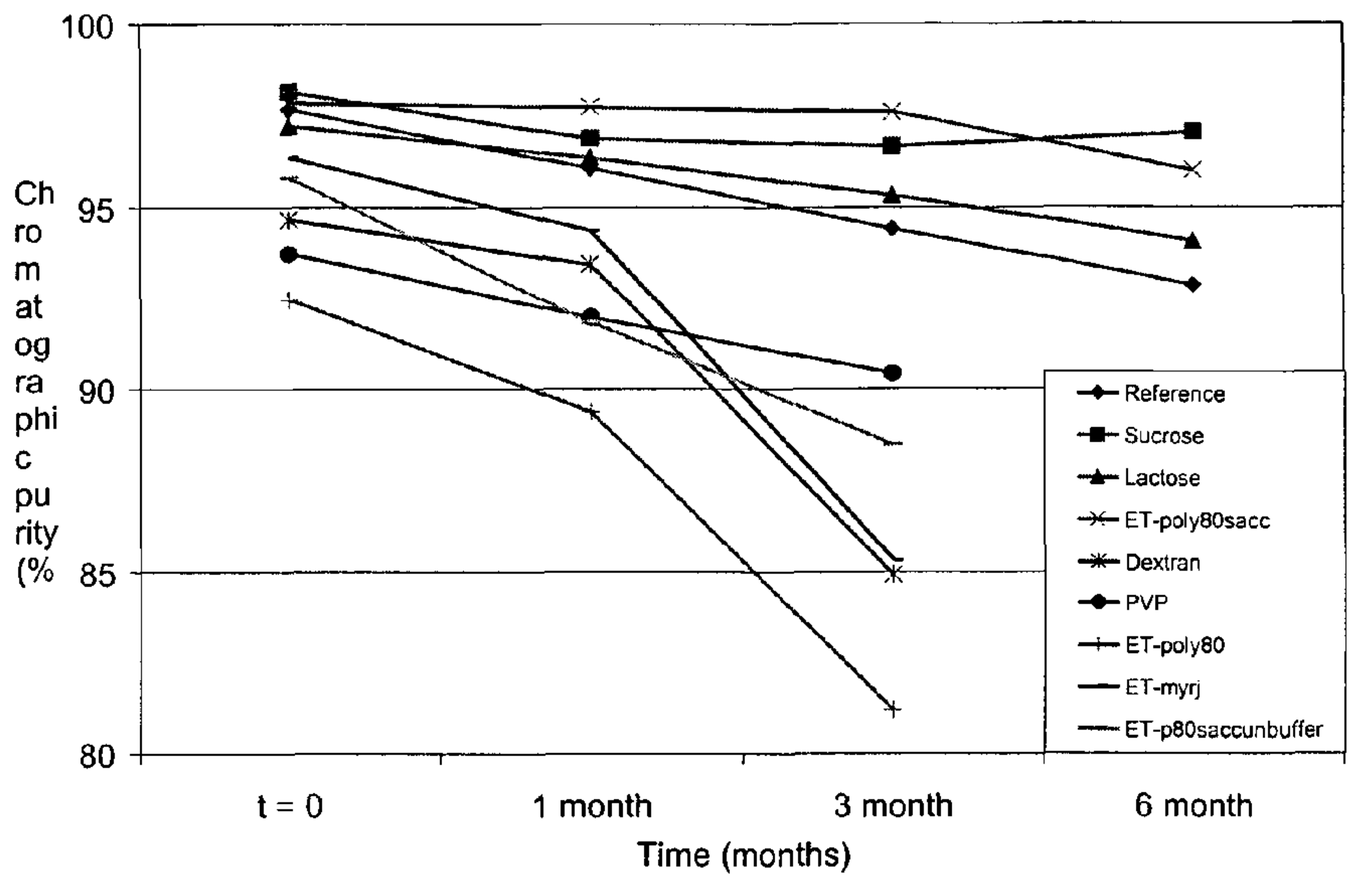


Figure 1

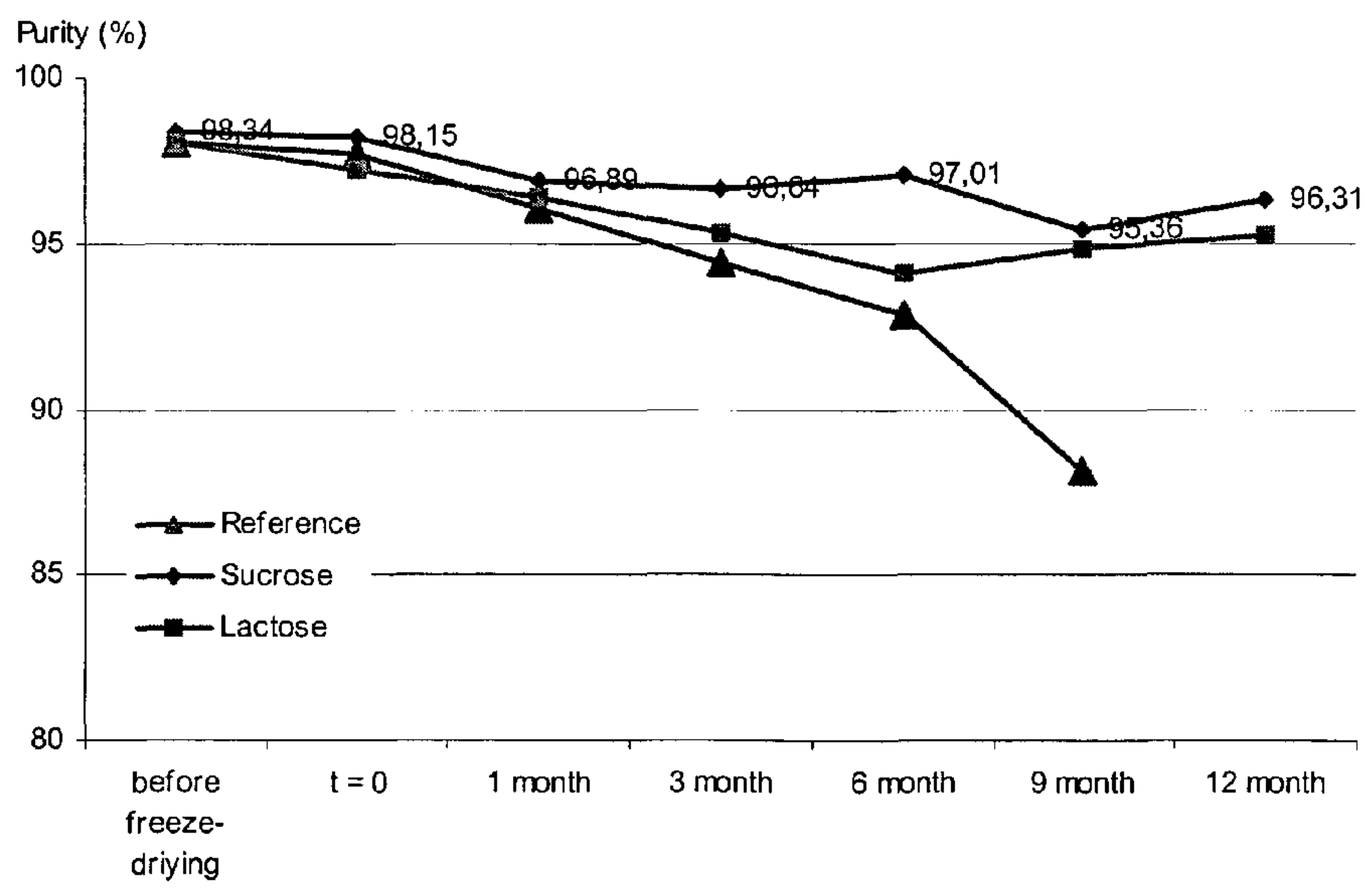


Figure 2

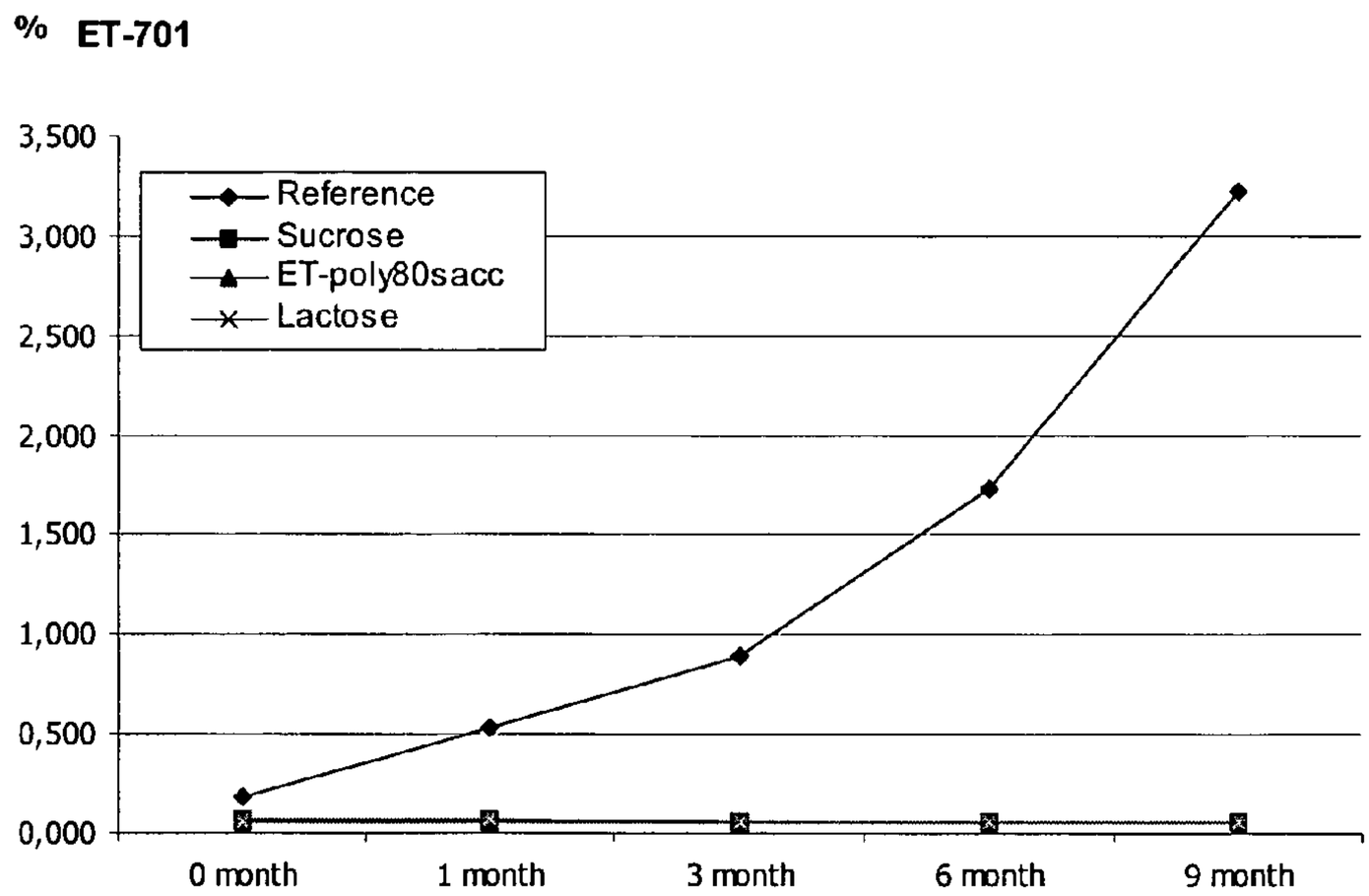


Figure 3

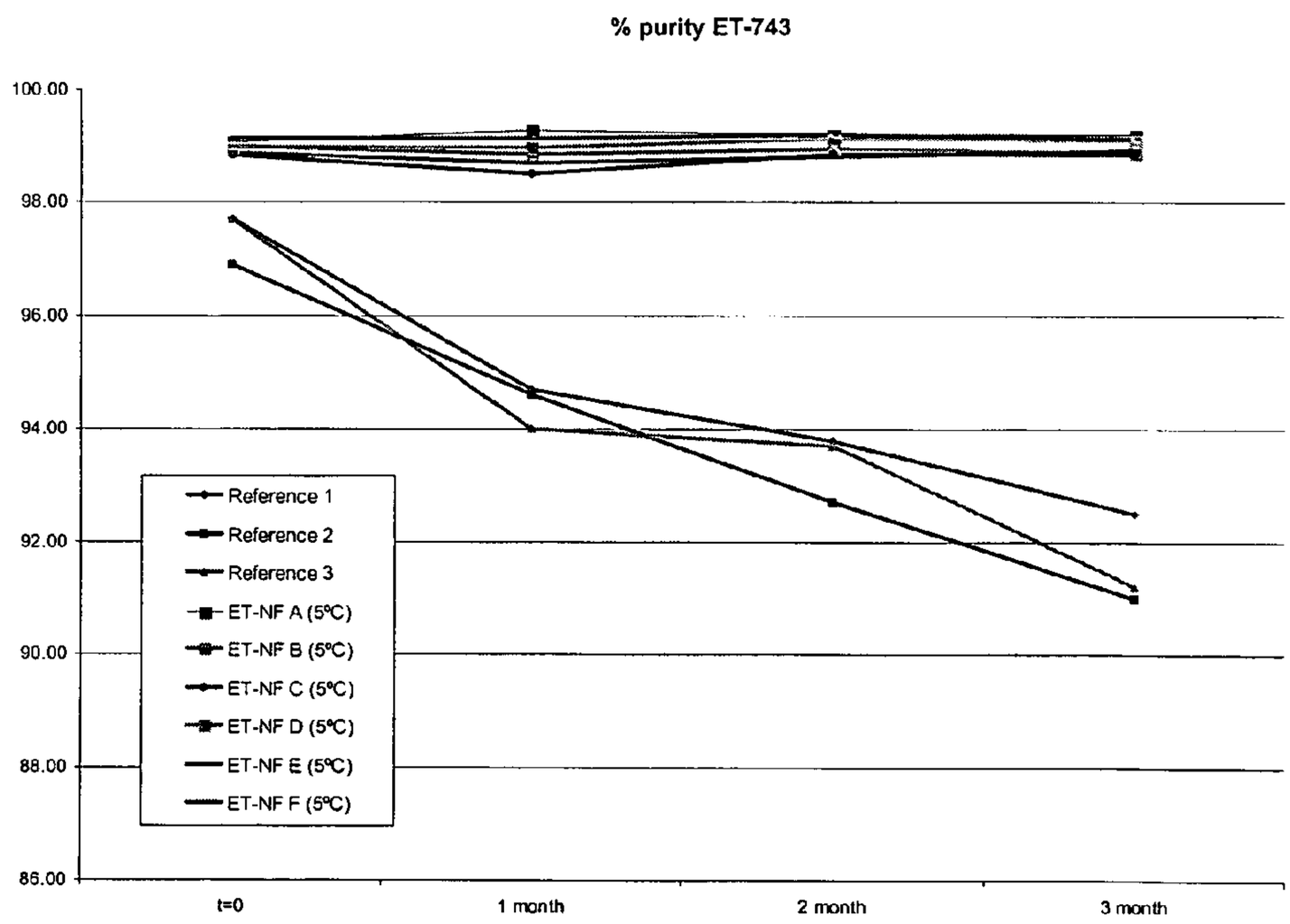


Figure 4

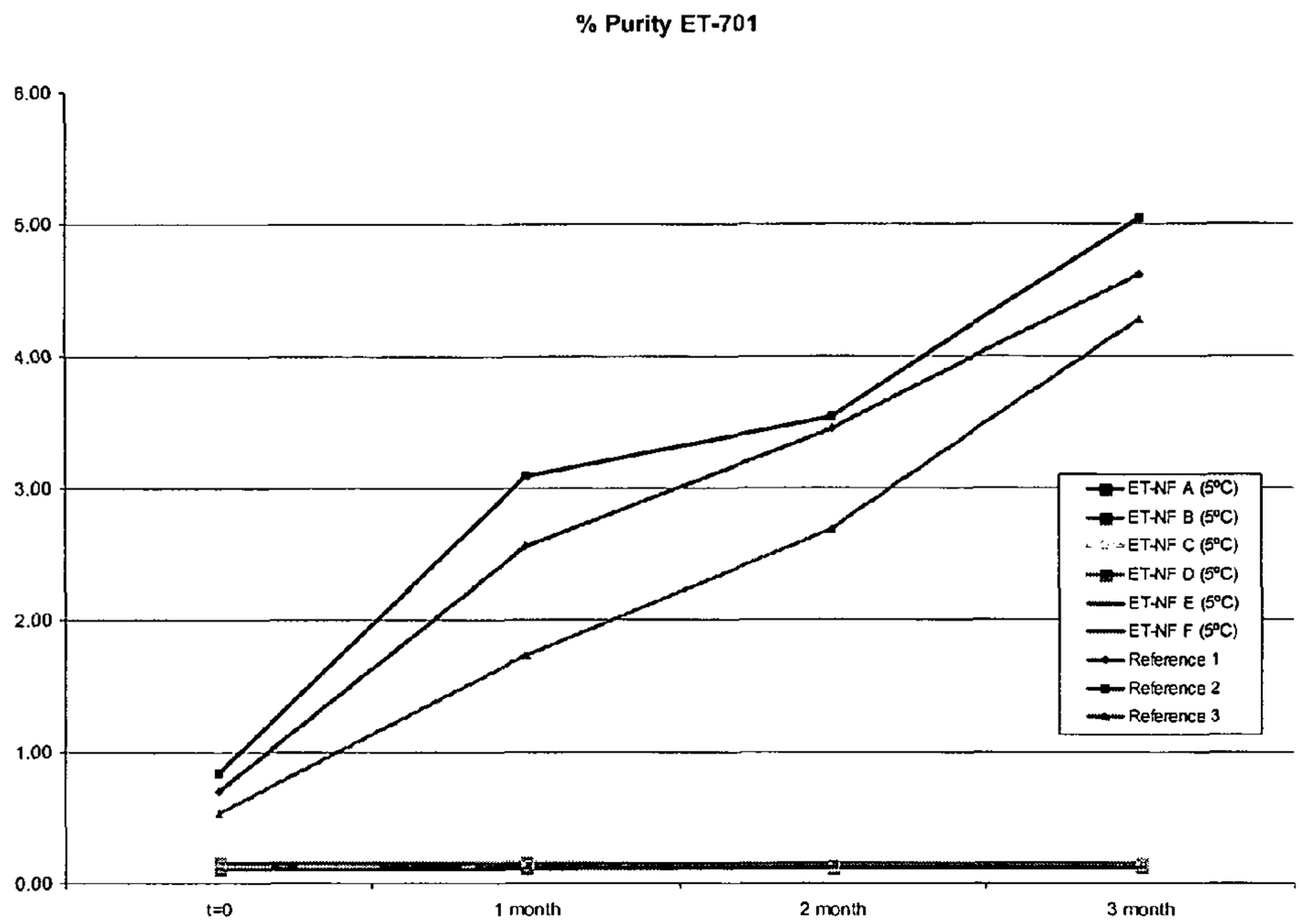
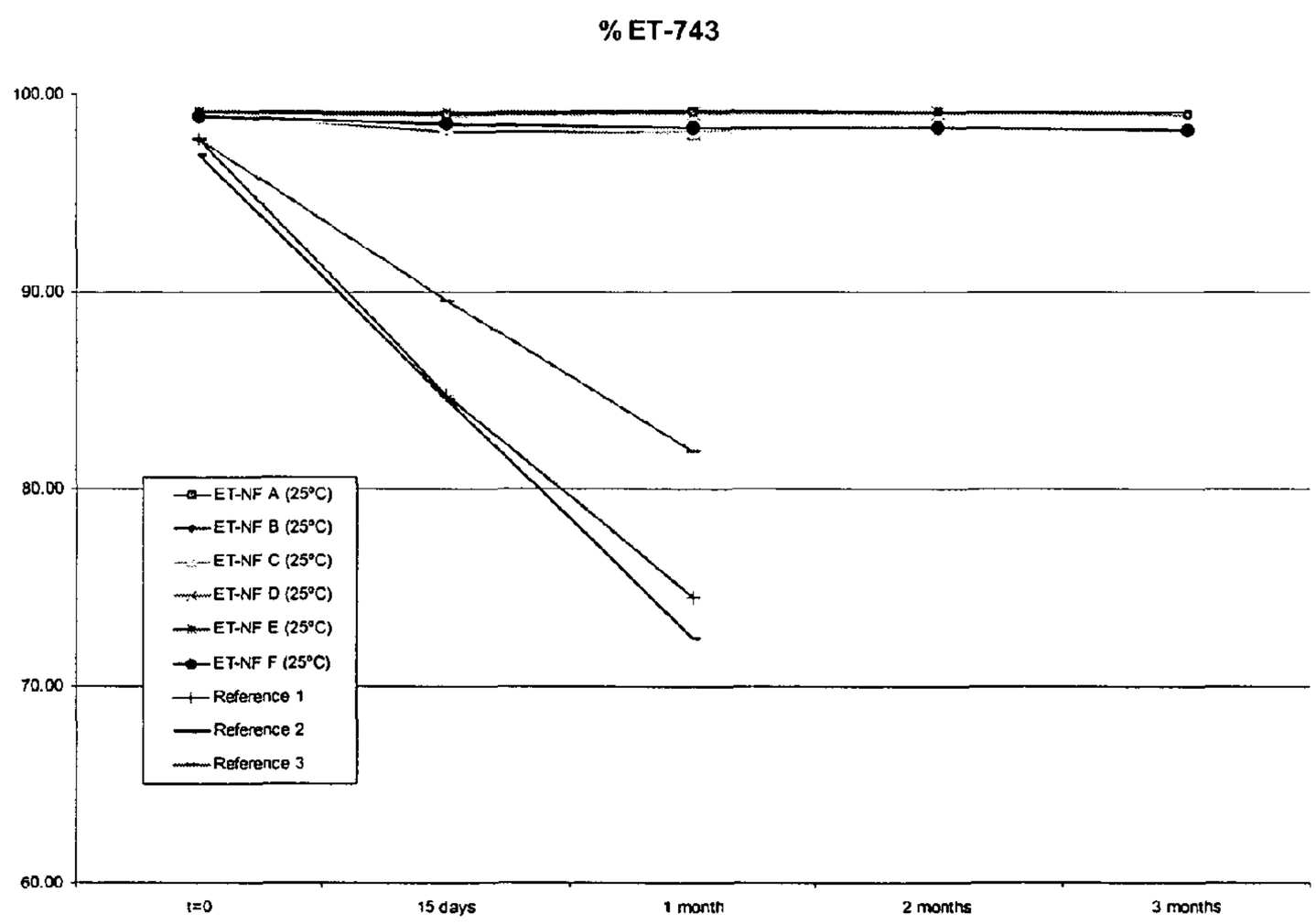


Figure 5



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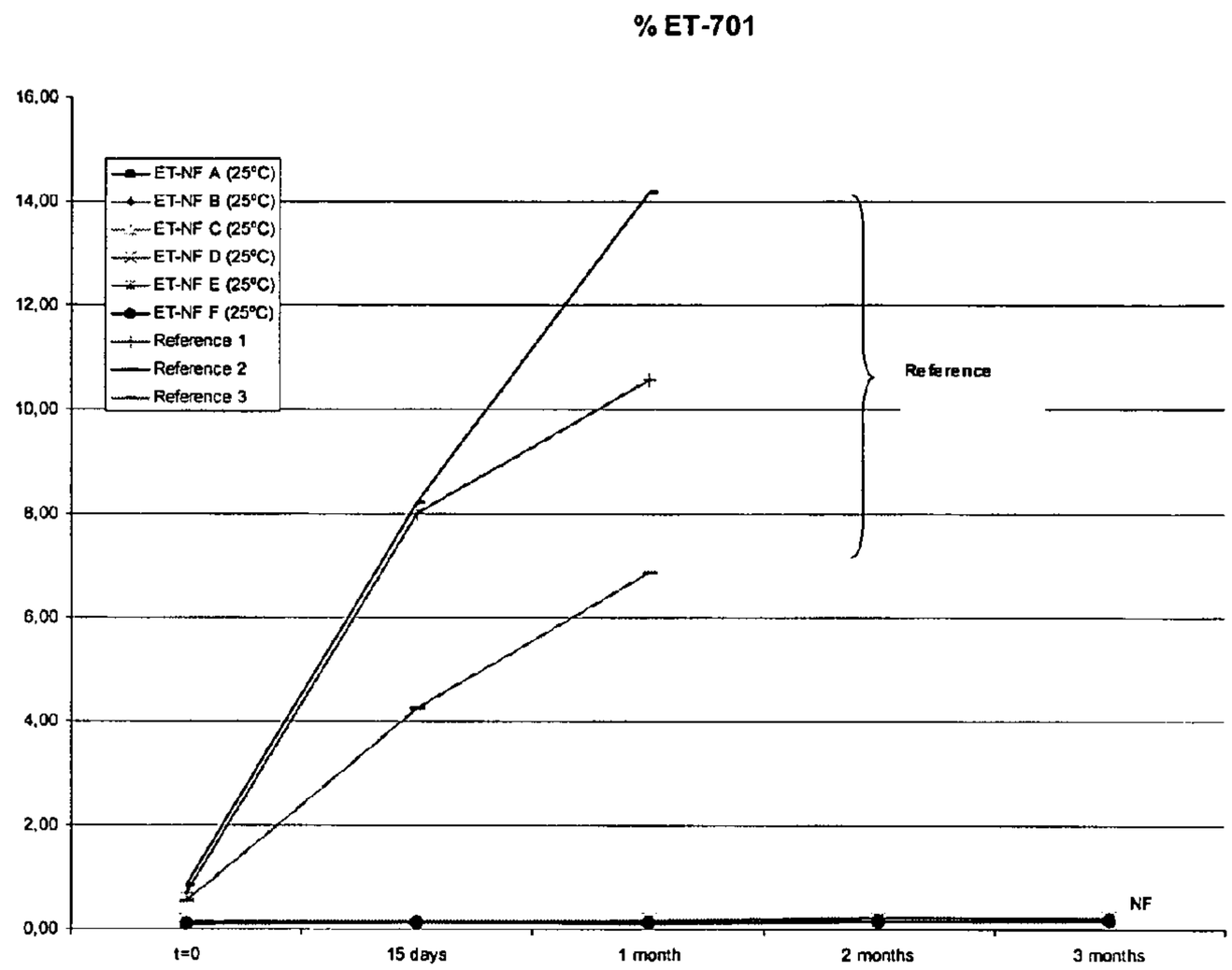


Figure 7

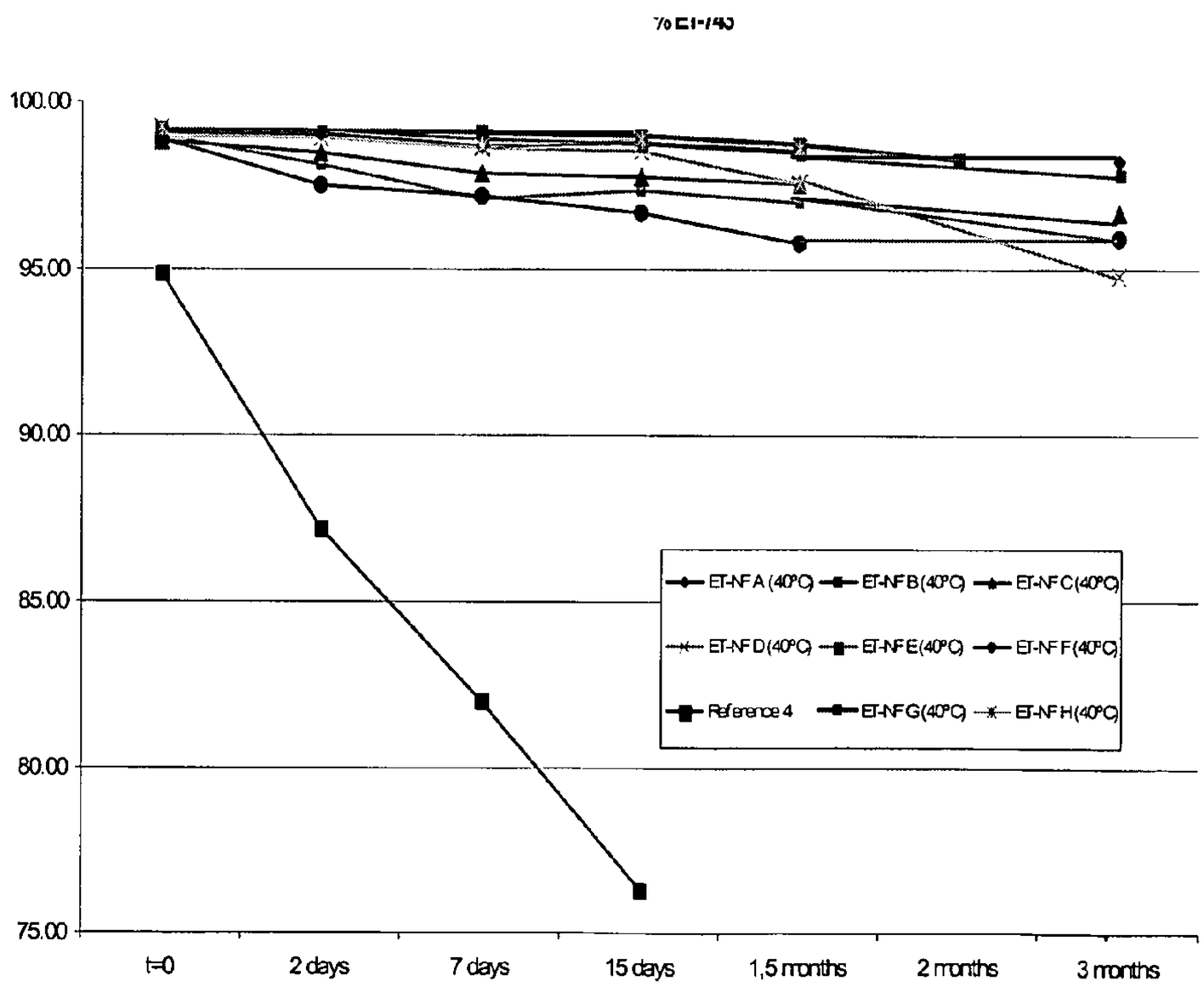


Figure 8

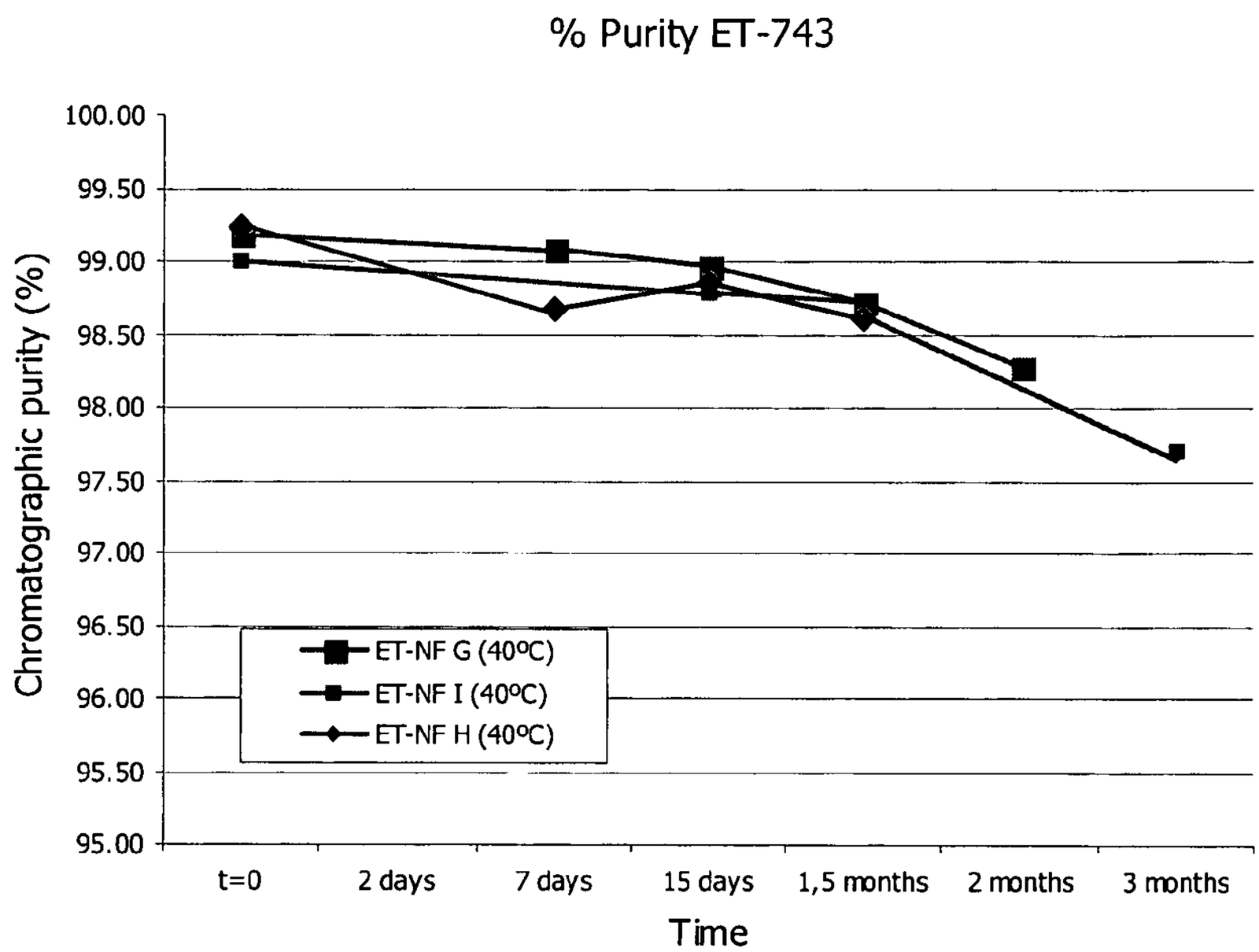

% Purity ET-743
Chromatographic purity (%)
100.00
99.50
99.00
98.50
98.00
97.50
97.00
96.50
96.00
95.50
95.00
ET-NF G (40ºC)
ET-NF I (40ºC)
ET-NF H (40ºC)
t=0
2 days
7 days
15 days
1,5 months
2 months
3 months
Time

Figure 9

PHARMACEUTICAL FORMULATIONS OF ECTEINASCIDIN COMPOUNDS

RELATED APPLICATIONS

Priority is claimed under 35 U.S.C. §119(e) for U.S. Provisional Application Ser. No. 60/623,813, filed Oct. 29, 2004. The entire disclosure of the priority application is considered as being part of the disclosure and is incorporated herein by reference.

The present invention relates to formulations. More particularly it relates to compositions and formulations of ecteinascidins, such as ecteinascidin 743.

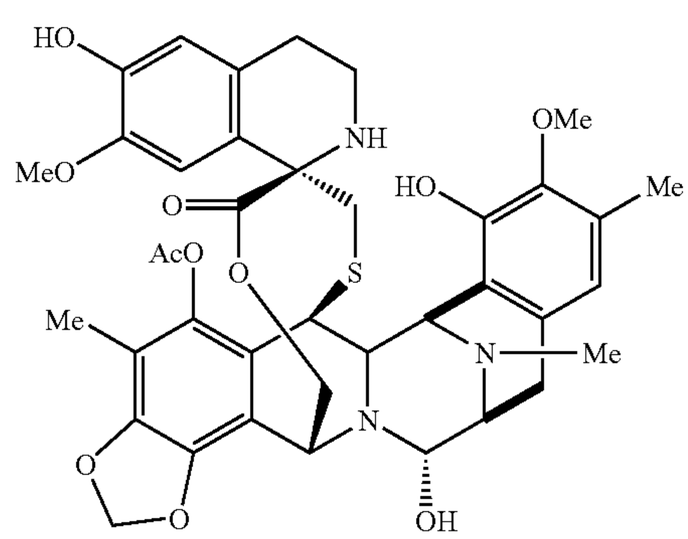
BACKGROUND OF THE INVENTION

Ecteinascidins have been identified, structurally characterized and synthetic methods for making them have been described. See for example, R. Sakai, et al., 1992, *Prco. Natl. Acad. Sci. USA* 89, pages 11456-11460, "Additional antitumor ecteinascidins from Caribbean tunicate: Crystal structures and activities in vivo"; R. Menchaca, et al., 2003, *J. Org. Chem.* 68(23), pages 8859-8866, "Synthesis of natural ecteinascidins (ET-729, ET-745, ET-759B, ET-736, ET-637, ET-594) from cyanosafracin B"; and I. Manzanares, et al., 2001, *Curr. Med. Chem.—Anti-Cancer Agents,* 1, pages 257-276, "Advances in the Chemistry and Pharmacology of Ecteinascidins, A Promising New Class of Anticancer Agents"; and references therein. These references describe ecteinascidins. Examples of ecteinascidins are provided by ET-743, ET-729, ET-745, ET-759A, ET-759B, ET-759C, ET-770, ET-815, ET-731, ET-745B, ET-722, ET-736, ET-738, ET-808, ET-752, ET-594, ET-552, ET-637, ET-652, ET-583, ET-597, ET-596, ET-639, ET-641, and derivatives thereof, such as acetylated forms, formylated forms, methylated forms, and oxide forms, such as N-oxide forms.

The structural characterizations of such ecteinascidins are not given again explicitly herein because from the detailed description provided in such references and citations therein; any person of ordinary skill in this technology is capable of obtaining such information directly from the sources cited here and related sources.

At least one of the ecteinascidin compounds, ET-743, has been extensively studied, and it will be referred to specifically herein to illustrate features of this invention.

Ecteinascidin 743 (ET-743) is a tetrahydroisoquinoline alkaloid isolated from the marine tunicate *Ecteinascidia turbinata* and has the following structure:



ET-743

A pharmaceutical composition comprising ET-743 in combination with a pharmaceutically acceptable carrier, diluent or excipient is claimed in U.S. Pat. No. 5,256,663.

A recent review of ET-743, its chemistry, mechanism of action and preclinical and clinical development can be found in van Kesteren, Ch. et al., 2003, *Anti-Cancer Drugs,* 14 (7), pages 487-502: "Yondelis (trabectedin, ET-743): the development of an anticancer agent of marine origin", and references therein.

ET-743 possesses potent antineoplastic activity against a variety of human tumour xenografts grown in athymic mice, including melanoma and ovarian and breast carcinoma.

In clinical phase I studies of ET-743, promising responses were observed in patients with sarcoma and breast and ovarian carcinoma. Therefore this new drug is currently under intense investigation in several phase II clinical trials in cancer patients with a variety of neoplastic diseases.

As it is explained in WO 0069441, incorporated in full by reference, ET-743 is supplied and stored as a sterile lyophilised product, having ET-743, mannitol and a phosphate buffer. A preferred formulation is one obtained from 0.9% sodium chloride or other suitable infusion vehicle, 250 μg of ET-743 with 250 mg of mannitol, 34 mg of monopotassium phosphate, and phosphoric acid to adjust the pH. This formulation is then reconstituted and diluted for intravenous injection.

ET-743 is a complex chemical entity, as revealed by its structural features. In addition, ET-743 exhibits limited aqueous solubility, and its stability, particularly in biocompatible forms and formulations, is difficult to predict and achieve. These characteristics challenge the ordinary skills and conventional methodologies in this technology, particularly when it comes to the preparation of ET-743 formulations that are to be readily used for medical purposes. Such uses preferably rely on formulations whose characteristics include one or more of the following: biocompatibility, stability under ambient conditions, or under conditions that are as near to ambient conditions as possible, with a shelf life that is as long as possible, and easy reconstitutability to form reconstituted solutions that are as stable under ambient, or near ambient conditions, for as long as possible.

However, conventional formulations and methodologies for preparing such formulations do not provide desirable features and characteristics such as those referred to above. For example, the cited review of 2003 by van Kesteren Ch. et al. reports that

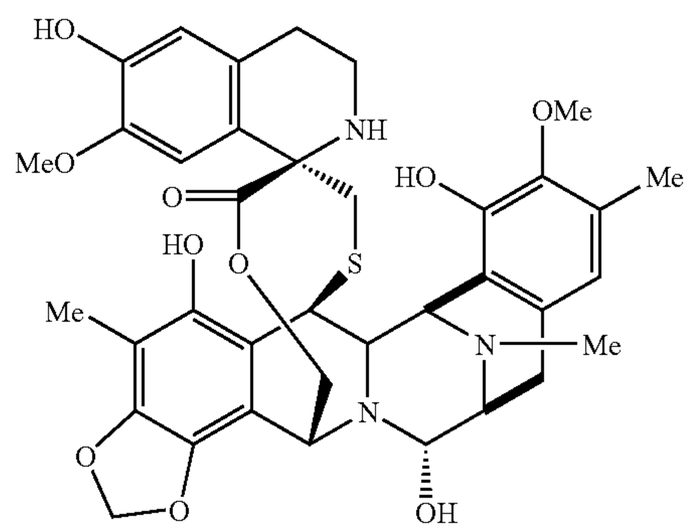
> ET 743 has limited aqueous solubility. However, by adjustment of the pH to 4, adequate concentrations of ET 743 could be reached. Instability of ET 743 in aqueous solution necessitated lyophilization in order to increase the storage stability of the pharmaceutical product. ET-743 is currently formulated as a sterile lyophilized product containing 250 pg active substance per dosage unit, 250 mg mannitol as a bulking agent and 0.05 M phosphate buffer at pH 4 in order to solubilize ET-743. This formulation is unstable with long-term storage at refrigerated and room temperature, and should therefore be stored between −15 and −25° C., protected from light. Reconstitution is performed by adding 5 ml Water for Injection, with subsequent dilution in normal saline before i.v. infusion. The reconstituted solution is stable at ambient temperature for up to 24 h.

In practice this product containing 250 μg of ET-743 is manufactured by freeze-drying 5 ml of solution containing ET-743, mannitol, phosphate buffer and water in a moulded vial. Moulded vials containing 1 mg ET-743 are also manufactured by freeze-drying 20 ml of the solution.

Freeze-drying typically involves freezing the solution, reducing the pressure for a period of primary drying to remove water vapour from the frozen material by sublimation and give a semi-dried mass, and increasing the temperature for a period of secondary drying to remove residual water from the semi-dried mass. The vials are then sealed.

The above-described conventional ET-743 formulation suffers from several disadvantages. One of them is that the lyophilised ET-743 formulation has to be stored at about −20° C. to prevent decomposition of the ET-743 in order to achieve a shelf life of at least 18 months.

In addition, ET-743 formulations face the problem of formation of relatively large amounts of ET-701 as impurity. ET-701 is the main impurity produced during the lyophilisation process and during storage of the ET-743 formulation. It comes from the hydrolysis of ET-743 and has the following structure:



ET-701

Formation of impurities, however, diminishes or even forestalls the ability to standardize formulations. It is consequently desirable to provide formulations and methods for making the same that provide embodiments whose composition does not readily and unpredictably change by the uncontrolled formation of impurities.

Furthermore, another disadvantage of the above-described conventional ET-743 formulation methodology is that in order to obtain the lyophilised formulation it is necessary to freeze-dry a relatively large amount of solution with fill volumes in the order of 5 to 20 ml. In contrast, it would be desirable to develop a manufacturing methodology for formulations with compounds as complex as ET-743 that permits the making of formulations with higher active substance concentrations, so that the volumes to be handled are consequently reduced. Time and energy are needed in conventional methods for the step of freeze-drying, in view of the relatively high fill volumes of 5 or 20 ml. Along with the time and energy, there is also the risk of decomposition of the ET-743, particularly in the secondary drying.

In view of the potential of ET-743 formulations as antitumoral agents, there is a need to provide a formulation that can solve problems that conventional formulations and manufacturing methodologies do not address or do not completely solve. These problems include the problem of stability of ET-743. Embodiments of ET-743 formulations should preferably exhibit favourable freeze-drying properties, should preferably be susceptible of ready reconstitution, and they should preferably exhibit dilution properties, such as upon dilution with infusion fluid, while presenting as many as the desirable characteristics of formulations for medical use as referred to herein. As indicated above, embodiments of ET-743 formulations should be stable during long term storage. In addition, the formulation and its manufacturing methodology should satisfy biocompatibility standards and should thus allow for the effective use of a formulation vehicle that is non-toxic, at least at the concentrations used for infusion.

A general review of excipient-drug interactions in parental formulations is provided by Akers, M J, in Journal of Pharmaceutical Sciences, 91, 2002, 2283-2300. This reference provides, inter alia, a section on bulking agents and lyoprotectants, including this subject matter in the context of lyophilisation.

It is envisaged that the methodologies and formulations developed in the context of this invention are applicable to other ecteinascidins, in addition to ET-743.

OBJECTS OF THE INVENTION

It is an object of this invention to provide stable formulations of ecteinascidins, and methods of making such formulations.

It is a specific object of this invention to provide a new stable formulation of ET-743. In particular, a formulation is needed which has greater storage stability. There is especially a need to avoid the formation of impurities. In particular, it is desirable to provide embodiments of formulations that are substantially free of ET-701.

Furthermore, other objects of this invention concern the development of manufacturing methodologies that permit the preparation of ET-743 formulations with ET-743 concentrations that are higher than those achieved by conventional means. Additional objects concern the development of processes for improving the solubility of chemical entities as complex as ET-743, eventually increasing the ET-743 concentration in the solution for lyophilising, and thus reducing the fill volume in the vials before lyophilising the formulation.

SUMMARY OF THE INVENTION

According to the present invention there is provided ET-743 compositions which comprise ET-743 and a disaccharide, and methods for preparing such compositions. Preferred embodiments of such compositions are of pharmaceutical purity.

Other embodiments of this invention are provided by compositions that comprise an ecteinascidin and a disaccharide.

Some embodiments of such compositions are provided by lyophilised formulations which comprise an ecteinascidin such as ET 743 and a disaccharide. Methods for preparing such formulations are provided.

The invention provides methods of reducing, or even substantially eliminating, the formation of impurities in ET-743 formulations. Some embodiments include methodology for reducing, or even substantially eliminating, ET-701 formation in ET-743 formulations.

The invention also provides methodology for more effectively handling formulations of an ecteinascidin such as ET-743, including methods for making higher concentration formulations and methods for reducing the fill volume of a vial when producing a lyophilised formulation.

The present invention also provides methods of solubilising complex chemical entities, such as ecteinascidins, including but not limited to ET-743. Such methods allow for the manufacturing of a more concentrated solution of ET-743 in bulk solution for lyophilising, leading to reduced fill volumes.

DETAILS OF THE INVENTION

We have found in the context of this invention that disaccharides stabilize ecteinascidin formulations. Ecteinascidins,

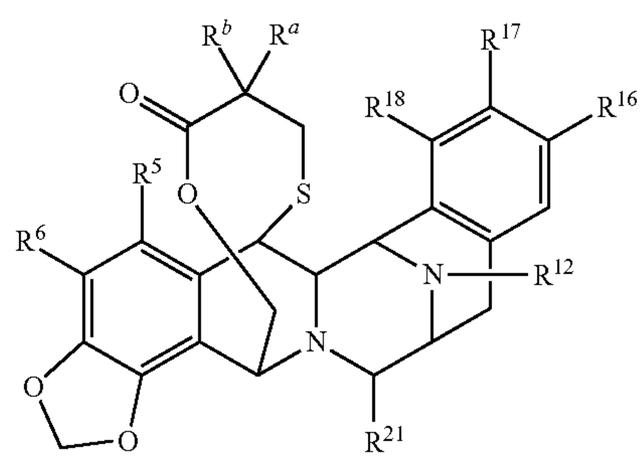
including ET-743, are complex chemical entities whose behaviour in formulations is not predictable in terms of the behaviour of other unrelated chemical substances. Such behaviour is even more difficult to predict when at least one ecteinascidin is included as the active substance in a formulation that is to satisfy biocompatibility standards, including medical standards. We have further found in this regard that the use of disaccharides as bulking agents can drastically reduce the formation of impurities during the lyophilisation process and storage of ET-743 compositions.

When embodiments of this invention are to provide ET-743 formulations that are substantially free of other ecteinascidins such as ET-701, or at least with a content of ET-701 as low as possible, then ET-701 is regarded as an impurity whose presence in the formulation is to be at least reduced.

In addition, the use of disaccharides also improves the storage conditions allowing long term storage of the lyophilised formulation in a wide temperature range, including refrigeration conditions and room temperature. The term "stable" as used herein in, for example the expression "a stable ET-743 formulation", refers to a formulation that satisfies stability characteristics as reported herein and equivalents thereof, that are not possessed by conventional formulations and that are not achieved when the formulation is prepared by conventional manufacturing methodologies.

Examples of embodiments of the present invention are provided by novel pharmaceutically acceptable compositions comprising an ecteinascidin such as ET-743 and a disaccharide.

As noted in the introduction, ecteinascidins have been widely described. They may have the following general formula (I):



wherein:

R^5 is OH, alkoxy or alkanoyloxy;

R^6 is hydrogen, alkyl, alkenyl, alkynyl or aryl;

R^{12} is hydrogen, alkyl, alkenyl, alkynyl or aryl;

R^{16} is hydrogen, alkyl, alkenyl, alkynyl or aryl;

R^{17} is OH, alkoxy or alkanoyloxy;

R^{18} is OH, alkoxy or alkanoyloxy;

R^{21} is H, OH, CN or another nucleophilic group; and

R^a is hydrogen and R^b is optionally substituted amino, or

R^a with R^b form a carbonyl function =O, or

R^a, R^b and the carbon to which they are attached form a tetrahydroisoquinoline group.

In these compounds the substituents can be selected in accordance with the following guidance:

Alkyl and alkoxy groups preferably have from 1 to 12 carbon atoms. One more preferred class of alkyl and alkoxy groups has from 1 to about 6 carbon atoms, and most preferably 1, 2, 3 or 4 carbon atoms. Methyl, ethyl and propyl including isopropyl are particularly preferred alkyl groups in the compounds of the present invention. Methoxy, ethoxy and propoxy including isopropoxy are particularly preferred alkyl groups in the compounds of the present invention. Another more preferred class of alkyl and alkoxy groups has from 4 to about 12 carbon atoms, yet more preferably from 5 to about 8 carbon atoms, and most preferably 5, 6, 7 or 8 carbon atoms. As used herein, the term alkyl, unless otherwise modified, refers to both cyclic and noncyclic groups, although cyclic groups will comprise at least three carbon ring members.

Preferred alkenyl and alkynyl groups in the compounds of the present invention have one or more unsaturated linkages and from 2 to about 12 carbon atoms. One more preferred class of alkenyl or alkynyl groups has from 2 to about 6 carbon atoms, and most preferably 2, 3 or 4 carbon atoms. Another more preferred class of alkenyl or alkynyl groups has from 4 to about 12 carbon atoms, yet more preferably from 5 to about 8 carbon atoms, and most preferably 5, 6, 7 or 8 carbon atoms. The terms alkenyl and alkynyl as used herein refer to both cyclic and noncyclic groups.

Suitable aryl groups in the compounds of the present invention include single and multiple ring compounds, including multiple ring compounds that contain separate and/or fused aryl groups. Typical aryl groups contain from 1 to 3 separated or fused rings and from 6 to about 18 carbon ring atoms. Specially preferred aryl groups include substituted or unsubstituted phenyl, naphthyl, biphenyl, phenanthryl and anthracyl.

Suitable alkanoyloxy and alkanoyl groups have from 2 to about 20 carbon atoms, more preferably from 2 to about 8 carbon atoms, still more preferably from 2 to about 6 carbon atoms, even more preferably 2 carbon atoms. Another preferred class of alkanoyloxy groups has from 12 to about 20 carbon, yet more preferably from 14 to about 18 carbon atoms, and most preferably 15, 16, 17 or 18 carbon atoms.

The groups above mentioned may be substituted at one or more available positions by one or more suitable groups such as OR', =O, SR', SOR', SO_2R', NO_2, NHR', $N(R')_2$, =N—R', NHCOR', $N(COR')_2$, $NHSO_2R'$, CN, halogen, C(=O)R', CO_2R', OC(=O)R' wherein each of the R' groups is independently selected from the group consisting of H, OH, NO_2, NH_2, SH, CN, halogen, =O, C(=O)H, $C(=O)CH_3$, CO_2H, substituted or unsubstituted C_1-C_{12} alkyl, substituted or unsubstituted C_2-C_{12} alkenyl, substituted or unsubstituted C_2-C_{12} alkynyl and substituted or unsubstituted aryl. Suitable halogen substituents in the compounds of the present invention include F, Cl, Br and I.

Preferred compounds of the invention are those of general formula (I) wherein one or more of the following definitions will apply:

R^5 is an alkanoyloxy;

R^6 is methyl;

R^{12} is methyl;

R^{16} is methyl;

R^{17} is methoxy;

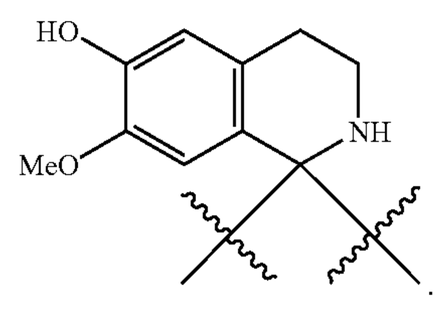
R^{18} is OH;

R^{21} is H, OH or CN; and

R^a is hydrogen and R^b is an amido group, or

R^a with R^b form =O, or

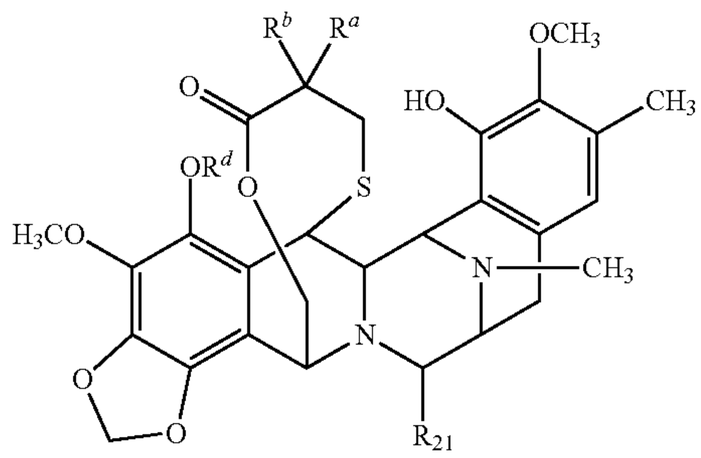
R^a, R^b and the carbon to which they are attached form a group of formula (II):



Examples of compounds for the present invention include natural ecteinascidins, such as ecteinascidin 743 and other 1,4 bridged fused ecteinascidin compounds disclosed for

example in U.S. Pat. Nos. 5,089,273, 5,478,932, 5,654,426, 5,721,362, 6,124,293, 5,149,804, Ser. No. 09/546,877, U.S. Pat. No. 5,985,876 and WO 01/77115.

Compounds of the following formula (III) are particularly preferred:

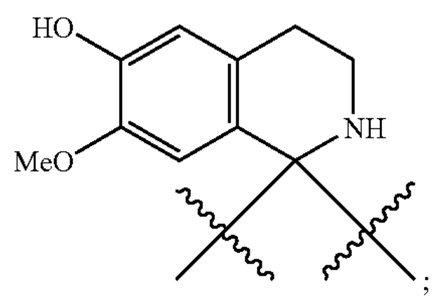


where

R^a is hydrogen and R^b is amido of formula —NHR^f— where R^f is alkanoyl, or

R^a with R^b form =O, or

R^a, R^b and the carbon to which they are attached form a group of formula (II):

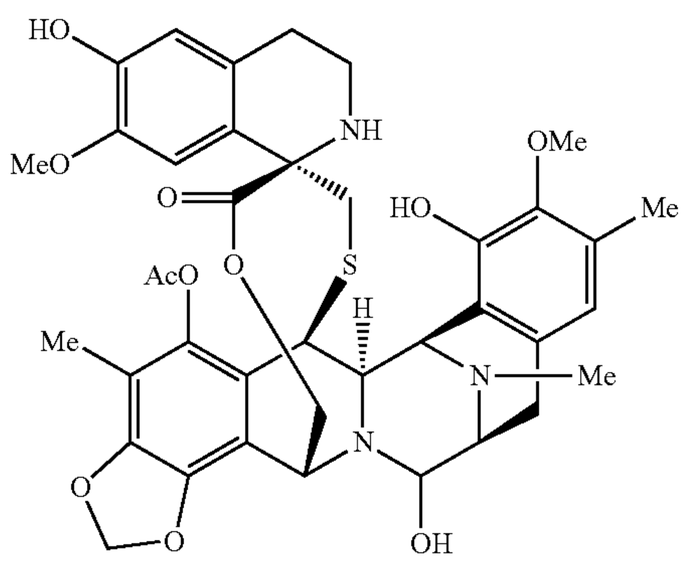


R^d is alkanoyl; and

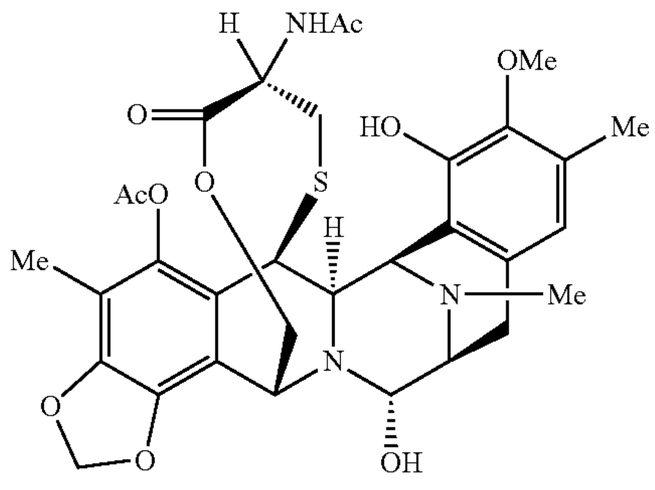
R^{21} is H, OH or CN.

The alkanoyl groups can be acetyl or higher, for example up to C_{20}.

Thus, preferred compounds of this invention include:

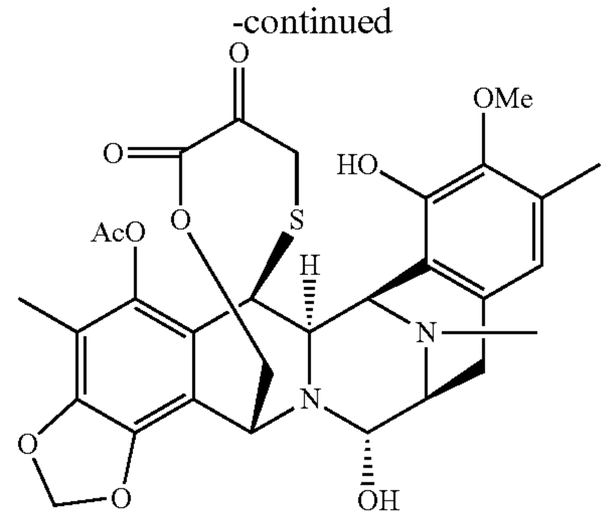


ET743

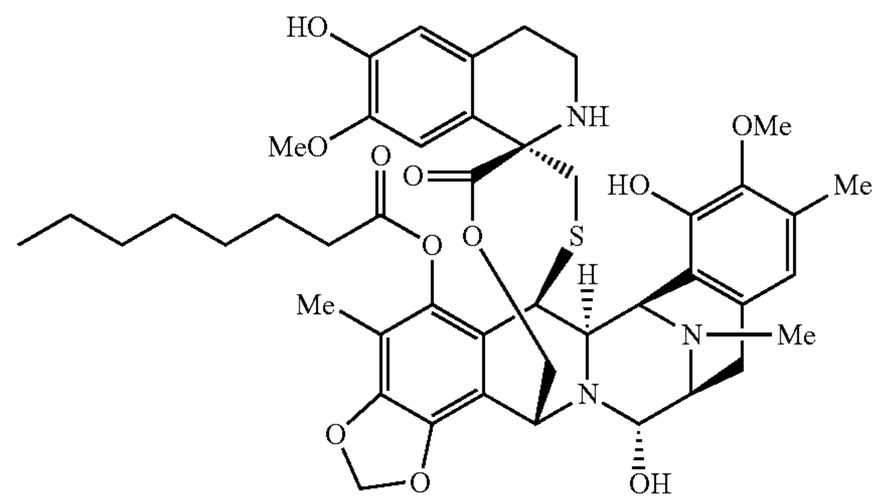


ET637 Derivative A

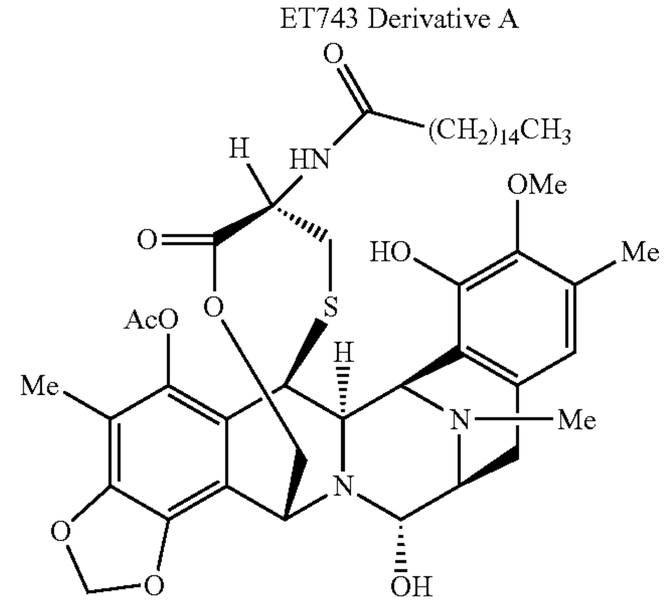
-continued



ET594

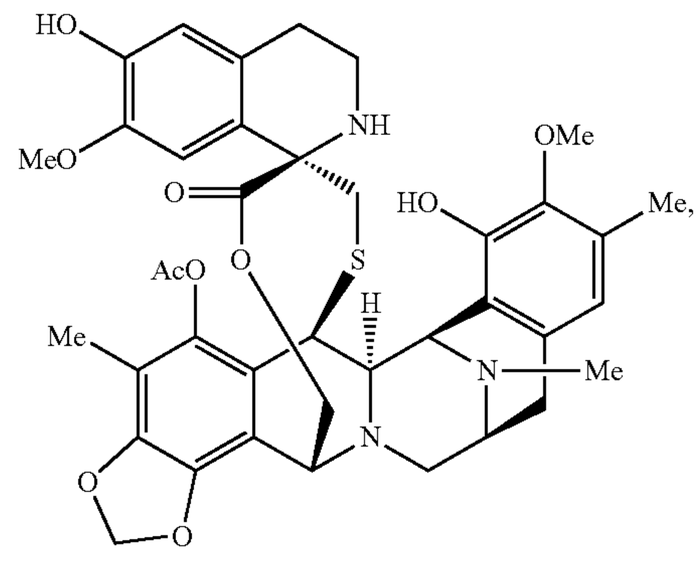


ET743 Derivative A



ET637 Derivative B

or



ET745

and related compounds with different acyl groups.

Ecteinascidin 743, also known as ET743 or ecteinascidin 743 is particularly preferred.

Examples of suitable disaccharides for the compositions of this invention include lactose, trehalose, sucrose, and combinations thereof. Additional examples of disaccharides that can be used in some embodiments of this invention include at least one of maltose, isomaltose, cellobiose, isosaccharose,

isotrehalose, sorbose, turanose, melibiose, gentiobiose, and mixtures thereof. Sucrose is currently preferred.

In other embodiments of the invention, the composition comprises an ecteinascidin such as ET-743 and a lactose-free disaccharide. In other embodiments of the invention, the composition comprises an ecteinascidin such as ET-743 and a trehalose-free disaccharide. In other embodiments of the invention, the composition comprises an ecteinascidin such as ET-743 and a sucrose-free disaccharide. In other embodiments of the invention, the composition comprises an ecteinascidin such as ET-743 and a maltose-free disaccharide. In other embodiments of the invention, the composition comprises an ecteinascidin such as ET-743 and an isomaltose-free disaccharide. In other embodiments of the invention, the composition comprises an ecteinascidin such as ET-743 and a cellobiose-free disaccharide. In other embodiments of the invention, the composition comprises an ecteinascidin such as ET-743 and an isosaccharose-free disaccharide. In other embodiments of the invention, the composition comprises an ecteinascidin such as ET-743 and an isotrehalose-free disaccharide. In other embodiments of the invention, the composition comprises an ecteinascidin such as ET-743 and a sorbose-free disaccharide. In other embodiments of the invention, the composition comprises an ecteinascidin such as ET-743 and a turanose-free disaccharide. In other embodiments of the invention, the composition comprises an ecteinascidin such as ET-743 and a melibiose-free disaccharide. In other embodiments of the invention, the composition comprises an ecteinascidin such as ET-743 and a gentiobiose-free disaccharide.

Thus, in some embodiments, the composition of this invention contains less than 2% or less than 1% or less than 0.5% or less than 0.2% or less than 0.1% by weight of at least one of, preferably each of, lactose, trehalose, sucrose, maltose, isomaltose, cellobiose, isosaccharose, isotrehalose, sorbose, turanose, melibiose, and gentiobiose.

The terms "mixtures thereof" and "combinations thereof" as used herein refer to at least two entities that provide the antecedent basis for the terms "mixtures thereof" or "combinations thereof". By way of illustration, but not as a limitation, the terms "product comprising at least one of A, B, C, and mixtures thereof" refer to embodiments of the product for which any one of the following is satisfied: A is in the product; B is in the product; C is in the product; A and B are in the product; A and C are in the product; B and C are in the product; and A, B and C are in the product.

Furthermore, it is understood that terms such as "reacting", "forming", and related terms, applied to a chemical entity herein refer to any one of: (a) the chemical entity as such, and (b) the chemical entity in the form in which such entity is present in the reaction medium. Analogously, to name a chemical entity or to give its formula in the context of an operation or reaction step, or to name it or give its formula as being in a medium, whether solid or liquid, including products, formulations, and combinations, refers herein to any one of: (a) the entity as such, and (b) the entity in the form in which such entity is present in the medium. For example, naming an acidic chemical entity herein refers to whichever form or forms such entity is present in the context in which it is named. By way of illustration, but not as a limitation, naming the chemical entity "sodium chloride" or providing its chemical formula refers herein to the entity NaCl as such diatomic molecule, if such is the form in which sodium chloride is present in the relevant medium; it also refers to the collection of undissociated and/or dissociated chemical species if sodium chloride in the relevant medium is entirely or partially dissociated, including species in such medium that are solvated, part of cages, associated with other species, etc.

Any compound referred to herein is intended to represent such specific compound as well as certain variations or forms. In particular, compounds referred to herein may have asymmetric centers and therefore exist in different enantiomeric forms. All optical isomers and stereoisomers of the compounds referred to herein, and mixtures thereof, are considered within the scope of the formulations and methodologies of this invention. Thus any given compound referred to herein is intended to represent any one of a racemate, one or more enantiomeric forms, one or more diastereomeric forms, one or more atropisomeric forms, and mixtures thereof.

Furthermore, compounds referred to herein may exist as geometric isomers (i.e., cis and trans isomers), as tautomers, or as atropisomers. Additionally, any compound referred to herein is intended to represent hydrates, solvates, and polymorphs, and mixtures thereof when such forms exist in the medium. In addition, compounds referred to herein may exist in isotopically-labelled forms. All geometric isomers, tautomers, atropisomers, hydrates, solvates, polymorphs, and isotopically labelled forms of the compounds referred to herein, and mixtures thereof, are considered within the scope of the formulations and methodologies of this invention.

To provide a more concise description, some of the quantitative expressions given herein are not qualified with the term "about". It is understood that, whether the term "about" is used explicitly or not, every quantity given herein is meant to refer to the actual given value, and it is also meant to refer to the approximation to such given value that would reasonably be inferred based on the ordinary skill in the art, including equivalents and approximations due to the experimental and/or measurement conditions for such given value.

The active substance or substances in the context of this invention can be of natural, semisynthetic or synthetic origin, including combinations of origins. In embodiments where, the active substance is an ecteinascidin such as ET-743, the ET-743 can be of natural origin, isolated for example from a tunicate of the genus *Ecteinascidia*, preferably the species *Ecteinascidia turbinata*. The ET-743 can be of synthetic or semisynthetic origin. Reference is made for example to WO 0069862 and WO 0187895, both of which are incorporated in full by reference.

The ratio of the active substance to the bulking agent in embodiments of this invention is determined according to the solubility of the bulking agent and, when the formulation is freeze dried, also according to the freeze-dryability of the bulking agent. It is envisaged that this ratio (w/w) can be about 1:1 in some embodiments, about 1:5 in other embodiments, about 1:10 in still other embodiments, while other embodiments illustrate ratios in the range from about 1:10 to about 1:1. It is envisaged that other embodiments have such ratios in the range from about 1:10 to about 1:100, and still further embodiments have such ratios in the range from about 1:100 to about 1:1500. When the active compound is ET-743, the ratio (w/w) of ET-743 to bulking agent is typically from about 1:100 to about 1:1500, preferably from about 1:200 to about 1:800, more preferably from about 1:250 to about 1:600, and even more preferably about 1:400.

The lyophilised material is usually presented in a vial which contains a specified amount of ecteinascidin or active compound. When the active compound is ET-743, active amounts are illustrated by 250 μg and 1 mg.

The present invention is not limited by specific container forms or designs, as long as the container is acceptable for its

intended use and standards therefore. Embodiments of this invention are provided with a formulation contained in vials, preferably tubing vials.

The lyophilised formulations of this invention can be reconstituted and diluted to give a composition of this invention in the form of a solution ready for intravenous injection. The actual amounts of reconstituting fluid are not limiting features of embodiments of this invention. By way of illustrations, but not as limitations, embodiments of lyophilised formulations according to this invention are reconstituted with a volume of water. Most of such volumes do not exceed about 20 ml, with preferred volumes being in the range from about 1 ml to about 15 ml, more preferably in the range from about 1 ml to about 10 ml, and even more preferably in the range from about 1 ml to about 4 ml. When the active substance is embodied by ET-743, the reconstituted solution in such embodiments contains a concentration of ET-743 up to 500 Hg/ml, with concentrations of about 50 μg/ml, about 100 μg/ml, and about 250 μg/ml being preferred.

Reconstituted embodiments of the present invention can further be diluted if so desired, with this further dilution not being a limitation of the present invention. This further dilution is preferably carried out with an aqueous system which is usually 0.9% sodium chloride or 5% glucose. The reconstituted solution will be diluted depending on the concentration in the reconstituted solution and the desired concentration in the diluted solution.

Embodiments of ET-743 formulations according to this invention can be used in the treatment of a variety of cancers, including the treatment of any one of sarcoma, leiomyosarcoma, liposarcoma, osteosarcoma, ovarian cancer, breast cancer, melanoma, colorectal cancer, mesothelioma, renal cancer, endometrial cancer and lung cancer, and conditions with a plurality of such forms of cancer. It is understood that "treatment" in this context refers to an action that leads to an amelioration of the cancer condition(s). Embodiments of ET-743 formulations according to this invention can also be used in the treatment of refractory cancer conditions that have not responded favourably to other treatments. Furthermore, embodiments of formulations according to this invention can be used in the trials with laboratory tissues, including but not limited to clinical trials, analytical trials, and modelling assays.

Embodiments of this invention that comprise an ecteinascidin such as ET-743 are preferably administered by infusion. The infusing step is typically repeated on a cyclic basis, which may be repeated as appropriate over for instance 1 to 20 cycles. The cycle includes a phase of infusing ET-743 formulation, and usually also a phase of not infusing ET-743. Typically the cycle is worked out in weeks, and thus the cycle normally comprises one or more weeks of an ET-743 infusion phase, and one or more weeks to complete the cycle. A cycle of 3 weeks is preferred, but alternatively it can be from 1 to 6 weeks. The infusion phase can itself be a single administration in each cycle of say 1 to 72 hours, more usually of about 1, 3 or 24 hours; or an infusion on a daily basis in the infusion phase of the cycle for preferably 1 to 5 hours, especially 1 or 3 hours; or an infusion on a weekly basis in the infusion phase of the cycle for preferably 1 to 3 hours, especially 2 or 3 hours. A single administration at the start of each cycle is preferred. Preferably the infusion time is about 1, 3 or 24 hours.

The reconstituted and diluted solutions exemplify embodiments of this invention. A formulation that is reconstituted and diluted can be administered intra-venously using the available protocols. The dose will be selected according to the dosing schedule, having regard to the existing data on Dose Limiting Toxicity, on which see for example WO 0069441, WO 0236135 and WO 0339571, and van Kesteren, Ch. et al., 2003, Anti-Cancer Drugs, 14 (7), 487-502. These three WO patent specifications and this van Kesteren article are incorporated by specific reference.

Preferred dosing protocols include:

a) about 1.5 mg/m^2 body surface area, administered as an intravenous infusion over 24 hours with a three week interval between cycles;

b) about 1.3 mg/m^2 body surface area, administered as an intravenous infusion over 3 hours with a three week interval between cycles;

c) about 0.580 mg/m^2 body surface area, administered weekly as an intravenous infusion over 3 hours during 3 weeks and one week rest.

An ecteinascidin such as ET-743 can be used in combination with another drug. For example, it can be administered with another anti-tumour drug. The reader is referred to the list in WO 0069441 and WO 0236135, both of which are incorporated herein by specific reference. Examples of such other drugs include doxorubicin, cisplatin, paclitaxel, carboplatin, pegylated liposomal doxorubicin, docetaxel, capecitabine, and gemcitabine. Drugs with other modes of action can be used, including dexamethasone. Administration of the other drug can be before, during or after administration of the ecteinascidin such as ET-743.

Embodiments of formulations of this invention that contain an ecteinascidin such as ET-743 can be made by freeze-drying a composition of this invention in the form of a bulk solution including the ecteinascidin and disaccharide. Usually the bulk solution will be buffered, for example to a pH of about 4. Suitable buffering agents include phosphate buffer and citrate buffer. Other possible buffers can be used, such as phosphate/citrate buffer (a mixture of phosphate buffer and citrate buffer), lactate buffer, ascorbate buffer, tartaric/citrate buffer, bicarbonate/hydrochloric acid buffer, acetate buffer, succinate buffer and glycine/hydrochloric acid buffer. Mixtures of buffers can be used. Biocompatible buffers that permit the control of pH at a desired value provide additional embodiments of this invention.

Other components can be included in the bulk solution, for example surface-active agents such as polyoxyethylene 20 sorbitan monooleate or polyoxyl 40 stearate. Other possible surface-active agents include phospholipids, such as a lecithin; polyoxyethylene-polyoxypropylene copolymers, such as a Pluronic surfactant; polyoxyethylene esters of 12-hydroxysteraric acid, such as a Solutol surfactant; ethoxylates of cholesterol, such as diacyl glycerol, dialkyl glycerol; bile salts, such as sodium cholate, sodium deoxycholate; sucrose esters, such as sucrose monolaurate, sucrose monooleate; polyvinyl pyrrolidone (PVP); or polyvinyl alcohol (PVA).

The formulation is normally supplied as a vial containing the lyophilised product. This supply form, however, is not a limitation of the present invention. To provide a vial containing the lyophilised product, the bulk solution is added to a vial and freeze-dried. As mentioned herein, another object of the present invention is to provide a process for improving the solubility of an ecteinascidin such as ET-743 in order to increase the ecteinascidin concentration in the solution and reduce the fill volume in the vials before proceeding with the lyophilisation process. This methodology developed in the context of the present invention allows for the manufacturing of embodiments of bulk solution with active substance concentration that is higher than that obtained according to conventional methodologies. Reduced fill volumes in comparison with the conventional formulation with mannitol are therefore obtained with the present methodology. This reduction of fill volumes allows savings in time and energy during

the freeze-drying step. In addition, there is also a decrease in the risk of decomposition of ET-743, particularly in the secondary drying.

As noted hereinabove, ET 743 has limited aqueous solubility, see for example van Kesteren, Ch., et al., 2003, *Anti-Cancer Drugs,* 14 (7), pages 487-502. Conventional methodologies provide for the adjustment of the medium pH to 4 with buffer, to solubilise ET 743. This pH control is conventionally achieved with a 0.05 M phosphate buffer at pH 4. It was found in the context of this invention that ET-743 solubility is improved in the bulk solution by forming a pre-solution of the ET-743 in an acid. With this pre-dissolution the ET-743 concentration in the bulk solution and the vial can be increased and the fill volume in the vials can be reduced. In these embodiments of the present invention, the fill volume is usually reduced by about 80% with respect to that of the conventional fill volume. By way of illustration, but not as limitations, embodiments of this invention provide a fill volume of 1 ml for a vial containing 0.25 mg ET-743, and 4 ml for a vial containing 1 mg ET-743. The fill volume can optionally reduced further in other embodiments of this invention by increasing the ET-743 concentration.

Conventional methodology comprised the dissolution of ET-743, mannitol and 0.05 M phosphate buffer at pH 4 together with water for injection; the solubility of the bulk solution was limited due to the low solubility of ET-743 in this medium. It was found in the context of the present invention that pre-treatment of ET-743 in an acid solution improves the ET-743 solubility and allows to have bulking solutions with higher concentrations of ET-743. Thus, the present invention provides processes useful for improving the solubility of ET-743 in the bulking solution that comprise dissolving ET-743 in an acidic medium, mixing the medium with ET-743 with other components of the bulking solution, and, optionally, adjusting the pH. In some illustrative, but not limiting, embodiments of this invention, pH adjustment is accomplished with a phosphate buffer. The acidic medium suitably contains no or substantially no buffering components, and usually consists of aqueous acid.

Illustrative embodiments of bulk solution for freeze drying according to the present invention are provided by a solution of ET-743 buffered at pH 4 with potassium dihydrogen phosphate and phosphoric acid with sucrose as bulking agent.

An illustrative embodiment of the methodology according to this invention provides as follows: ET-743 is dissolved in 0.1N phosphoric acid. Then water for injection ("WFI"), potassium dihydrogen phosphate, sucrose and ET-743 (pre-dissolved in 0.1N phosphoric acid) are mixed. Dissolution is visually checked before continuing, and dissolution is considered complete when it is so appreciated visually The pH of the solution is checked and adjusted to a value in the range from about 1 to about 5, more preferably in the range from about 2 to about 4.5, even more preferably in the rane from about 3 to about 4.5, and most preferably to a pH of about 4.0 by slow addition of a suitable acid. A preferred embodiment of such acid is phosphoric acid, in which case a preferred concentration is about 0.1N. A suitable base is optionally added for pH control. A preferred embodiment of such base is potassium hydroxide, preferably in solution, in which case a preferred concentration is about 0.1N. The volume is finally adjusted by addition of a suitable, biocompatible fluid, preferably WFI. The bulk solution is then filled in vials according to the desired dose.

The freeze-drying is carried out in some embodiments of this invention by using reduced secondary drying times. A preferred protocol involves cooling to a temperature of about −40° C., primary drying at 40 to 80 μbar for 10 to 50 hours, and secondary drying at a lower pressure and at above 0° C. for 10 to 50 hours. In other protocols in the context of this invention cooling to temperatures below −40° C. is performed.

Embodiments of this invention comprise lyophilization by cooling product below −40° C. The primary drying is performed at a temperature from about −20° C. to about −26° C. and a pressure of about 60 μbar for approximately 15 to 40 hours. The secondary drying is carried out at a temperature from about 20° C. to about 30° C. and a pressure of about 100 μbar for approximately 20 to 40 hours.

Embodiments of lyophilised formulations of this invention are suitable for storage at temperatures significantly higher than conventional formulation storage temperatures. Examples of storage temperatures for formulations according to this invention are around +5° C. These temperatures are readily provided by ordinary refrigerators.

DRAWINGS OF THE INVENTION

FIG. **1**. Comparative stability study. ET-743 purity evaluation after 6 months storage at 5° C.

FIG. **2**. Comparative stability study. ET-743 purity evaluation after 12 months at 5° C.

FIG. **3**. ET-701 impurity production in different formulations stored during 9 months at 5° C.

FIG. **4**. Comparative ET-743% purity evolution of the new formulations and 3 batches of reference formulation, stored during 3 months at 5° C.

FIG. **5**. ET-701 impurity production in different formulations stored during 3 months at 5° C.

FIG. **6**. Comparative ET-743% purity evolution of the new formulations and 3 batches of reference formulation, stored during 3 months at 25° C./65% RH.

FIG. **7**. ET-701 impurity production in different formulations, stored during 3 months at 25° C./65% RH.

FIG. **8**. Comparative ET-743% purity evolution of the new formulations stored at 40° C./70% RH during 3 months.

FIG. **9**. Comparative ET-743% purity evolution of the new formulations stored at 40° C./70% RH during 3 months.

EXAMPLES

Example 1

This example discloses a comparative stability study of 8 new formulations with the conventional ET-743 formulation (with mannitol). Lactose and sucrose were used to illustrate the present invention. A reference formulation was carried out using mannitol. Other known bulking agents such as dextran (Dextran 40) and povidone (Kollidon 12, PVP) were tried for comparison. Surface-active agents polyoxyl 40 stearate (Myrj 45) or polyoxyethylene 20 sorbitan monooleate (Polysorbate 80) were used in some formulations, and buffer was omitted in some formulations.

Bulk solutions were prepared and freeze-dried by a standardised procedure. A volume of 150 ml of each formulation was prepared:

The amount of potassium phosphate for the final solution (1.02 g) was weighed and dissolved in 90% of final volume (135 ml) of water. Then, the pH was adjusted to pH 4.0 with 0.1N phosphoric acid.

7.85 mg of ET-743 were added to a compounding glass vessel and dissolved by magnetical stirring in ⅔ volume (90 ml) of the potassium phosphate solution for approximately 1 h (dissolution was checked visually).

The amount of bulking agent and surfactant were added and dissolved in ⅓ volume of the potassium phosphate solution. Then, the solution was added to the ET-743 solution and the agitation was maintained for 1 additional hour.

The solution was brought to final weight with water (a density of 1.019 g/cc was adopted for all formulations).

The solution was filtered through a 0.22 μm cellulose filter.

The solution was filled into 25 ml glass vials at 5 ml/vial and maintained at −20° C. until lyophilization process.

Lyophilization was performed according to the following table I:

TABLE I

Freezing time to −48° C.:	4 h
Primary drying:	48 h
Secondary drying:	44 h

After freeze-drying, the vials were sealed. The vials were transferred to a refrigerated area (−20° C.).

The composition for each vial was as follows (Table II), noting that the water evaporates during the freeze-drying procedure.

TABLE II

	Reference	Sucrose	Dextran	PVP	Lactose
ET-743	0.250 mg	0.250 mg	0.250 mg	0.250 mg	0.250 mg
Mannitol	250 mg				
Sucrose		500 mg			
Dextran 40			500 mg		
Kollidon 12				375 mg	
Lactose					500 mg
Potassium dihydrogen phosphate	34 mg	34 mg	34 mg	34 mg	34 mg
Phosphoric acid	q.s. pH 4.0	q.s. pH 4.0	q.s. pH 4.0	q.s. pH 4.0	q.s. pH 4.0
Water for injection	q.s. 5 ml	q.s. 5 ml	q.s. 5 ml	q.s. 5 ml	q.s. 5 ml

	ET-poly80	ET-myrj	ET-poly80sacc	ET-p80saccunbuffer
ET-743	0.250 mg	0.250 mg	0.250 mg	0.250 mg
Mannitol	250 mg	250 mg		
Sucrose			500 mg	500 mg
Polysorbate 80	50 mg		50 mg	50 mg
Myrj 45		50 mg		
Potassium dihydrogen phosphate	34 mg	34 mg	34 mg	
Phosphoric acid	q.s. pH 4.0	q.s. pH 4.0	q.s. pH 4.0	
Water for injection	q.s. 5 ml	q.s. 5 ml	q.s. 5 ml	q.s. 5 ml

Stability testing was carried at a temperature of 5±3° C.

The purity evaluation of the nine formulations at 5° C. during 3 months is shown in table III and FIG. **1**. In the case of the formulations displaying a higher stability (Reference, Sucrose, Lactose and ET-poly80sacc), the stability testing was prolonged until 9 months, and in the case of Lactose and Sucrose formulations the stability testing was even prolonged until 12 months due to their high stability. Data in table III and FIG. **2** show that formulations containing sucrose and lactose displayed an improved stability with a purity decrease of only 2%. This decrease is significantly lower than the decrease observed in the other assayed formulations.

TABLE III

	ET-743 purity (%)			
	Reference	Sucrose	Lactose	ET-poly80sacc
Before freeze-drying	98.04	98.34	98.06	97.96
t = 0	97.67	98.15	97.21	97.85
1 month	96.06	96.89	96.35	97.72
3 months	94.37	96.64	95.33	97.58
6 months	92.81	97.01	94.07	95.98
9 months	88.17	95.36	94.83	96.01
12 months		96.31	95.22	

TABLE III-continued

	ET-743 purity (%)				
	Dextran	PVP	ET-poly80	ET-myrj	ET-p80saccunbuffer
Before freeze-drying	96.18	97.62	96.45	97.46	97.45
t = 0	94.64	93.72	92.46	96.36	95.80
1 month	93.42	91.96	89.38	94.36	91.84
3 months	84.90	90.40	81.18	85.31	88.46

As shown in table IV and FIG. **3**, the main degradation product of the reference formulation, ET-701, was dramatically reduced when ET-743 was formulated in the presence of sucrose or lactose.

TABLE IV

	ET-701 (%)			
	Reference	Sucrose	Lactose	ET-poly80sacc
t = 0	0.188	0.066	0.060	0.050
1 month	0.533	0.063	0.076	0.060
3 months	0.890	0.057	0.054	0.050
6 months	1.732	0.050	0.062	0.050
9 months	3.225	0.050	0.050	0.050

It was found in the context of this invention that disaccharides improve the stability of ET-743 in comparison with mannitol. Embodiments of such disaccharides include lactose, sucrose and mixtures thereof. The stability of the formulations comprising disaccharides is also improved in comparison with other formulations containing other conventional bulking agents such as dextran and povidone. Embodiments of disaccharide formulations according to this invention were determined to be stable for at least 12 months at 5° C. Embodiments of formulations according to this invention have impurity content that is significantly reduced with respect to that of conventional formulations. Presence of ET-701 is accordingly reduced. Embodiments of this inven-

tion comprise at least one surface-active agent, such as Polysorbate 80. These embodiments exhibited favorable ET-743 solubility properties and stabilization characteristics. The presence of at least one surface-active agent, however, is not a limiting feature of this invention, and other embodiments do not comprise such agent(s).

Example 2

The purpose of this study was to compare the stability of the standard formulation of ET743 with five new formulations. This study evaluated the stability of the formulations at +5° C.

The composition of tested formulations were the following (Table V), noting that the water evaporates during the freeze-drying procedure:

TABLE V

	Reference	ETtreal	ETP80treal	ETP80treal 250	ETP80sacc 250	ETP80trealgly 250
ET-743	0.250 mg	0.250 mg	0.250 mg	0.250 mg	0.250 mg	0.250 mg
Mannitol	250 mg					
Trehalose		500 mg	100 mg	100 mg		200 mg
Sucrose					100 mg	
Polysorbate 80			10 mg	10 mg	10 mg	150 mg
Glycine						15 mg
Potassium dihydrogen phosphate	34 mg	34 mg	34 mg	6.8 mg	6.8 mg	
Phosphoric acid	q.s. pH 4.0	q.s. pH 4.0	q.s. pH 4.0	q.s. pH 4.0	q.s. pH 4.0	
Hydrochloric acid						q.s. pH 4.5
Water for injection	q.s. 5 ml	q.s. 5 ml	q.s. 5 ml	q.s. 1 ml	q.s. 1 ml	q.s. 1 ml

Bulk solutions were prepared and freeze-dried using the following particular protocols:

Formulations ETtreal, ETP80treal and Reference

A weight of 100 g of each formulation was prepared as follows: The amount of potassium phosphate for the final solution was weighed and dissolved in 90% of final volume (90 ml) of water. Then, the pH was adjusted to pH 4.0 with 0.1N phosphoric acid.

The amount of ET-743 (5.0 mg) was added to a compounding glass vessel and dissolved by magnetical stirring in ⅔ volume (60 ml) of the potassium phosphate solution for approximately 1 h (dissolution was checked visually).

The amount of bulking agent and surfactant were added and dissolved in ⅓ volume of the potassium phosphate solution. Then, the solution was added to the ET-743 solution and the agitation was maintained for 1 additional hour.

The solution was brought to final weight with water.

The solution was filtered through a 0.22 μm cellulose filter, taking an aliquot before filtration for IPC.

The solution was filled into 25 ml vials at 5 ml/vial and maintained at −20° C. until the lyophilization process.

Formulations ETP80sacc250, ETP80treal250, ETP80trealgy250

A weight of 30 g of each formulation was prepared as follows:

The amount of potassium phosphate or glycine for the final solution was weighed and dissolved in 90% of final volume (27 ml) of water. Then, the pH was adjusted to pH 4.0 with 0.1N phosphoric acid or 0.1N HCl.

The amount of polysorbate 80 was weighed and added to ⅓ volume of the buffer solution.

The amount of ET-743 (7.5 mg) was added to a compounding glass vessel and dissolved by magnetical stirring in ⅔ volume (60 ml) of the potassium phosphate solution for approximately 1 h (dissolution was checked visually).

The amount of bulking agent was added and dissolved in ⅔ volume of the buffer solution. Then, the solution was added to the ET-743 solution and the agitation was maintained for 10 min.

The solution was brought to final weight with water.

The solution was filtered through a 0.22 μm cellulose filter.

The solution was filled into 10 ml vials at 1 ml/vial and maintained at −20° C. until lyophilization process.

Lyophilization process in all six formulations was performed according to the following table VI:

TABLE VI

Freezing time to −48° C.:	2 h 30 min
Primary drying:	29 h
Secondary drying:	36 h

After freeze-drying, the vials were sealed. The vials were transferred to a refrigerated area (−20° C.).

Stability testing was carried at a temperature of 5±3° C.

All the formulations were more stable at 5° C. than the reference formulation. No major differences were noted between the new formulations. Table VII discloses the ET-743 chromatographic purity of the formulations under study:

TABLE VII

	ET-743 purity (%)					
	Reference	ETtreal	ETP80treal	ETP80treal 250	ETP80sacc 250	ETP80trealgly 250
Before filtration	98.63	98.30	97.10	97.67	97.22	98.3
After filtration	98.62	98.46	97.27	97.67	97.06	98.46

TABLE VII-continued

	ET-743 purity (%)					
	Reference	ETtreal	ETP80treal	ETP80treal 250	ETP80sacc 250	ETP80trealgly 250
After freeze-dried	97.96	98.18	97.65	97.37	97.79	98.18
1 month	97.35	98.22	97.78	96.39	97.40	98.22
3 months	97.65	98.53	98.25	96.61	96.66	98.53
6 months	95.28	97.38	96.93	97.01	97.06	97.38

Example 3

Six formulations ET-NF A, ET-NF B, ET-NF C, ET-NF D, ET-NF E and ET-NF F were manufactured and used for further study of stability at different temperatures.

Sucrose and lactose were selected as bulking agent. Two different buffers were used: sodium citrate buffer 0.1M pH 4 and potassium phosphate 0.05M buffer pH 4. Two different ET-743 concentrations in the bulk solution were tested: 0.250 mg/ml and 0.100 mg/ml. Two different freeze-dried cycles were used depending on the filling volume (4 ml vs 10 ml). A batch of at least 125 vials was manufactured for each formulation.

For each vial the composition of the bulk solution was as follows (Table VIII), noting that the water evaporates during the freeze-drying procedure:

TABLE VIII

Ingredient	ET-NF A	ET-NF B	ET-NF C	ET-NF D	ET-NF E	ET-NF F
ET-743	1 mg	1 mg	1 mg	1 mg	1 mg	1 mg
Sucrose	400 mg	—	—	1000 mg	1000 mg	—
Lactose	—	400 mg	1000 mg	—	—	200 mg
Citric acid monohydrate	43.6 mg	43.6 mg	109 mg	109 mg	—	43.6 mg
Sodium citrate dihydrate	45.12 mg	45.12 mg	112.8 mg	112.8 mg	—	45.12 mg
Potassium dihydrogen phosphate	—	—	—	—	68 mg	—
Phosphoric acid	—	—	—	—	q.s pH 4	—
Water for injection	4 ml	4 ml	10 ml	10 ml	10 ml	4 ml

Bulk solutions were prepared and freeze-dried using the following particular protocols:

Formulations ET-NF A, ET-NF B and ET-NF F

Preparation of 2 L of citric acid approximately 0.2M: 76.96 g of citric acid were dissolved in a volumetric flask of 2 L and the solution was brought to the final volume with water for injection. The final molarity of the solution of citric acid was 0.183M.

Preparation of 2 L of sodium citrate approximately 0.2M: 117.64 g of sodium citrate were dissolved in a volumetric flask of 2 L and the solution was brought to the final volume with water for injection. The final molarity of the solution of sodium citrate was 0.175M.

Preparation of 4 L of citrate buffer pH 4 approximately 0.1M: 1125 mL of citric acid solution 0.2M were mixed with 875 mL of sodium citrate solution 0.2M in a volumetric flask of 4 L. The solution was brought to the final volume with water for injection. pH of the solution was checked and adjusted to pH 4. The final molarity of the solution of citrate buffer was 0.089M.

143.83 mg of ET-743 was added to a compounding glass vessel and dissolved by magnetical stirring in approximately 80% of required total volume of citrate buffer 0.1 M for approximately 1 h (dissolution was checked visually).

Then, the amount of sucrose or lactose (55 g sucrose formulation A and B, and 27.5 g lactose formulation F) was added and the mixture was stirred for an additional period of approximately 1 h until dissolution.

After checking the pH, the solution was brought to the final volume by adding citrate buffer 0.1 M pH 4. Re-adjusting to pH 4 with citric acid was needed for formulation A. Density of the final solution: 1.04 g/l. Final weight 572 mg.

The solution was filtered through a 0.22 μm PVDF filter.

The solution was filled into 25 ml vials using an automatic pump and silicone platinum-cured tubing 3.2 mm. Standard filling volume was 4 ml. The fill volume was checked at regular intervals (each 15 vials), and fill volume adjusted if required.

After filling lyophilization stoppers were placed and the vials were loaded in the lyophiliser at 5° C.

Lyophilization process was performed according to the following table IX:

TABLE IX

Cycle	Step	Pressure	Setpoint T (° C.)	Slope (min)	Holding time
Loading	Shelves T[a]	atm	5° C.		
Freezing	Freeze 1	atm	−50° C.	1 h 50 min 0.5° C./min	
	Freeze 2	atm	−50° C.		6 h
Vacuum	Ch vacuum	0.5 mb			
Sublimation	1° drying	0.080 mb	−27° C.	45 min 0.5° C./min	
	1° drying	0.080 mb	−27° C.		58 h
2nd drying	2° drying	<0.020 mb	25° C.	3 h 30 min 0.25° C./min	
	2° drying	<0.020 mb	25° C.		40 h
	stoppering	0.010 mb	25° C.		

The vials were sealed. A final reconciliation was performed. The vials were transferred to a refrigerated area (−20° C.).

Formulations ET-NF C and ET-NF D

Preparation of 1 L of citric acid approximately 0.2M: 38.48 g of citric acid were dissolved in a volumetric flask of 1 L and the solution was brought to the final volume with water for injection. The final molarity of the solution of citric acid was 0.183 M.

Preparation of 1 L of sodium citrate approximately 0.2M: 58.82 g of sodium citrate were dissolved in a volumetric flask of 1 L and the solution was brought to the final volume with water for injection. The final molarity of the solution of sodium citrate was 0.175 M.

Preparation of 2 L of citrate buffer pH 4 approximately 0.1M: 850 ml of citric acid solution 0.2M were mixed with 650 ml of sodium citrate solution 0.2M in a volumetric flask of 2 L. The solution was brought to the final volume with water for injection. pH of the solution was checked and adjusted to pH 4. The final molarity of the solution of citrate buffer was 0.089 M.

141.21 mg of ET-743 was added to a compounding glass vessel and dissolved by magnetical stirring in approximately 80% of total volume of citrate buffer 0.1 M for approximately 1 h (dissolution was checked visually).

The amount of sucrose or lactose (135 g) was added and the mixture was stirred for an additional period of approximately 1 h until dissolution.

After checking the pH, the solution was brought to the final volume by adding citrate buffer 0.1 M pH 4. No re-adjusting of pH was needed. Density of the final solution: 1.04 g/l. Final weight 1404 mg.

The solution was filtered through a 0.45 μm PVDF filter.

The solution was filled into 25 ml vials using an automatic pump and silicone platinum-cured tubing 3.2 mm. Standard filling volume was 10 ml.

The fill volume was checked at regular intervals (each 15 vials), and fill volume adjusted if required.

After filling lyophilization stoppers were placed and the vials loaded in the lyophiliser at 5° C.

Lyophilization process was performed as before for Formulations ET-NF A, ET-NF B and ET-NF F (Table IX).

Due to the large volume in vials, the cycle proposed failed to give an adequate liophilization and collapse was produced. To avoid a new manufacture, all the vials were reconstituted with 10 ml of purified water, purity profile of some reconstituted solutions was checked, stoppers were replaced for two hole stoppers and the following new cycle was used (Table X):

TABLE X

Cycle	Step	Pressure	Setpoint T (° C.)	Slope (min)	Holding time
	Freeze 2	atm	−50° C.		6 h
Vacuum	Ch vacuum	0.5 mb			
Sublimation	1° drying	0.080 mb	−23° C.	45 min 0.5° C./min	
	1° drying	0.080 mb	−23° C.		80 h
2nd drying	2° drying	<0.020 mb	0° C.	10 h 0.045° C./min	
	2° drying		25° C.	62 h 0.006° C./min	
	stoppering	0.010 mb	25° C.		

After freeze-drying, the vials were sealed. A final reconciliation was performed. The vials were transferred to a refrigerated area (−20° C.).

Formulation ET-NF E

141.21 mg of ET-743 was added to a compounding glass vessel and dissolved by magnetical stirring in 1080 ml wfi+ 3,240 mL phosphoric acid 1N for approximately 1 h (dissolution was checked visually).

The amount of sucrose (135 g) and potassium phosphate (9.18 g) was added and the mixture was stirred for an additional period of approximately 1 h until total dissolution of the molecule.

After checking the pH and re-adjusting to pH 4 with phosphoric acid 1N, the solution was brought to the final volume by adding water for injection. Density of the final solution: 1.04 g/l. Final weight 1404 mg.

The solution was filtered through a 0.45 μm PVDF filter.

The solution was filled into 25 ml vials using an automatic pump and silicone platinum-cured tubing 3.2 mm. Standard filling volume was 10 ml. The fill volume was checked at regular intervals (each 15 vials), and fill volume adjusted if required.

After filling lyophilization stoppers were placed and the vials loaded in the lyophiliser at 5° C.

Lyophilization process was performed as before for Formulations ET-NF A, ET-NF B and ET-NF F (Table 1X).

Due to the large volume in vials, the cycle proposed failed to give an adequate liophilization and collapse was produced. To avoid a new manufacture, all the vials were reconstituted with 10 ml of purified water, purity profile of some reconstituted solutions was checked, stoppers were replaced for two hole stoppers and a new cycle was used as in the case of formulations ET-NF C and ET-NF D (Table X).

After freeze-drying, the vials were sealed. A final reconciliation was performed. The vials were transferred to a refrigerated area (−20° C.).

The desired ET-743 concentration was reached in all cases and the impurity profile was similar between formulations. No differences in ET-743 concentration, and impurity profiles were observed during manufacture (before and after filtration, or after filling). The colour of the bulk solution was slight yellowish in those formulations containing lactose.

Formulations with 4 ml filling or 10 ml filling were initially freeze-dried following the indicated protocol. Whereas formulations with 4 ml filling were correctly lyophilised, formulations with 10 ml filling collapsed. A pressure variation in secondary desiccation indicated collapse and boiling of the freeze-dried cake. Vials of formulations ET-NF C, ET-NF D and ET-NF E were reconstituted with 10 ml of purified water. Purity profile of formulations was checked. As no modification in the purity profile was observed in compare with bulk solutions, it was decided to re-lyophilise the vials using the indicated revised freeze-dried cycle. Batches lyophilised as described, resulted in good aspect without collapse but some bottom contraction.

The ET-743 content of vials was within specifications (95%-105%). Impurities profiles showed similarity between formulations and those profiles are comparable with impurities of bulk solutions. Residual water content was lower or equal than 2% with the largest values being those of formulations of 10 ml filling.

pH of the reconstituted solutions were between pH 4 and pH 4.2 in all cases. Solutions were clear and colourless without visible foreign matter or precipitation. Reconstitution time was similar for all formulations and less than 30 s.

Example 4

The purpose of the study was to investigate the stability of ET-743 in the different formulations ET-NF A, ET-NF B,

ET-NF C, ET-NF D, ET-NF E, and ET-NF F at 1 mg/vial under different temperature conditions.

A batch of 130 vials of each formulation ET-NF A, ET-NF B, ET-NF C, ET-NF D, ET-NF E and ET-NF F, 1 mg ET-743/vial, was manufactured according to example 3.

Stability testing was carried at a temperature of 5° C., 25° C./65% RH and 40° C./70% RH.

FIG. **4** and table XI show the ET-743 purity evolution of the new formulations during storage at 5° C., in comparison with three conventional formulations (containing ET-743, mannitol and phosphate buffer).

TABLE XI

	ET-743 purity (%)				
	Reference 1	Reference 2	Reference 3	ET-NF A	ET-NF B
t = 0	97.70	96.90	97.70	99.06	99.00
1 month	94.70	94.60	94.00	99.28	98.86
2 month	93.80	92.70	93.70	99.21	98.97
3 month	92.50	91.00	91.20	99.22	98.88

	ET-743 purity (%)			
	ET-NF C	ET-NF D	ET-NF E	ET-NF F
t = 0	98.83	98.98	99.15	98.88
1 month	98.53	99.03	99.14	98.71
2 month	98.88	99.15	99.21	98.80
3 month	98.86	99.12	99.16	98.92

FIG. **6** and table XII show the ET-743 purity evolution of the new formulations during storage at 25° C./65% RH, in comparison with three conventional formulations (containing ET-743, mannitol and phosphate buffer).

TABLE XII

	ET-743 purity (%)				
	Reference 1	Reference 2	Reference 3	ET-NF A	ET-NF B
t = 0	97.70	96.90	97.70	99.06	99.00
15 days	84.70	84.50	89.50	98.97	98.09
1 month	74.50	72.40	81.90	99.08	98.13
2 month				99.10	98.38
3 month				98.98	98.15

	ET-743 purity (%)			
	ET-NF C	ET-NF D	ET-NF E	ET-NF F
t = 0	98.83	98.98	99.15	98.88
15 days	98.35	98.90	99.11	98.31
1 month	98.01	99.03	99.13	98.32
2 month	98.35	98.99	99.10	98.28
3 month	98.33	98.93	99.09	98.14

FIG. **8** and table XIII show the ET-743 purity evolution of the new formulations during storage at 40° C./70% RH, in comparison with a conventional formulation (containing ET-743, mannitol and phosphate buffer).

TABLE XIII

	ET-743 purity (%)						
	Reference 4	ET-NF A	ET-NF B	ET-NF C	ET-NF D	ET-NF E	ET-NF F
t = 0	94.92	99.06	99.00	98.83	98.98	99.15	98.88
2 days	87.17	99.02	98.10	98.48	98.95	99.12	97.53
7 days	82.04	98.68	97.08	97.86	98.63	98.89	97.20
15 days	76.33	98.75	97.33	97.77	98.55	98.71	96.66
1.5 month		98.52	96.99	97.53	97.54	98.47	95.79
3 month		98.24	95.92	96.67	94.79	97.84	95.91

In addition, FIG. **5** and table XIV show the ET-701 impurity production of the new formulations during storage at 5° C., in comparison with three conventional formulations (containing ET-743, mannitol and phosphate buffer).

TABLE XIV

	ET-701 (%)				
	Reference 1	Reference 2	Reference 3	ET-NF A	ET-NF B
t = 0	0.70	0.84	0.53		0.11
1 month	2.56	3.09	1.73		0.12
2 month	3.45	3.54	2.69	0.12	0.13
3 month	4.61	5.04	4.28	0.13	0.13

	ET-701 (%)			
	ET-NF C	ET-NF D	ET-NF E	ET-NF F
t = 0	0.15	0.16	0.15	0.11
1 month	0.12	0.12	0.14	0.11
2 month	0.16	0.14	0.15	0.12
3 month	0.16	0.15	0.15	0.12

FIG. **7** and table XV show the ET-701 impurity production of the new formulations during storage at 25° C./65% RH, in comparison with three conventional formulations (containing ET-743, mannitol and phosphate buffer).

TABLE XV

	ET-701 (%)				
	Reference 1	Reference 2	Reference 3	ET-NF A	ET-NF B
t = 0	0.70	0.84	0.53	0.09	0.11
15 days	7.99	8.20	4.22	0.14	0.12
1 month	10.56	14.18	6.86	0.11	0.12
2 month				0.15	0.16
3 month				0.17	0.17

	ET-701 (%)			
	ET-NF C	ET-NF D	ET-NF E	ET-NF F
t = 0	0.15	0.16	0.15	0.11
15 days	0.18	0.17	0.16	0.13
1 month		0.18	0.18	
2 month	0.18	0.19	0.24	0.15
3 month	0.22	0.23	0.24	0.18

As shown in these figures, the stability of the new formulations was higher than the stability of the conventional for-

mulations. In addition, the main degradation product of the conventional formulations, ET-701, was dramatically reduced in the new formulations.

Example 5

Three new formulations based on sucrose as bulking agent were used for further stability studies. These new formulations differ in the phosphate buffer molarity (0.05M vs 0.1M) and the filling volume or, in other words, in the ET-743 concentration in the bulk solution (10 ml vs 4 ml).

The manufacture of a batch of at least 50 vials of each of these formulations is described. A summary of formula description per vial is as follows (Table XVI), noting that the water evaporates during the freeze-drying procedure:

TABLE XVI

	ET-NF G		ET-NF H		ET-NF I	
Ingredient	Concentration (mg/ml)	Final composition	Concentration (mg/ml)	Final composition	Concentration (mg/ml)	Final composition
ET-743	0.250	1 mg	0.100	1 mg	0.250	1 mg
Sucrose	100	400 mg	100	1 g	100	400 mg
Potassium dihydrogen phosphate	6.8	27.2 mg	13.6	136 mg	13.6	54.4 mg
Phosphoric acid	q.s to pH 4.0	q.s pH 4.0	q.s to pH 4.0	q.s pH 4.0	q.s to pH 4.0	q.s pH 4.0
Water for injection	q.s. to 1 ml	4 ml	q.s. to 1 ml	10 ml	q.s. to 1 ml	4 ml

A volume of 240 ml for formulations ET-NF-G and ET-NF-I was prepared:

62.76 mg of ET-743 were added to a compounding glass vessel and dissolved by magnetical stirring in a solution of 192 ml wfi+1N phosphoric acid (576 μl for NF G, 816 μl for NF I) for approximately 1 h (dissolution is checked visually).

The amounts of sucrose (24 g) and potassium phosphate (1.63 g for NF G; 3.26 g for NF I) were added and the mixture was stirred for an additional period of approximately 1 h until dissolution.

After checking the pH and re-adjusting to pH 4.00 if necessary with IN phosphoric acid, the solution was brought to the final volume by adding water for injection. Density of the final solution: 1.04 g/l. Final weight 249.6 g. The solution was filtered through a 0.45 μm PVDF filter.

The solution was filled into 25 ml vials using an automatic pump and silicone platinum-cured tubing 3.2 mm. Standard filling volume was 4 ml.

The fill volume was checked at regular intervals (each 15 vials), and adjusted if required.

After filling lyophilization stoppers were placed and the vials loaded in the lyophiliser at 5° C.

Lyophilization process was performed according to the following parameters (Table XVII):

TABLE XVII

Cycle	Step	Pressure	Setpoint T (° C.)	Slope (min)	Holding time
	Shelves T[a]	atm	5° C.		
Freezing	Freeze 1	atm	−50° C.	1 h 50 min 0.5° C./min	
	Freeze 2	atm	−50° C.		6 h
Vacuum	Ch vacuum	0.5 mb			
Sublimation	1° drying	0.080 mb	−27° C.	45 min 0.5° C./min	
	1° drying	0.080 mb	−27° C.		58 h
2nd drying	2° drying	<0.020 mb	25° C.	3 h 30 min 0.25° C./min	
	2° drying		25° C.		40 h
	end	0.010 mb	25° C.		

After freeze-drying, the vials were sealed. A final reconciliation was performed. The vials were transferred to a refrigerated area (−20° C.).

A volume of 600 ml of the formulation ET-NF-H was prepared as follows:

62.76 mg of ET-743 were added to a compounding glass vessel and dissolved by magnetical stirring in a solution of 480 ml wfi+1.44 ml of 1N phosphoric acid for approximately 1 h (dissolution was checked visually).

The amounts of sucrose (60 g) and potassium phosphate (8.16 g) were added and the mixture was stirred for an additional period of approximately 1 h until dissolution.

After checking the pH and re-adjusting to pH 4.00 if necessary with 1N phosphoric acid, the solution was brought to the final volume by adding water for injection. Density of the final solution: 1.04 g/l. Final weight 624 g.

The solution was filtered through a 0.45 μm PVDF filter.

The solution was filled into 25 ml vials using an automatic pump and silicone platinum-cured tubing 3.2 mm. Standard filling volume was 10 ml.

The fill volume was checked at regular intervals (each 15 vials), and adjusted if required.

After filling lyophilization stoppers were placed and the vials loaded in the lyophiliser at 5° C.

Lyophilization process was performed according to the following parameters (Table XVIII):

TABLE XVIII

Cycle	Step	Pressure	Setpoint T (° C.)	Slope (min)	Holding time
	Shelves T[a]	atm	5° C.		
Freezing	Freeze 1	atm	−50° C.	1 h 50 min 0.5° C./min	
	Freeze 2	atm	−50° C.		6 h
Vacuum	Ch vacuum	0.5 mb			
Sublimation	1° drying	0.080 mb	−27° C.	45 min 0.5° C./min	
	1° drying	0.080 mb	−27° C.		62 h
2nd drying	2° drying	<0.020 mb	25° C.	3 h 30 min 0.25° C./min	
	2° drying		25° C.		50 h
	end	0.010 mb	25° C.		

After freeze-drying, the vials were sealed. A final reconciliation was performed. The vials were transferred to a refrigerated area (−20° C.).

The ET-743 content was within specifications (95%-105%). Impurities profiles showed similarity for all the formulations and comparable with impurities of bulk solutions. Residual water content was lower than 2%, being the highest value for the formulation of 10 ml filling.

The pH of the reconstituted solutions was between pH 4 and pH 4.28 in all cases. Solutions were clear and colourless without visible foreign matter or precipitation. Reconstitution time was similar for all formulations and less than 30 s.

Stability testing was carried during 3 months at a storage temperature of 40° C./70% RH.

FIG. **9** and table XIX show the ET-743 purity evolution of the new formulations during storage at 40° C./70% RH.

TABLE XIX

	ET-743 purity (%)		
	ET-NF G	ET-NF H	ET-NF I
t = 0	99.18	99.23	99.00
7 days	99.04	98.68	
15 days	98.97	98.86	98.79
1.5 month	98.72	98.61	98.40
2 month	98.29		
3 month			97.71

In addition, in FIG. **8** the stability data is shown in comparison with a conventional formulation (containing ET-743, mannitol and phosphate buffer).

The results obtained in Examples 4 and 5 indicate that all the formulations comprising a disaccharide as bulking agent are more stable than conventional formulations containing mannitol as bulking agent. Formulation ET-NF-G is a preferred formulation.

Embodiments of formulations according to this invention were tested after storage under a plurality of storage conditions (including temperatures of −20° C., 4° C., and 25° C./60% RH) at various storage times (including storage times of 3 months, 6 months, and 9 months). The assay results indicated that at least 99.5% of ET-743 remained after 9 months of storage at −20° C., at least 99% of ET-743 remained after 9 months of storage at 4° C., and at least 97% of ET-743 remained after 9 months of storage at 25° C./60% RH. Total impurities, including ET-701, ET-745, and other impurities did not exceed 1.66% after 9 months of storage at 25° C./60% RH. In addition, the level of ET-701 impurity did not exceed 0.21% after 9 months of said storage conditions.

All the references cited herein are incorporated by reference in their entirety. The features and advantages of this invention are apparent in light of the disclosure provided herein. Based on this disclosure, modifications and adaptations to various conditions and usages can be made, thus generating embodiments within the scope of this invention.

The invention claimed is:

1. A lyophilised anti-tumor composition comprising a single active anti-tumor compound and a disaccharide selected from sucrose, lactose and a combination thereof, wherein the anti-tumor compound is ET-743 and wherein the disaccharide is present in a sufficient amount to inhibit conversion of the ET-743 into ET-701, such that the ET-743 composition comprises less than 2% ET-701 after storage of the ET-743 composition at 5° C. for 3 months.

2. The lyophilised anti-tumor composition according to claim **1**, wherein said disaccharide is lactose.

3. The lyophilised anti-tumor composition according to claim **1**, wherein said disaccharide is sucrose.

4. The lyophilised anti-tumor composition according to claim **1**, wherein the ratio (w/w) of ET-743 to disaccharide is from about 1:100 to about 1:1500.

5. The lyophilised anti-tumor composition according to claim **4**, wherein the ratio (w/w) of ET-743 to disaccharide is from about 1:250 to about 1:600.

6. The lyophilised anti-tumor composition according to claim **5**, wherein the ratio (w/w) of ET-743 to disaccharide is about 1:400.

7. The lyophilised anti-tumor composition according to claim **1**, which further comprises a buffering agent.

8. The lyophilised anti-tumor composition according to claim **7**, wherein said buffering agent is selected from phosphate buffer, citrate buffer, glycine/hydrochloric acid buffer, and mixtures thereof.

9. The lyophilised anti-tumor composition according to claim **1**, which further comprises a surface-active agent.

10. The lyophilised anti-tumor composition according to claim **9**, wherein the surface-active agent is selected from polyoxyethylene 20 sorbitan monooleate, polyoxyl 40 stearate, and mixtures thereof.

11. The lyophilised anti-tumor composition according to claim **1**, wherein the lyophilised formulation is in a vial.

12. The lyophilised anti-tumor composition according to claim **11**, wherein ET-743 is present in an amount of about 250 micrograms.

13. The lyophilised anti-tumor composition according to claim **12**, wherein said vial contains a composition comprising: about 0.25 mg ET-743, about 100 mg sucrose, and about 6.8 mg potassium dihydrogen phosphate.

14. The lyophilised anti-tumor composition according to claim **11**, wherein ET-743 is present in an amount of about 1 mg.

15. The lyophilised anti-tumor composition according to claim **14**, wherein said vial contains a formulation comprising: about 1.0 mg ET-743, about 400 mg sucrose, and about 27.2 mg potassium dihydrogen phosphate.

16. A method of making a lyophilised composition of ET-743 according to claim **1**, comprising freeze-drying a bulk solution that comprises ET-743 and a disaccharide wherein said disaccharide is selected from sucrose, lactose and a combination thereof.

17. A method of reducing the formation of ET-701 in a composition of ET-743, comprising freeze-drying a bulk solution that comprises ET-743 and a disaccharide to yield a composition according to claim **1** wherein said disaccharide is selected from sucrose, lactose and a combination thereof.

18. A method of preparing a bulk solution for lyophilising to yield a composition according to claim **1**, comprising dissolving ET-743 in an acidic medium, mixing the pre-dissolved ET-743 with other components of the bulk solution and, optionally, adjusting the pH of the final solution.

19. A method of preparing a solution for intravenous infusion, comprising: providing a vial that comprises a lyophilised ET-743 composition according to claim **1**, adding water to form a reconstituted solution, and diluting said reconstituted solution with an aqueous system.

20. The lyophilised anti-tumor composition according to claim **3**, further comprising a buffering agent.

21. The lyophilised anti-tumor composition according to claim **20**, further comprising a surface-active agent.

22. A lyophilized anti-tumor composition comprising a single active anti-tumor compound and a disaccharide selected from sucrose, lactose and a combination thereof, wherein the anti-tumor compound is ET-743 and wherein the

disaccharide is present in a sufficient amount to inhibit conversion of the ET-743 into ET-701, such that the ET-743 composition comprises less than 2% ET-701 after storage of the ET-743 composition at 25° C. for 3 months.

23. The lyophilised anti-tumor composition according claim **22**, wherein the lyophilised composition is in a vial.

24. The lyophilised anti-tumor composition according to claim **22**, wherein the disaccharide is sucrose.

25. The lyophilised anti-tumor composition according to claim **22**, wherein the disaccharide is lactose.

26. The lyophilised anti-tumor composition according to claim **22**, further comprising a buffer.

* * * * *

EXHIBIT B

US007420051B2

(12) **United States Patent**
Francesch et al.

(10) **Patent No.: US 7,420,051 B2**
(45) **Date of Patent: Sep. 2, 2008**

(54) **SYNTHETIC PROCESS FOR THE MANUFACTURE OF AN ECTEINASCHIDIN COMPOUND**

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C07D 337/16	(2006.01)
C07D 487/00	(2006.01)

(52) **U.S. Cl.** **540/454**

(58) **Field of Classification Search** 540/454
See application file for complete search history.

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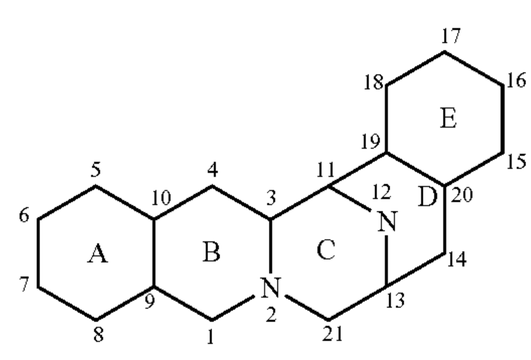
(57) **ABSTRACT**

Processes are provided for preparing compounds with a fused ring structure of formula (XIV). Such products include ecteinascidins and have a spiroamine-1,4-bridge. The process involving forming a 1,4 bridge using a 1-labile, 10-hydroxy, 18-protected hydroxyl, di-6,8-en-5-one fused ring compound. After formation of the 1,4 brige, C-18 protection is removed before spiroamine introduction.

(XIV)



16 Claims, No Drawings

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SYNTHETIC PROCESS FOR THE MANUFACTURE OF AN ECTEINASCHIDIN COMPOUND

CROSS REFERENCE TO RELATED APPLICATIONS

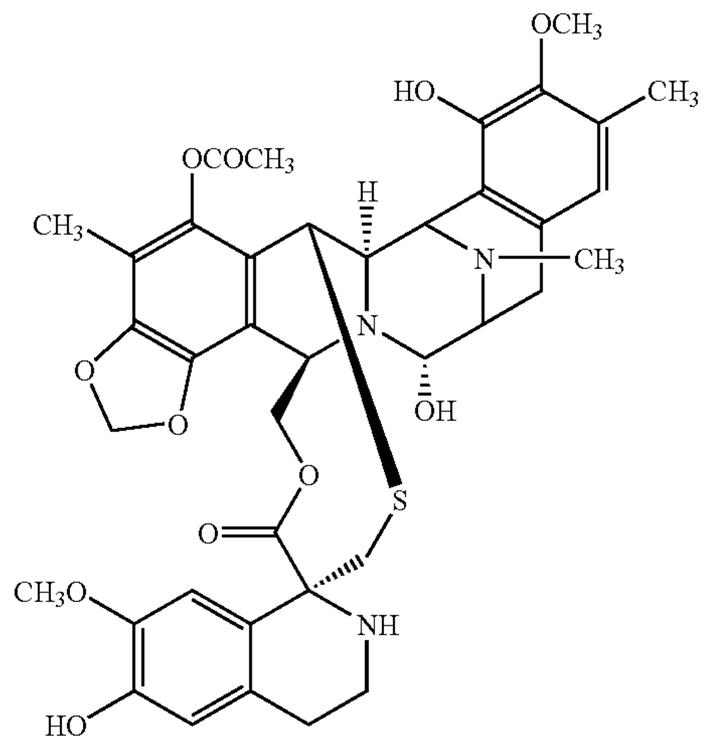
This application is a continuation of U.S. application Ser. No. 10/257,856, filed Mar. 31, 2003, now abandoned which is the National Stage of International Application No.: PCT/GB01/02120, filed on May 15, 2001, which claims the benefit of International Application No.: PCT/GB00/01852 filed on May 15, 2000. The contents of these prior applications are incorporated herein by reference in their entireties.

The present invention relates to synthetic processes, and in particular it relates to synthetic processes for producing ecteinascidin compounds.

BACKGROUND OF THE INVENTION

European Patent 309,477 relates to ecteinascidins 729, 743, 745, 759A, 759B and 770. The ecteinascidin compounds are disclosed to have antibacterial and other useful properties. Ecteinascidin 743 is now undergoing clinical trials as an antitumour agent.

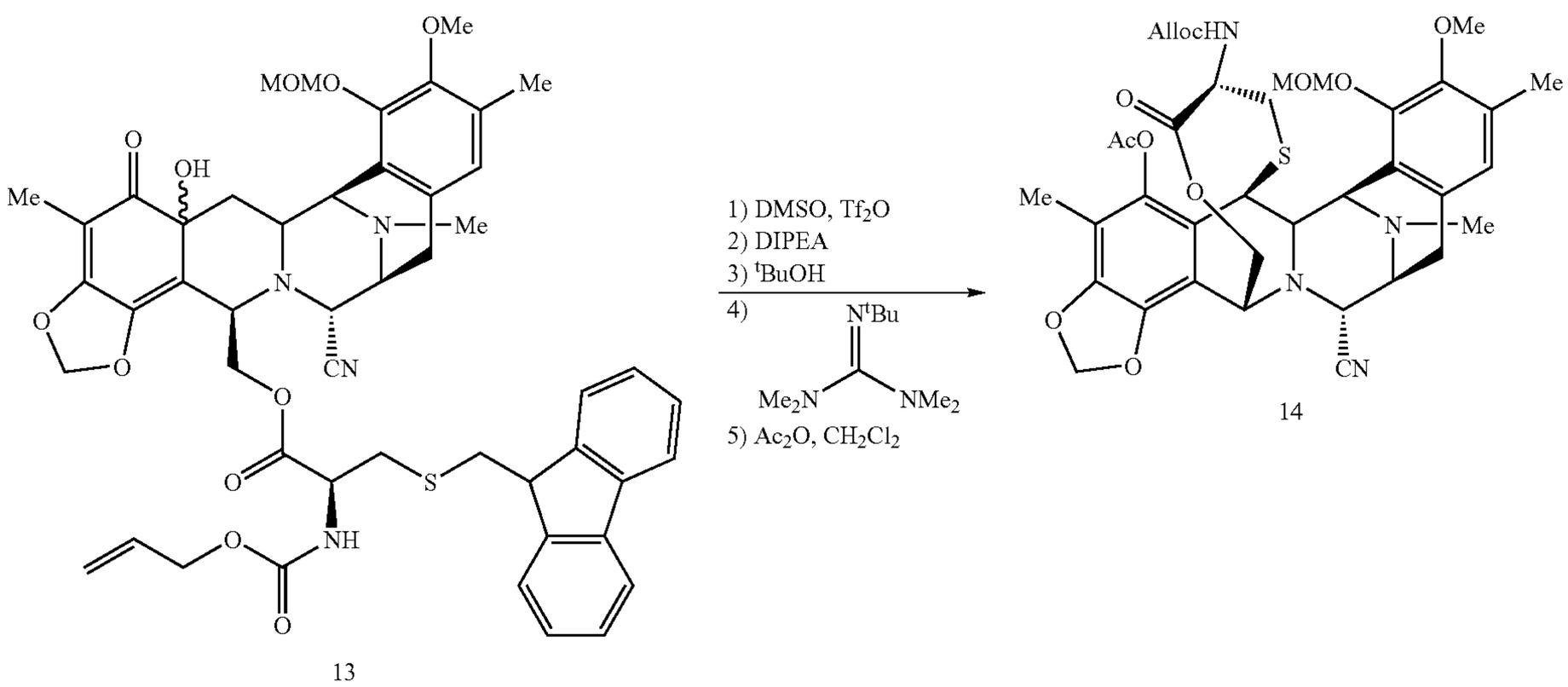
Ecteinascidin 743 has a complex tris(tetrahydroisoquinolinephenol) structure of the following formula (I):



It is currently prepared by isolation from extracts of the marine tunicate *Ecteinascidin turbinata*. The yield is low, and alternative preparative processes have been sought.

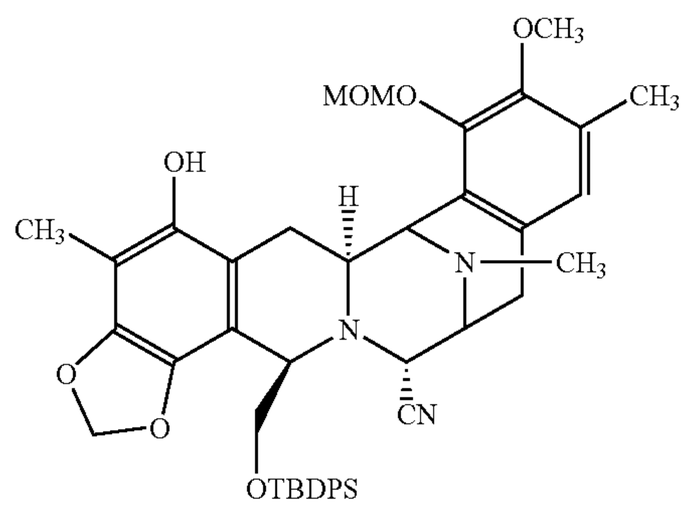
A synthetic process for producing ecteinascidin compounds is described in U.S. Pat. No. 5,721,362, see also WO 9812198 which is incorporated herein by reference in full. The claimed method is long and complicated, there being 38 Examples each describing one or more steps in the synthetic sequence to arrive at ecteinascidin 743.

In the known synthetic process, a 1,4 bridge is formed using a 1-labile, 10-hydroxy, 18-protected hydroxy, di-6,8-en-5-one fused ring compound. As shown in Example 33, a compound (13) is converted to compound (14):



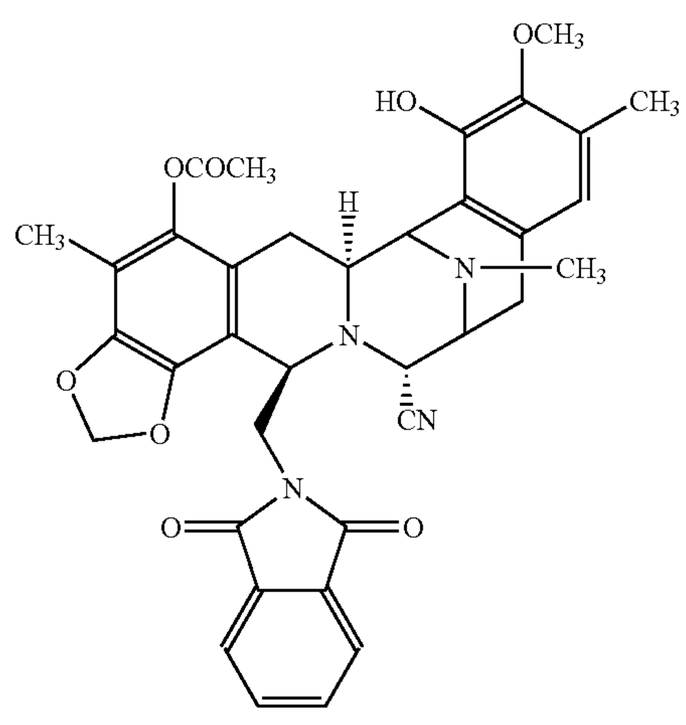
According to the known synthetic process, a spiroquinoline is then formed in the 1,4 bridge by the steps of Examples 34 to 36, and the 18-MOM protecting group is removed to give ecteinascidin 770 which can then be converted to ecteinascidin 743.

Claim 25 of U.S. Pat. No. 5,721,362 is directed at an intermediate phenol compound of a given formula (11), which we refer to also as Intermediate 11 or Int-11. It has the following bis(tetrahydroisoquinolinephenol) structure (II):

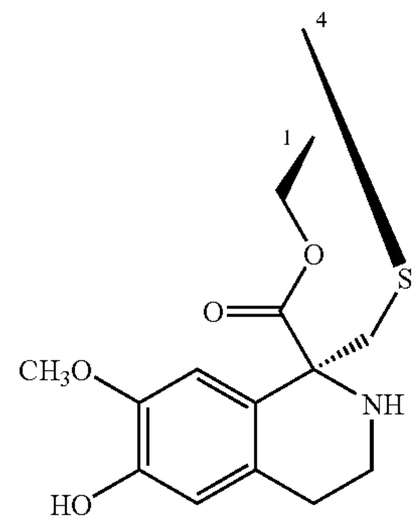


where MOM is a methoxymethyl substituent and TBDPS is a tert-butyldiphenylsilyl substituent.

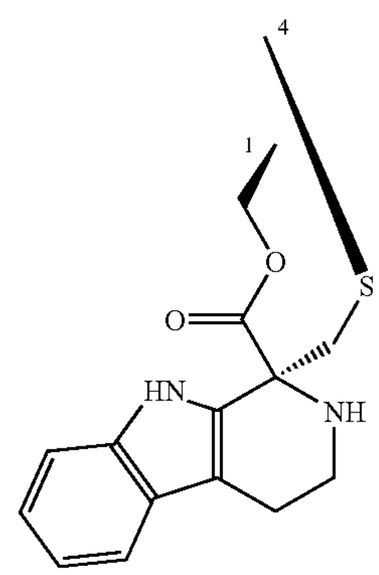
From Intermediate 11 it is possible to synthesise another interesting antitumour agent, phthalascidin, see Proc. Natl. Acad. Sci. USA, 96, 3496-3501, 1999. Phthalascidin is a bis(tetrahydroisoquinolinephenol) derivative of formula (III):



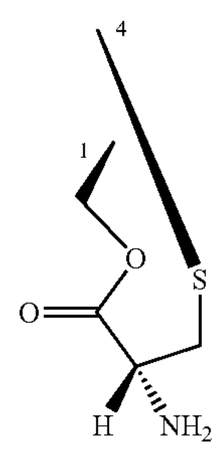
In ecteinascidins 743 and 770, the 1,4 bridge has the structure of formula (IV):



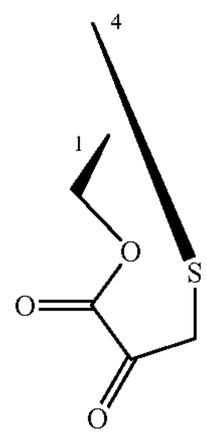
Other known ecteinascidins include compounds with a different bridged cyclic ring system, such as occurs in ecteinascidin 722 and 736, where the bridge has the structure of formula (V):



ecteinascidins 583 and 597, where the bridge has the structure of formula (VI):



and ecteinascidin 594 and 596, where the bridge has the structure of formula (VII):



The complete structure for these and related compounds is given in J. Am. Chem. Soc. (1996) 118, 9017-9023. This article is incorporated by reference.

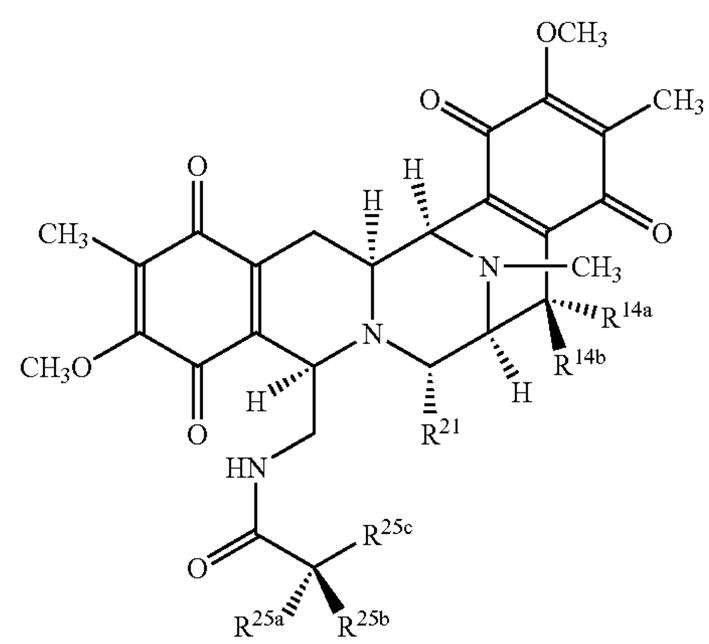
Other literature on the ecteinasdin compounds includes: Corey, E. J., *J. Am. Chem. Soc.*, 1996, 118 pp. 9202-9203; Rinehart, et al., *Journal of Natural Products*, 1990, "Bioactive Compounds from Aquatic and Terrestrial Sources", vol. 53, pp. 771-792; Rinehart et al., *Pure and Appl. Chem.*, 1990, "Biologically active natural products", vol 62, pp. 1277-1280; Rinehart, et al., *J. Org. Chem.*, 1990, "Ecteinascidins 729, 743, 745, 759A, 759B, and 770: potent Antitumour Agents from the Caribbean Tunicate *Ecteinascidia turninata*", vol. 55, pp. 4512-4515; Wright et al., *J. Org. Chem.*, 1990, "Antitumour Tetrahydroisoquinoline Alkaloids from the Colonial ascidian *Ecteinascidia turbinata*", vol. 55, pp. 4508-4512; Sakai et al., *Proc. Natl. Acad. Sci.* USA 1992, "Additional anitumor ecteinascidins from a Caribbean tunicate: Crystal structures and activities in vivo", vol. 89, 11456-11460; *Science* 1994, "Chemical Prospectors Scour the Seas for Promising Drugs", vol. 266, pp. 1324; Koenig, K. E., "Asymmetric Synthesis", ed. Morrison, Academic Press, Inc., Orlando, Fla., vol. 5, 1985, p. 71; Barton, et al., *J. Chem Soc. Perkin Trans.*, 1, 1982, "Synthesis and Properties of a Series of Sterically Hindered Guanidine bases", pp. 2085; Fukuyama et al., *J. Am. Chem. Soc.*, 1982, "Stereocontrolled Total Synthesis of (+)-Saframycin B", vol. 104, pp. 4957; Fukuyama et al., *J. Am. Chem. Soc.*, 1990, "Total Synthesis of (+)-Saframycin A", vol. 112, p. 3712; Saito, et al., *J. Org. Chem.*, 1989, "Synthesis of Saframycins. Preparation of a Key tricyclic Lactam Intermediate to Saframycin A", vol. 54, 5391; Still, et al., *J. Org. Chem.*, 1978, "Rapid Chromatographic Technique for Preparative Separations with Moderate Resolution", vol. 43, p. 2923; Kofron, W. G.; Baclawski, L. M., *J. Org. Chem.*, 1976, vol. 41, 1879; Guan et al., *J. Biomolec. Struc. & Dynam.*, vol. 10, pp. 793-817 (1993); Shamma et al., "Carbon-13 NMR Shift Assignments of Amines and Alkaloids", p. 206 (1979); Lown et al., *Biochemistry*, 21, 419-428 (1982); Zmijewski et al., *Chem. Biol. Interactions*, 52, 361-375 (1985); Ito, *CRC Crit. Rev. Anal. Chem.*, 17, 65-143 (1986); Rinehart et al., "Topics in Pharmaceutical Sciences 1989", pp. 613-626, D. D. Breimer, D. J. A. Cromwelin, K. K. Midha, Eds., Amsterdam Medical Press B. V., Noordwijk, The Netherlands (1989); Rinehart et al., "Biological Mass Spectrometry", 233-258 eds. Burlingame et al., Elsevier Amsterdam (1990); Guan et al., *Jour. Biomolec. Struct. & Dynam.*, vol. 10 pp. 793-817 (1993); Nakagawa et al., *J. Am. Chem. Soc*, 111: 2721-2722 (1989); Lichter et al., "Food and Drugs from the Sea Proceedings" (1972), Marine Technology Society, Washington, D.C. 1973, 117-127; Sakai et al., *J. Am. Chem. Soc.*, 1996, 118, 9017; Garcia-Rocha et al., *Brit. J. Cancer*, 1996, 73: 875-883; and Pommier et al., *Biochemistry*, 1996, 35: 13303-13309.

Further compounds are known which lack a bridged cyclic ring system. They include the bis(tetrahydroisoquinoline-quinone) antitumor-antimicrobial antibiotics safracins and saframycins, and the marine natural products renieramicins and xestomycin isolated from cultured microbes or sponges. They all have a common dimeric tetrahydroisoquinoline carbon framework. These compounds can be classified into four types, types I to IV, with respect to the oxidation pattern of the aromatic rings.

Type I, dimeric isoquinolinequinones, is a system of formula (VIII) most commonly occurring in this class of compounds, see the following table I.

TABLE I

Structure of Type I Saframycin Antibiotics.

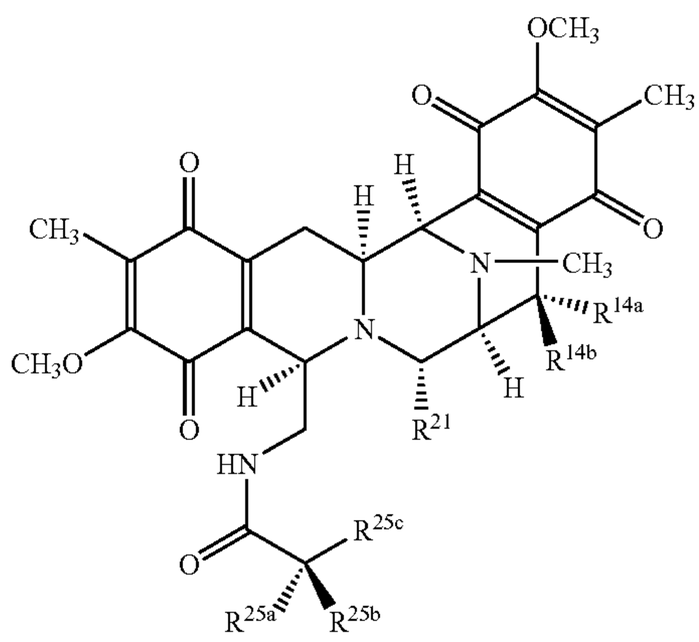


Substituents

Compound	R^{14a}	R^{14b}	R^{21}	R^{25a}	R^{25b}	R^{25c}
saframycin A	H	H	CN	O	O	CH_3
saframycin B	H	H	H	O	O	CH_3
saframycin C	H	OCH_3	H	O	O	CH_3
saframycin G	H	OH	CN	O	O	CH_3
saframycin H	H	H	CN	OH	CH_2COCH_3	CH_3
saframycin S	H	H	OH	O	O	CH_3
saframycin Y_3	H	H	CN	NH_2	H	CH_3
saframycin Yd_1	H	H	CN	NH_2	H	C_2H_5
saframycin Ad_1	H	H	CN	O	O	C_2H_5
saframycin Yd_2	H	H	CN	NH_2	H	H
saframycin Y_{2b}	H	Q^b	CN	NH_2	H	CH_3
saframycin Y_{2b-d}	H	Q^b	CN	NH_2	H	C_2H_5
saframycin AH_2	H	H	CN	H^a	OH^a	CH_3
saframycin AH_2Ac	H	H	CN	H	OAc	CH_3
saframycin AH_1	H	H	CN	OH^a	H^a	CH_3

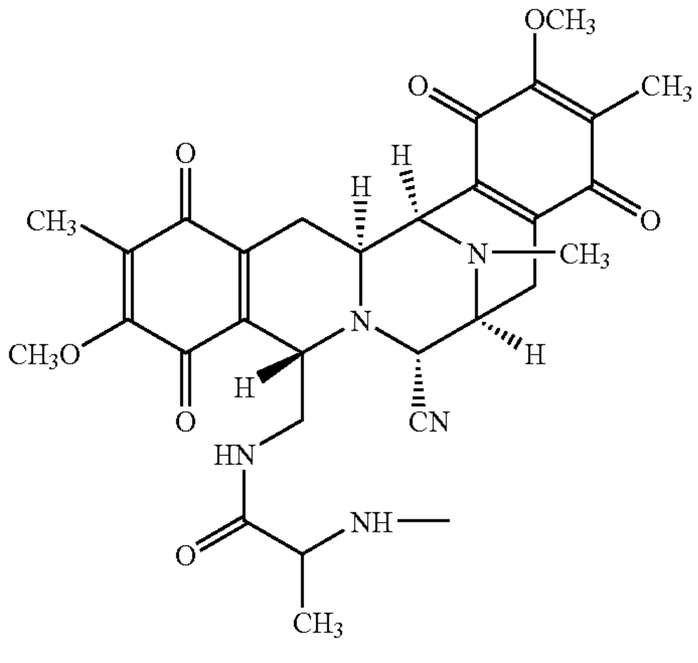
TABLE I-continued

Structure of Type I Saframycin Antibiotics.



Compound	Substituents R^{14a}	R^{14b}	R^{21}	R^{25a}	R^{25b}	R^{25c}
saframycin AH_1Ac	H	H	CN	OAc	H	CH_3
saframycin AR_3	H	H	H	H	OH	CH_3

[a]assignments are interchangeable.
[b]where the group Q is of formula (IX):

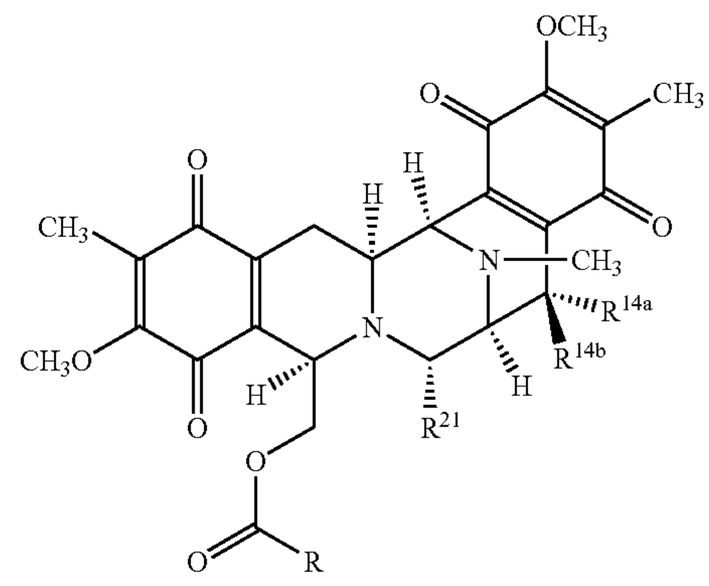


Type I aromatic rings are seen in saframycins A, B and C; G and H; and S isolated from *Streptomyces lavendulae* as minor components. A cyano derivative of saframycin A, called cyanoquinonamine, is known from Japanese Kokai JP-A2 59/225189 and 60/084,288. Saframycins Y_3, Yd_1, Ad_1, and Yd_2 were produced by *S. lavendulae* by directed biosynthesis, with appropriate supplementation of the culture medium. Saframycins Y_{2b} and $Y_{2b\text{-}d}$ dimers formed by linking the nitrogen on the C-25 of one unit to the C-14 of the other, have also been produced in supplemented culture media of *S. lavendulae*. Saframycins AR_1 (=AH_2,), a microbial reduction product of saframycin A at C-25 produced by *Rhodococcus amidophilus*, is also prepared by nonstereoselective chemical reduction of saframycin A by sodium borohydride as a 1:1 mixture of epimers followed by chromatographic separation [the other isomer AH_1 is less polar]. The further reduction product saframycin AR_3, 21-decyano-25-dihydro-saframycin A, (=25-dihydrosaframycin B) was produced by the same microbial conversion. Another type of microbial conversion of saframycin A using a *Nocardia* species produced saframycin B and further reduction by a *Mycobacterium* species produced saframycin AH^1Ac. The 25-O-acetates of saframycin AH_2 and AH_1 have also been prepared chemically for biological studies.

Type I compounds of formula (X) have also been isolated from marines sponges, see Table II.

TABLE II

Structures of Type I Compounds from Marine Sponges.



	Substituents R^{14a}	R^{14b}	R^{21}	R
renieramycin A	OH	H	H	—C(CH_3)═CH—CH_3
renieramycin B	OC_2H_5	H	H	—C(CH_3)═CH—CH_3
renieramycin C	OH	O	O	—C(CH_3)═CH—CH_3
renieramycin D	OC_2H_5	O	O	—C(CH_3)═CH—CH_3
renieramycin E	H	H	OH	—C(CH_3)═CH—CH_3
renieramycin F	OCH_3	H	OH	—C(CH_3)═CH—CH_3
xestomycin	OCH_3	H	H	—CH_3

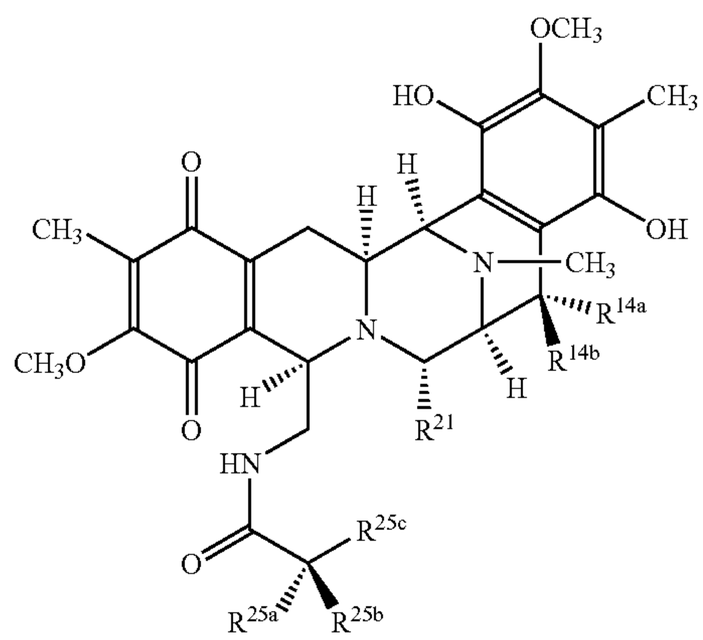
Renieramycins A-D were isolated from the antimicrobial extract of a sponge, a *Reniera* species collected in Mexico, along with the biogenetically related monomeric isoquinolines renierone and related compounds. The structure of renieramycin A was initially assigned with inverted stereochemistry at C-3, C-11, and C-13. However, careful examination of the ^1H NMR data for new, related compounds renieramycins E and F, isolated from the same sponge collected in Palau, revealed that the ring junction of renieramycins was identical to that of saframycins. This result led to the conclusion that the formerly assigned stereochemistry of renieramycins A to D must be the same as that of saframycins.

Xestomycin was found in a sponge, a *Xestospongia* species collected from Sri Lancan waters.

Type II compounds of formula (XI) with a reduced hydroquinone ring include saframycins D and F, isolated from *S. lavendulae*, and saframycins Mx-1 and Mx-2, isolated from *Myxococcus xanthus*. See table III.

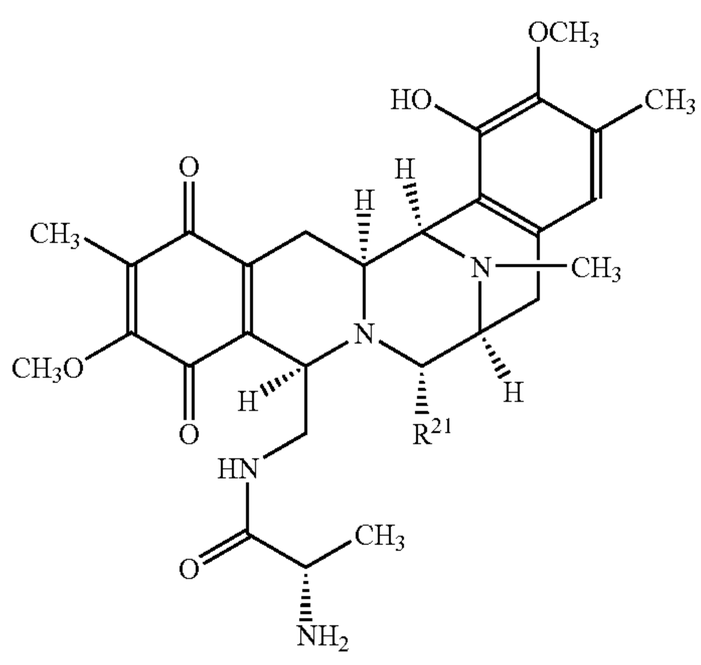
TABLE III

Type II Compounds



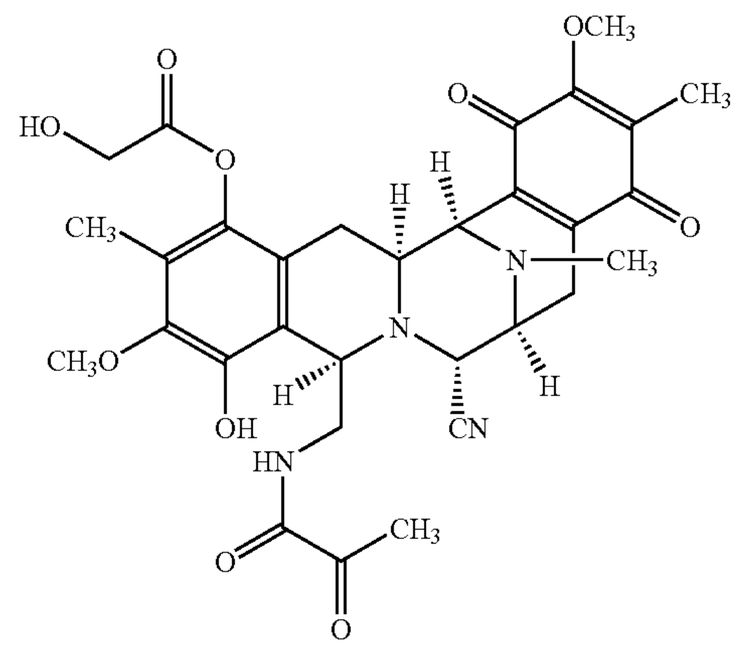
Compound	R^{14a}	R^{14b}	R^{21}	R^{25a}	R^{25b}	R^{25c}
	Substituents					
saframycin D	O	O	H	O	O	CH_3
saframycin F	O	O	CN	O	O	CH_3
saframycin Mx-1	H	OCH_3	OH	H	CH_3	NH_2
saframycin Mx-2	H	OCH_3	H	H	CH_3	NH_2

The type III skeleton is found in the antibiotics safracins A and B, isolated from cultured *Pseudomonas fluorescens*. These antibiotics of formula (XII) consist of a tetrahydroisoquinoline-quinone subunit and a tetrahydroisoquninolinephenol subunit.

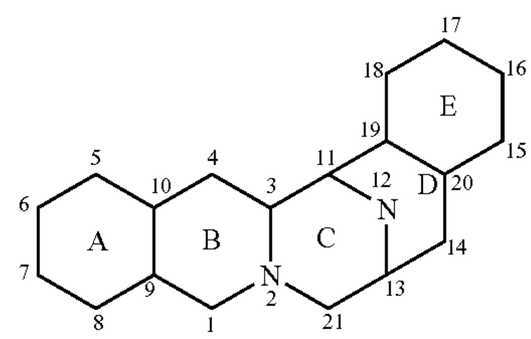


where R^{21} is —H in safracin A and is —OH in safracin B.

Saframycin R, the only compound classified as the Type IV skeleton, was also isolated from *S. lavendulae*. This compound of formula (XIII), consisting of a hydroquinone ring with a glycolic ester sidechain on one of the phenolic oxygens, is conceivably a pro-drug of saframycin A because of its moderate toxicity.



All these known compounds have a fused system of five rings (A) to (E) as shown in the following structure of formula (XIV):



The rings A and E are phenolic in the ecteinascidins and some other compounds, while in other compounds, notably the saframycins, the rings A and E are quinolic. In the known compounds, the rings B and D are tetrahydro, while ring C is perhydro.

OBJECT OF THE INVENTION

The need remains for alternative synthetic routes to the ecteinascidin compounds and related compounds. Such synthetic routes may provide more economic paths to the known antitumour agents, as well as permitting preparation of new active compounds.

SUMMARY OF THE INVENTION

This invention relates to synthetic processes for the formation of intermediates, derivatives and related structures of ecteinascidin or other tetrahydroisoquinolinephenol compounds.

In one aspect, the present invention provides a process for preparing an ecteinascidin product with a spiroamine-1,4-bridge. The process involving forming a 1,4 bridge using a 1-labile, 10-hydroxy, 18-protected hydroxy, di-6,8-en-5-one fused ring compound, where the fused ring is the formula (XIV). In the present invention, the C-18 protection is removed before spiroamine introduction.

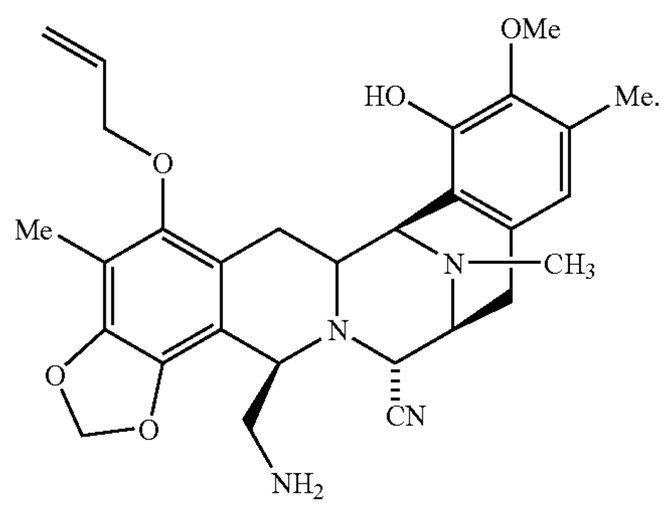
Suitable starting materials for the new synthetic processes include compounds related to the natural bis(tetrahydroisoquinoline) alkaloids. Such starting materials may be prepared either from the different classes of saframycin and safracin antibiotics available from different culture broths as detailed in WO 0069862 or by other synthetic or biochemical processes. In this respect, WO 0069862 is incorporated herein in

full by reference. The present PCT application claims priority from application PCT/GB 00/01852 which was published as WO 0069862. We incorporate that text by reference to the extent that there is disclosure therein which is not in the present specification.

PREFERRED EMBODIMENTS OF THE INVENTION

In one particular aspect, the present invention is directed at the use of the compound Intermediate 21 in a number of new synthetic processes for the preparation of ecteinascidin 743

and related compounds,



The Intermediate 21 has a 5-allyloxy group, where the allyl group serves to protect the 5-hydroxy group. It will be understood that other protecting groups can easily be employed, and that the present invention extends generally to the use of other such 5-protected hydroxy compounds.

Formation of Ecteinascidin 743 and Related Compounds

In general, the conversion of Intermediate 21, or a related compound, to an ecteinascidin product involves the following key transformations:

(a) Conversion of the NH_2 to OH by reaction, for example with sodium nitrite in acetic acid.

(b) E-ring phenol protection.

(c) Esterification by protecting the primary 1-hydroxy function with a protected cysteine sidechain.

(d) Deprotection of allyl group and oxidation.

(e) Creation of the bridged ring by cyclization reaction.

(f) Deprotections of E-ring phenol and the cysteine moiety

(g) Quinoline Introduction by Trans-amination and Petter Spengler reactions.

The high functionality of the intermediate compounds necessitates the use of protecting groups for the E-ring phenol and for the cysteine sidechain in order to prevent unwanted side reactions.

As such, a number of alternative intermediates can be generated dependent on the particular selection of protecting groups.

Different possible sequences are possible for combining these transformations dependent primarily on the protecting groups selected for the phenol ring and for the amine of the cysteine sidechain.

The total number of synthetic transformations is also a function of the protecting groups selected.

By way of illustration, the use of different combinations of protecting groups is described below for six typical routes for the preparation of ET-743 from Intermediate 21, also referred to herein as SF21.

Route	Phenol Protection	Cysteine Protection	Number of steps
1	MOM	Boc	12
2	MEM	Boc	10
3	MEM	Cbz	11
4	MOM	Alloc	13
5	MEM	Alloc	13
6	MOM	Cbz	15

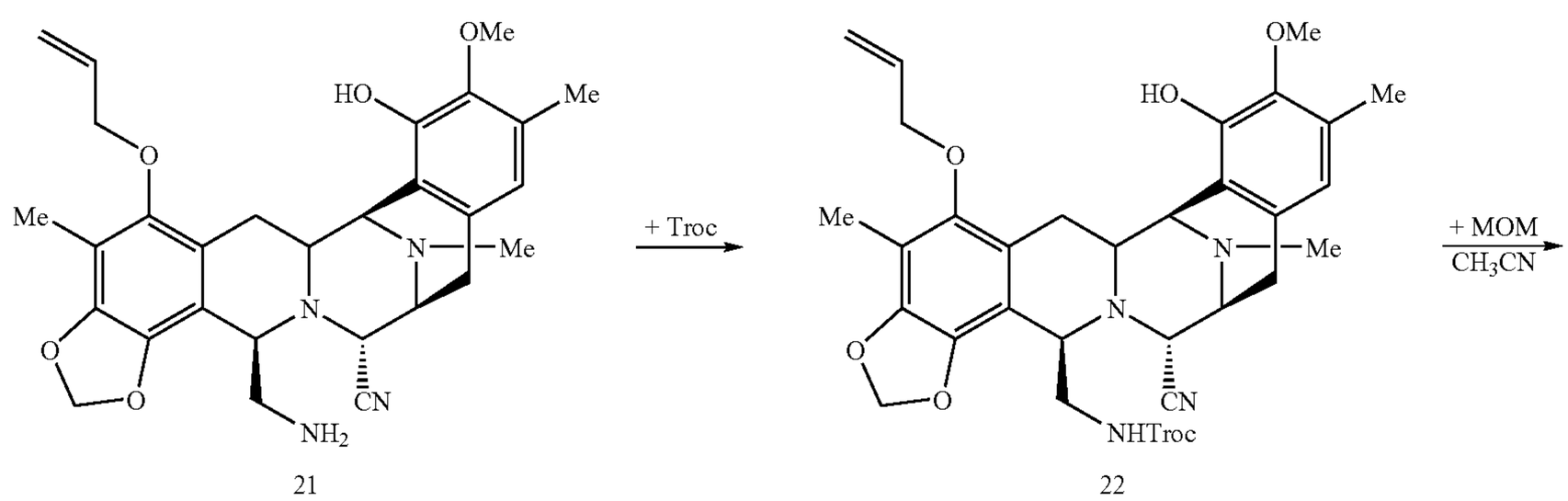
As the skilled artisan will readily appreciate, the reaction schemes described herein may be modified and/or combined in various ways, and the alternative sequences of steps and the compounds generated therefrom are part of this invention.

Additionally, the use of other protecting group strategies not detailed also part of this invention.

Process Details of Six Typical Synthetic Routes

Full reaction schemes for each route are in the following Schemes 1 to 6.

Scheme 1-ET-743: Hemisynthetic Alternative Route 1



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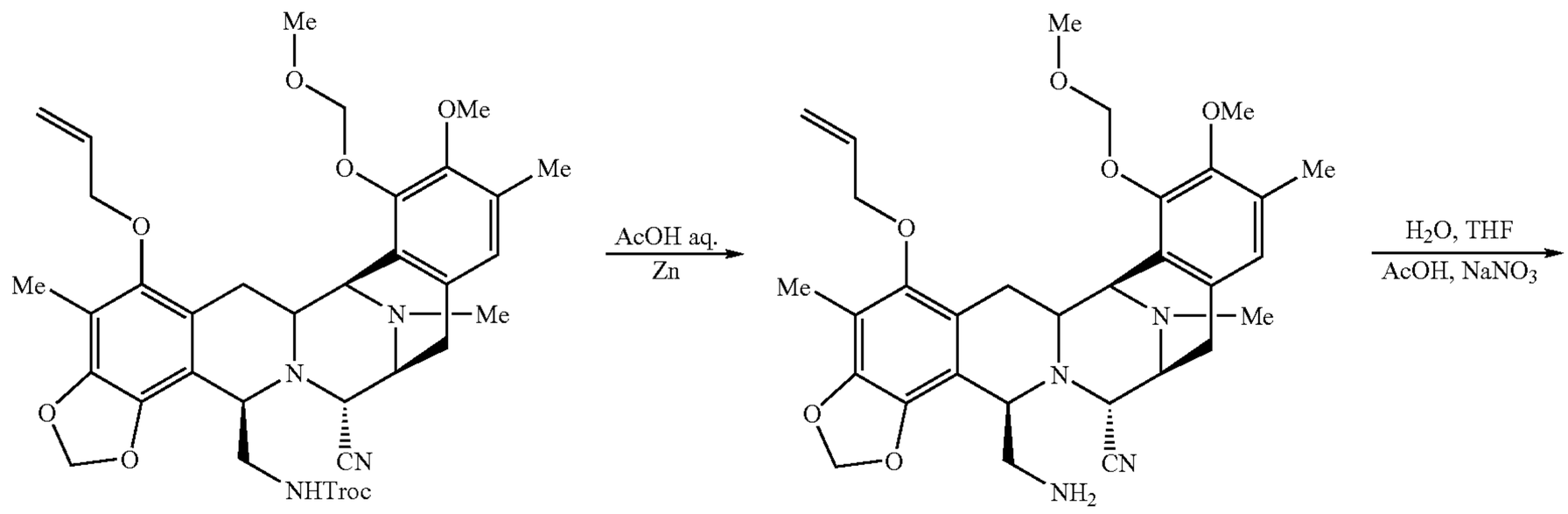

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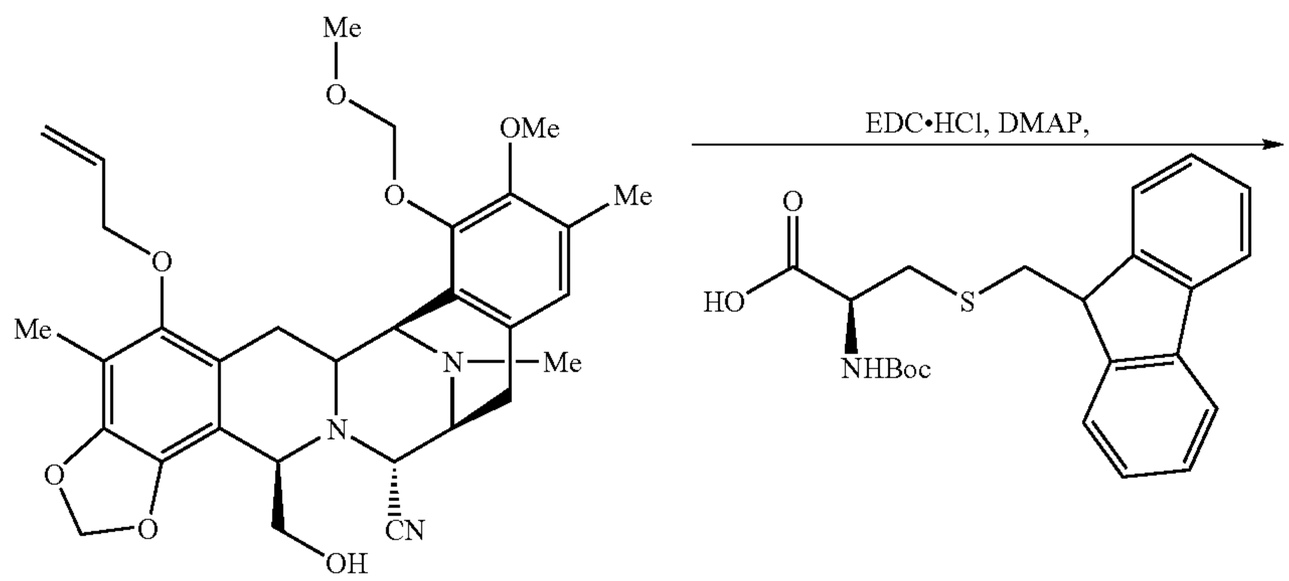

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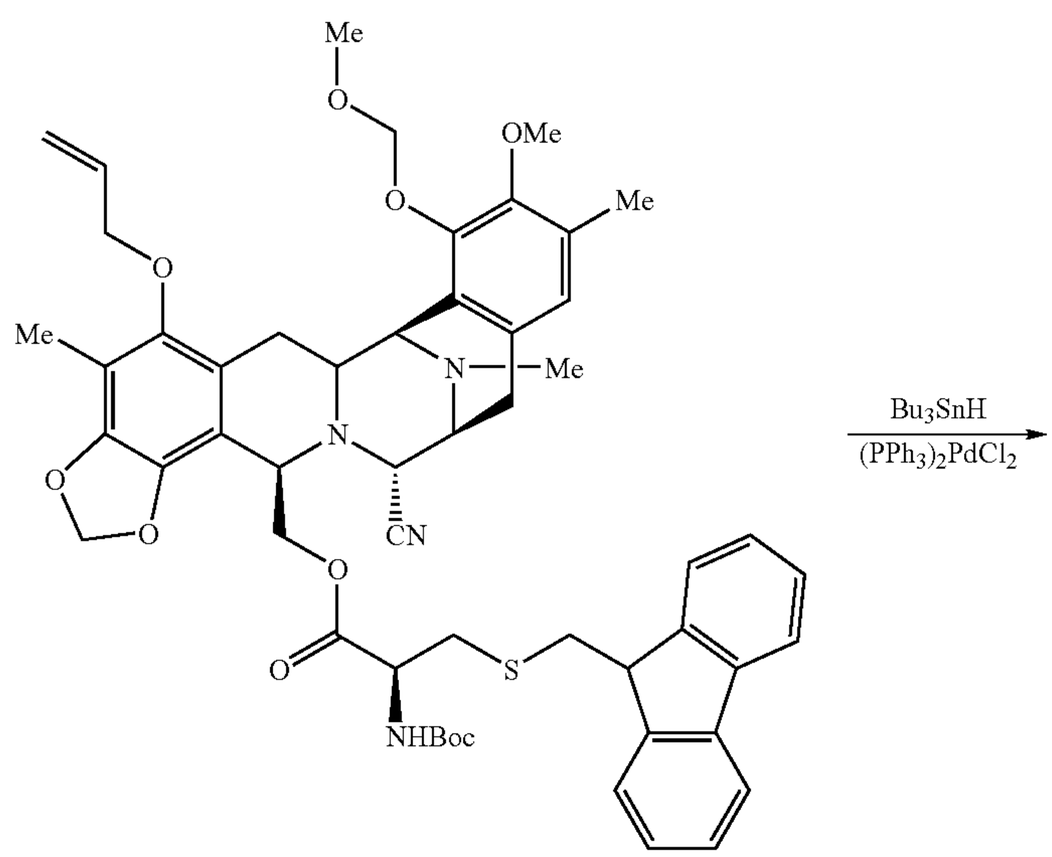

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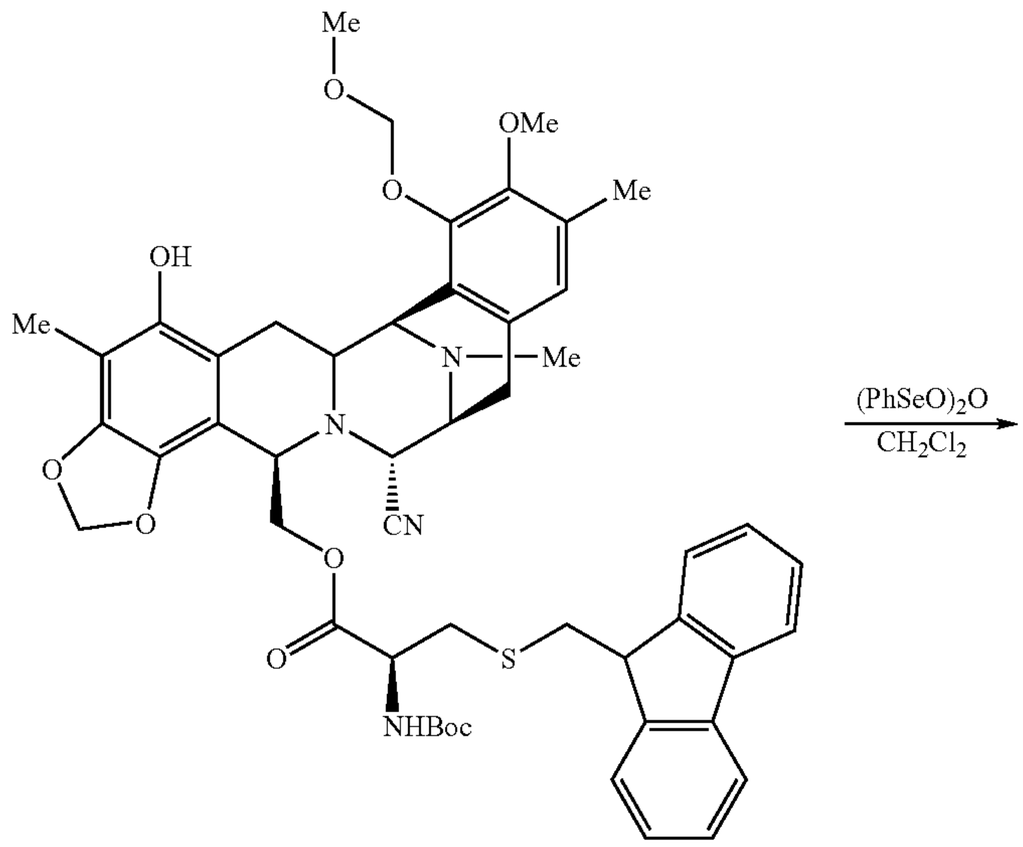

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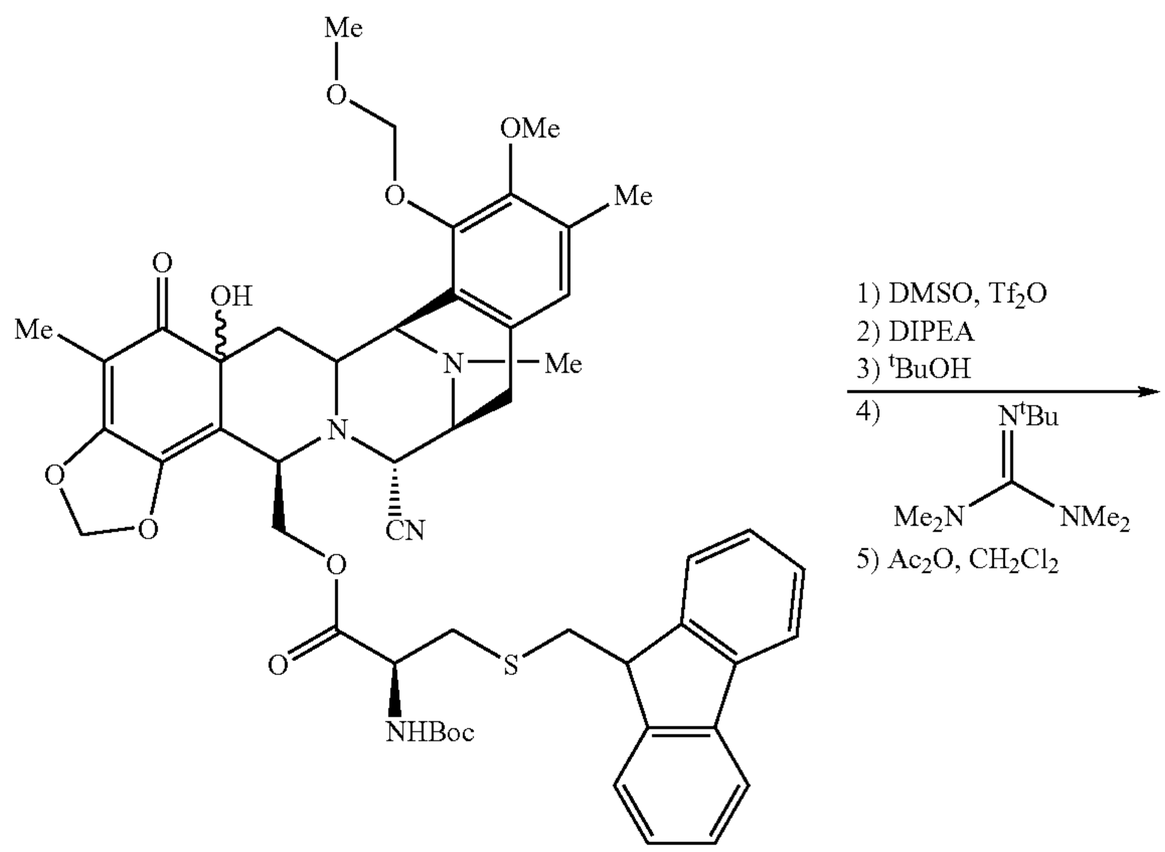

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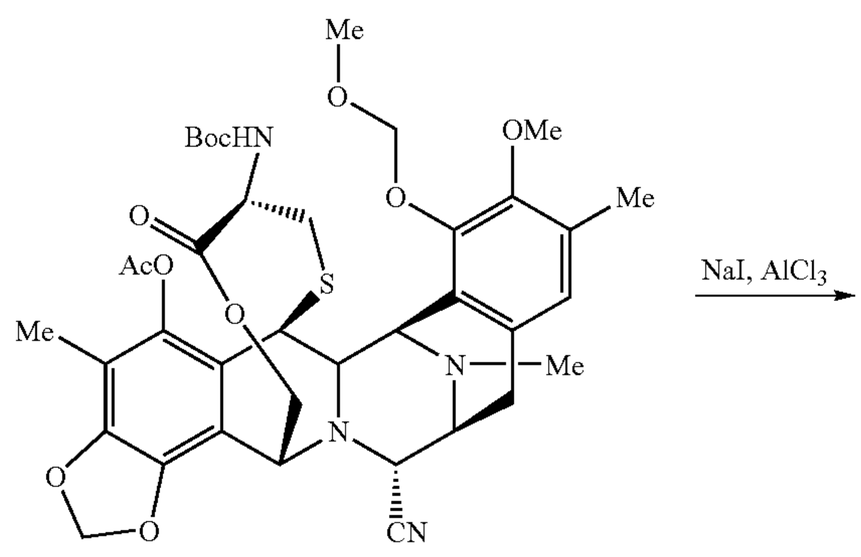

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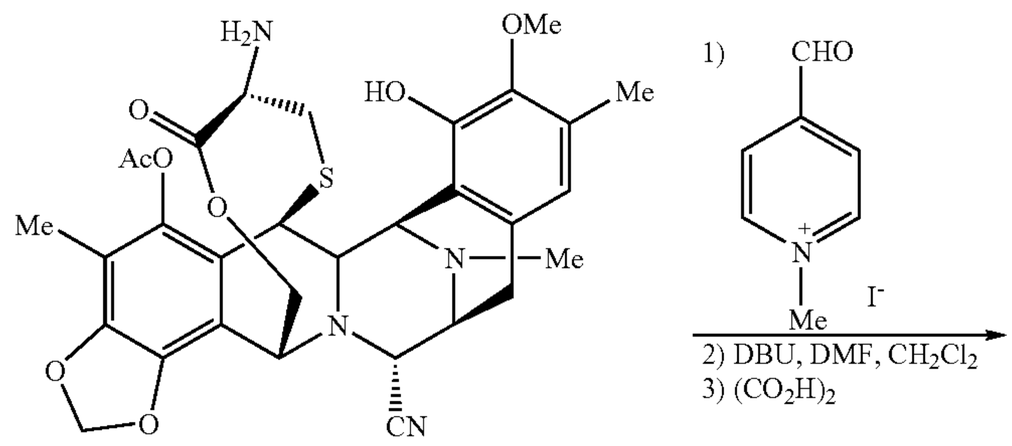

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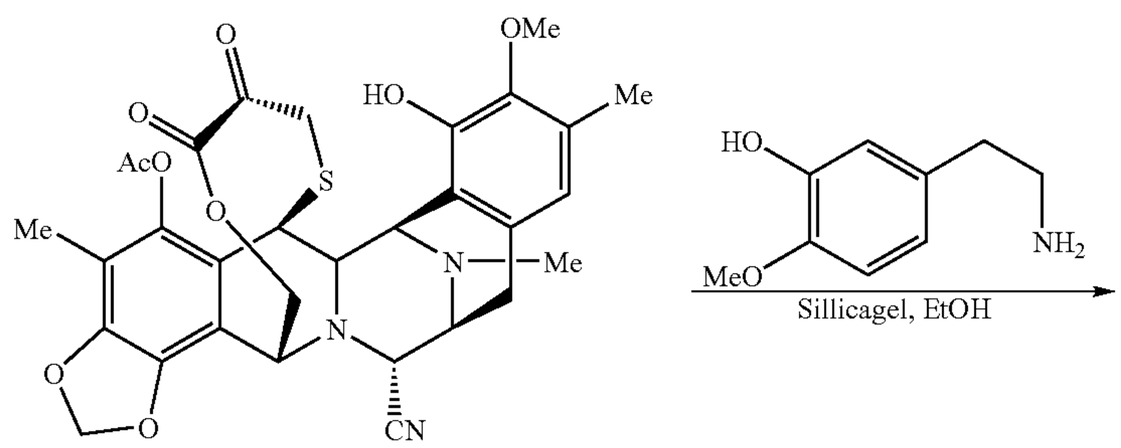

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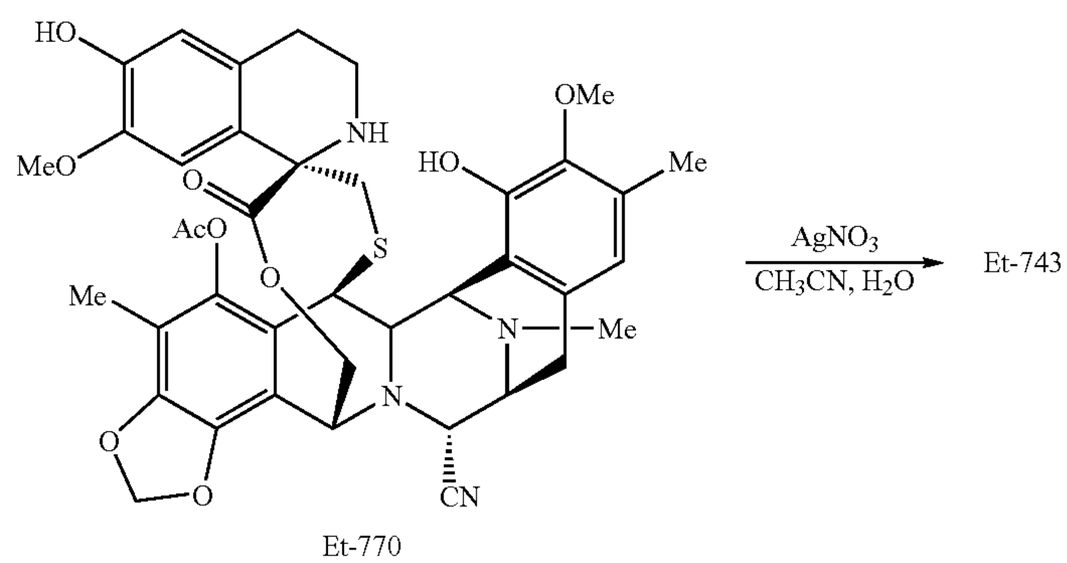

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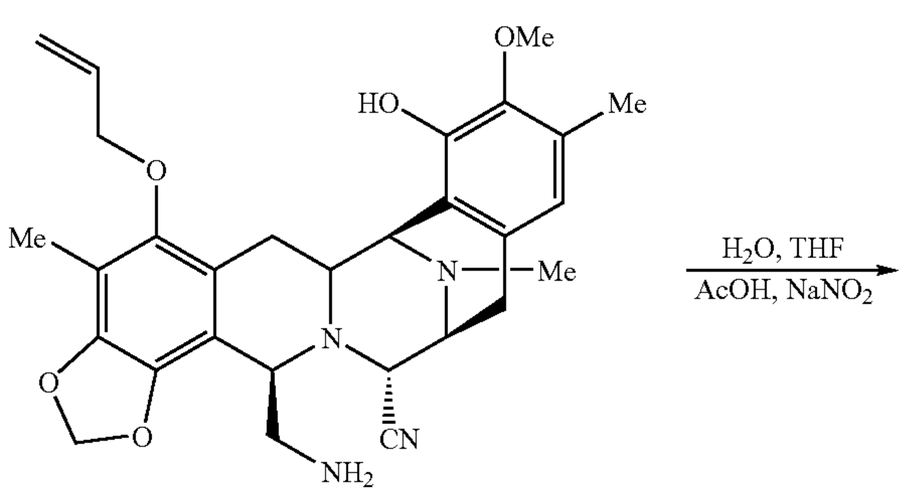
Scheme 2-ET-743: Hemisynthetic Alternative Route 2



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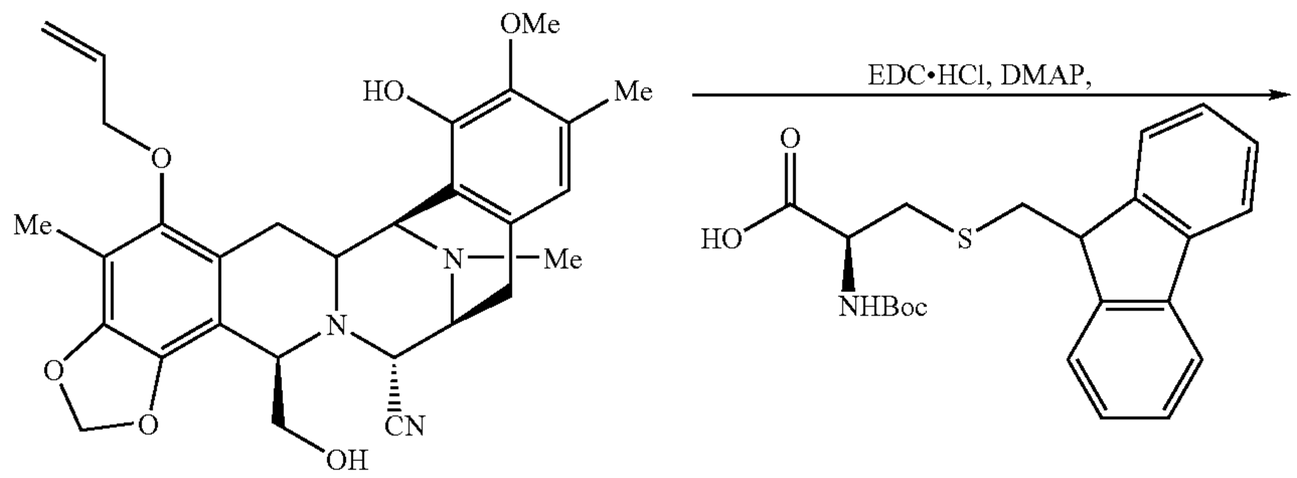

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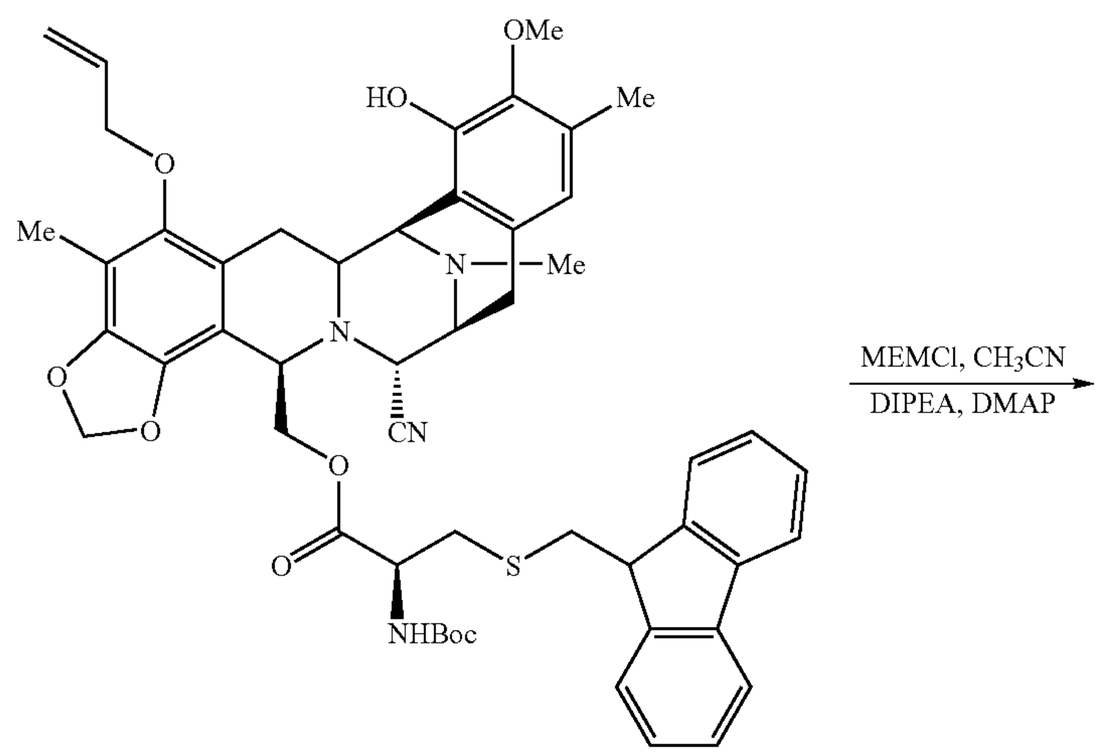

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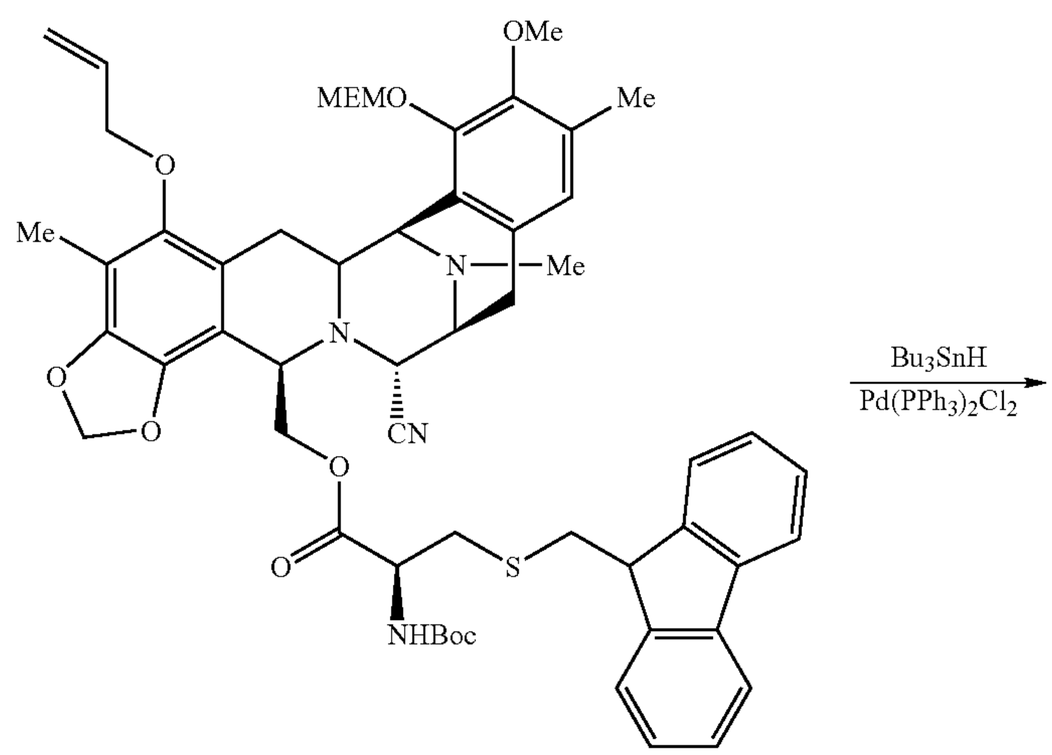

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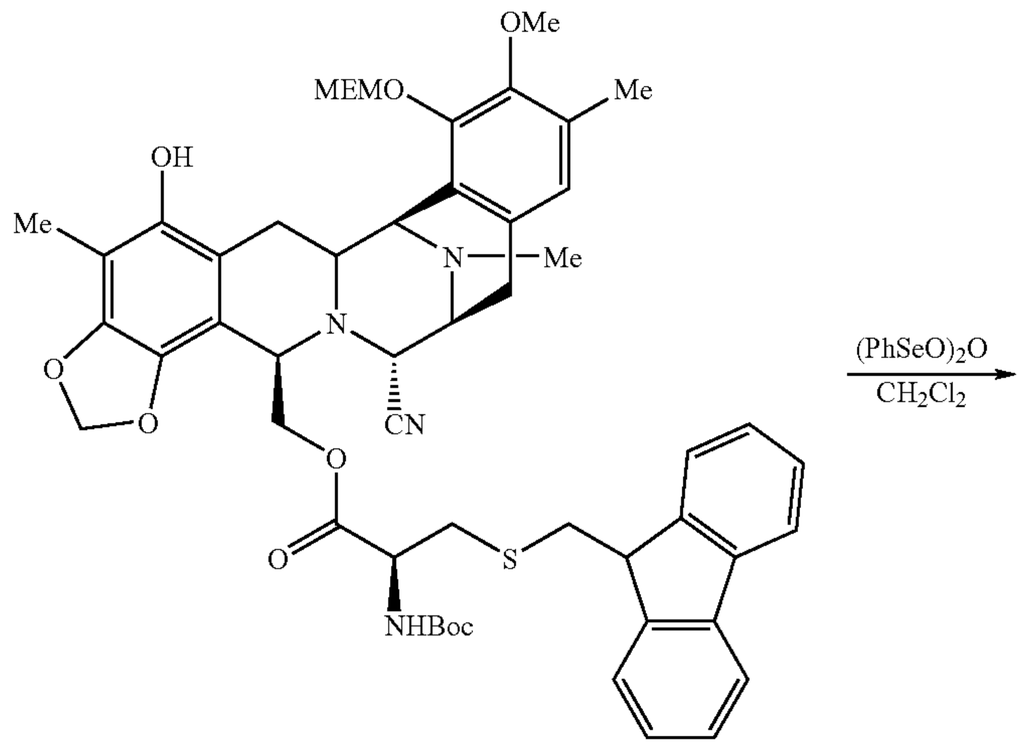

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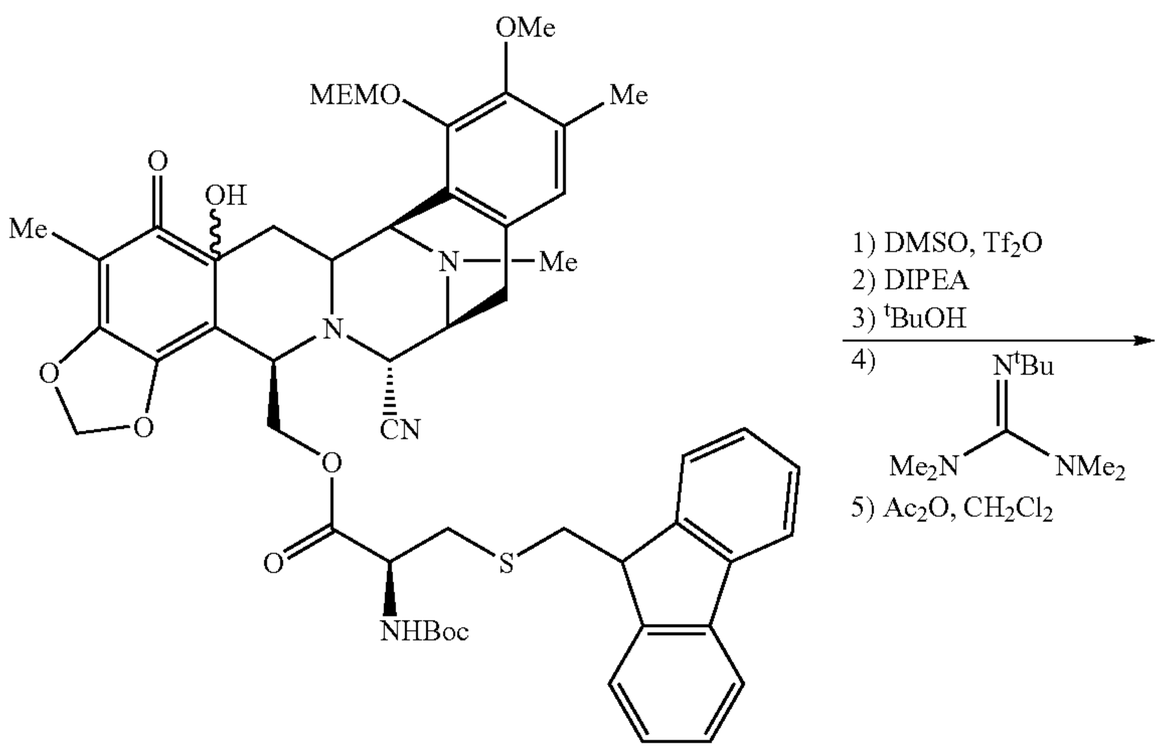

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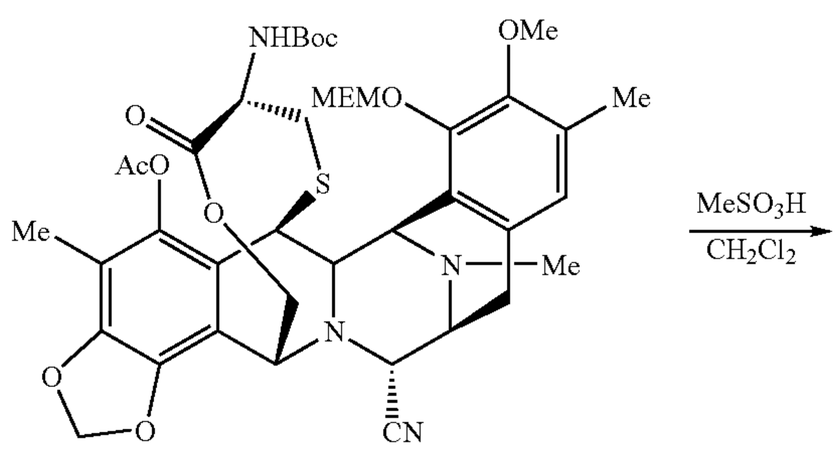

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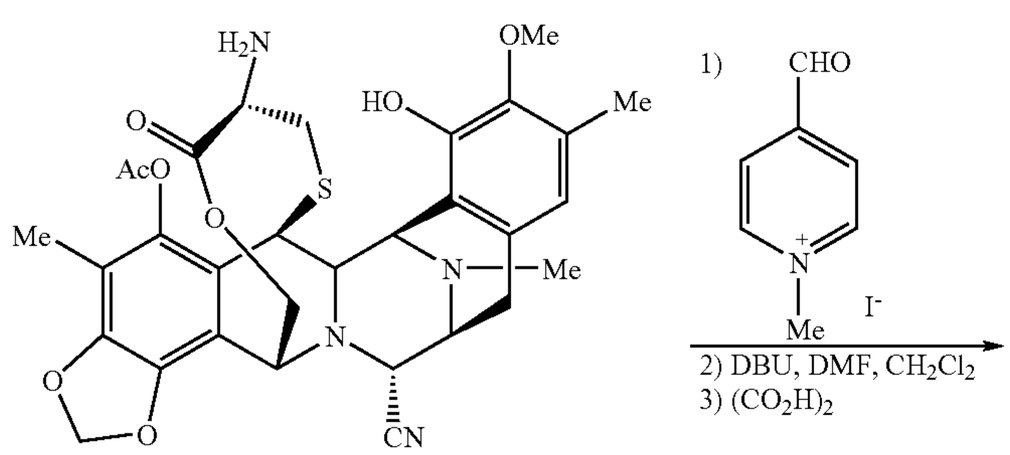

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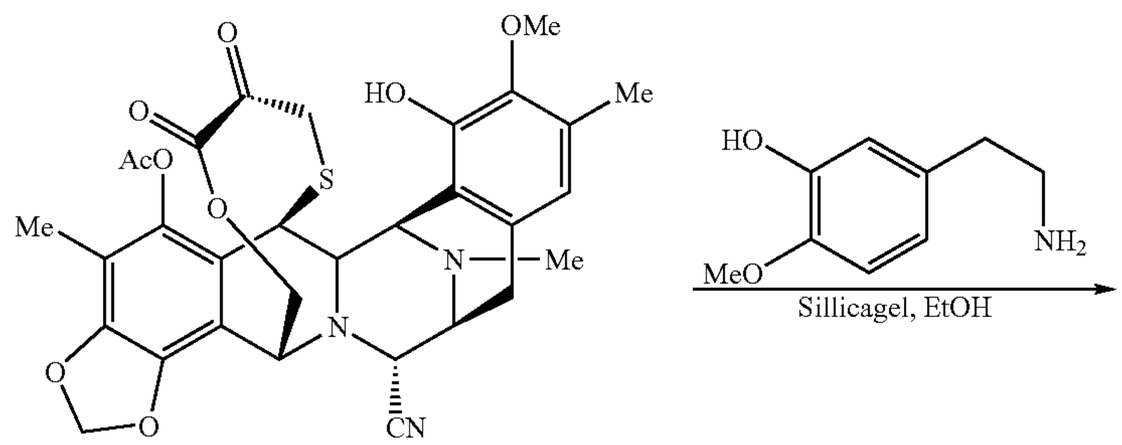

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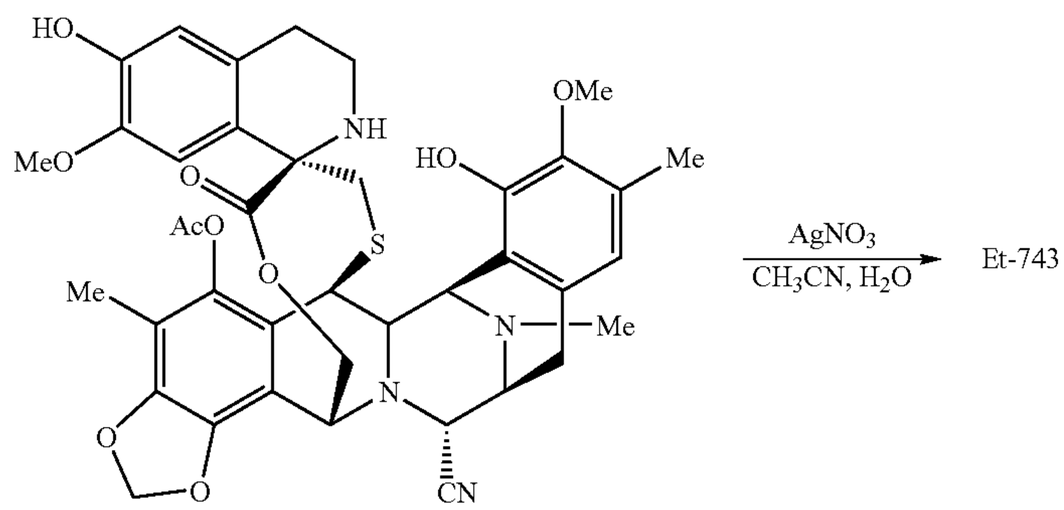

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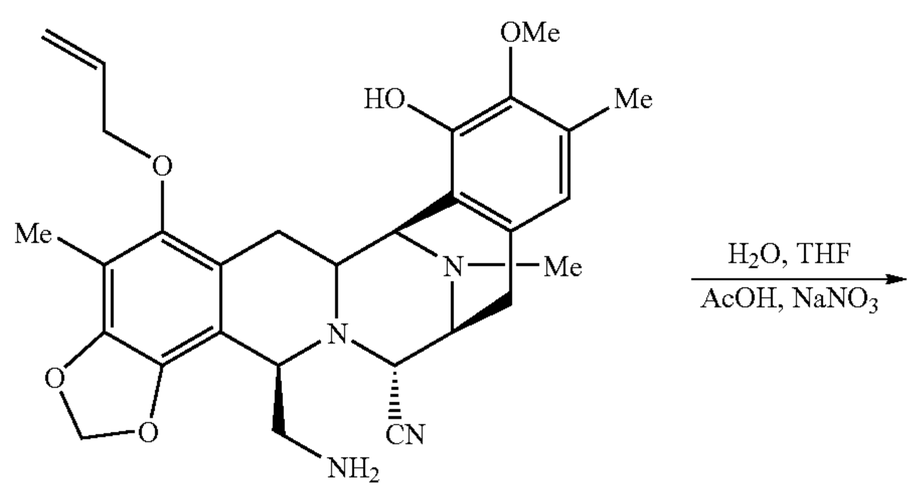
Scheme 3-ET-743: Hemisynthetic Alternative Route 3



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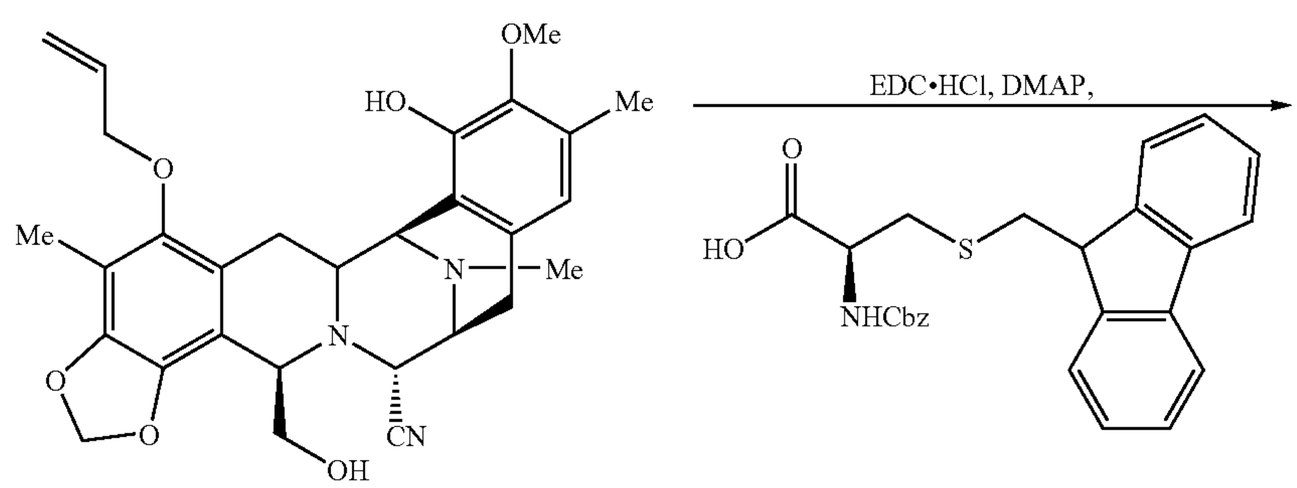

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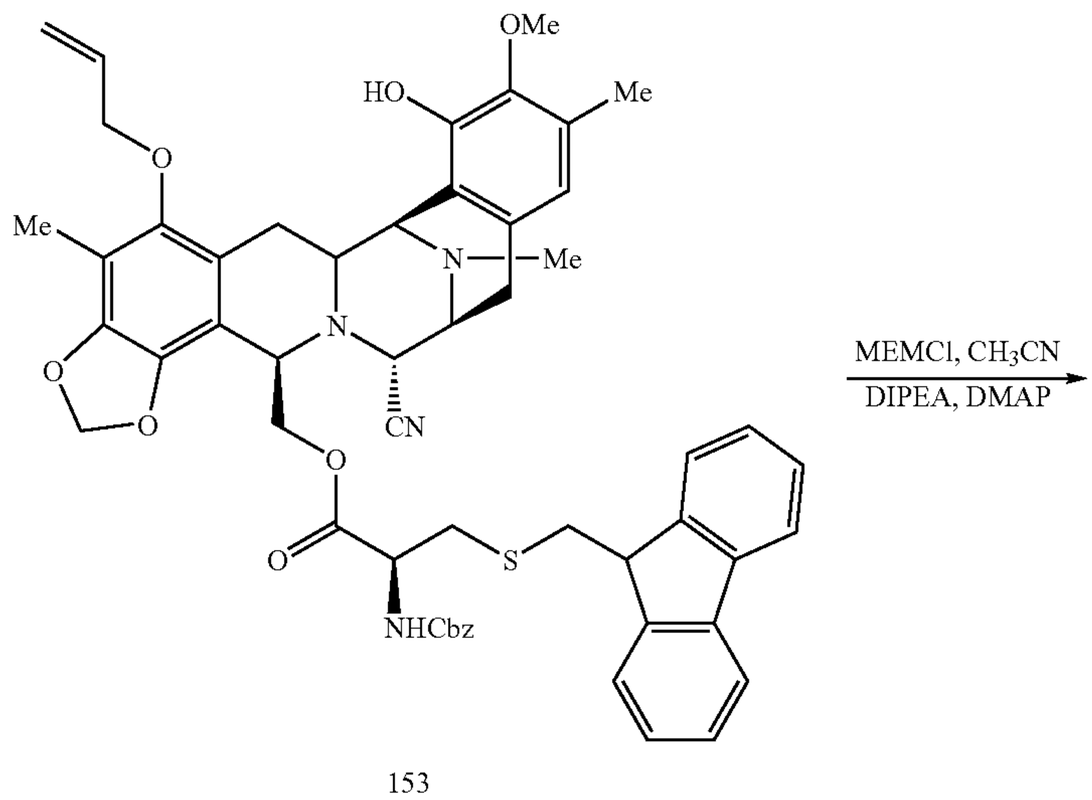

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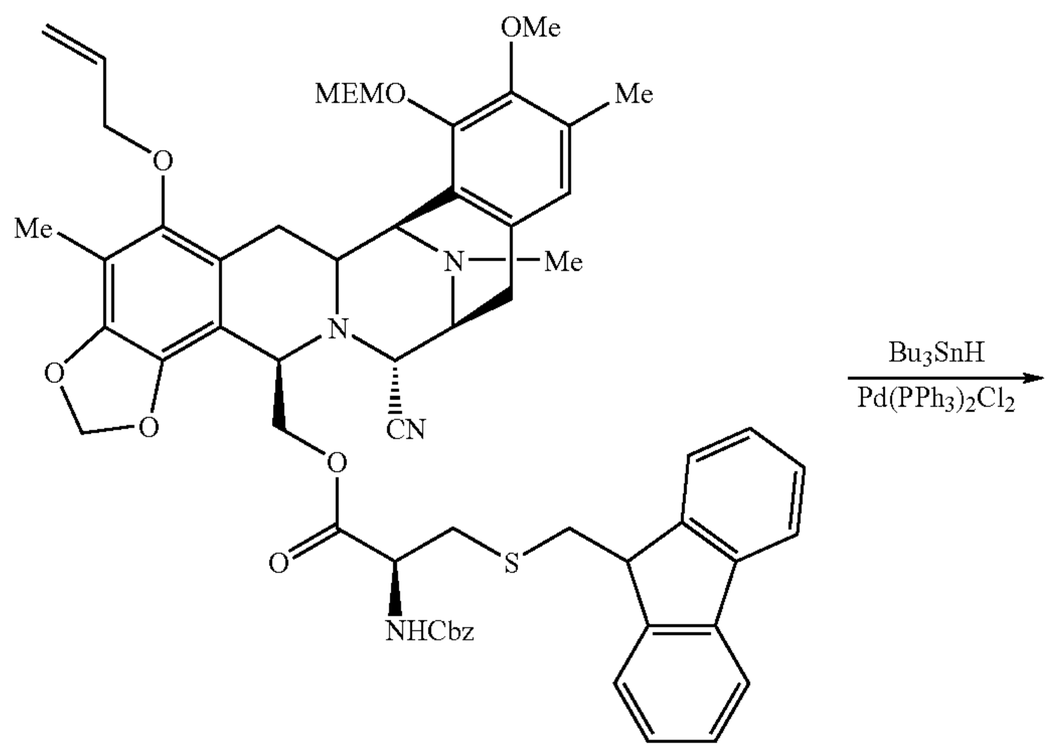

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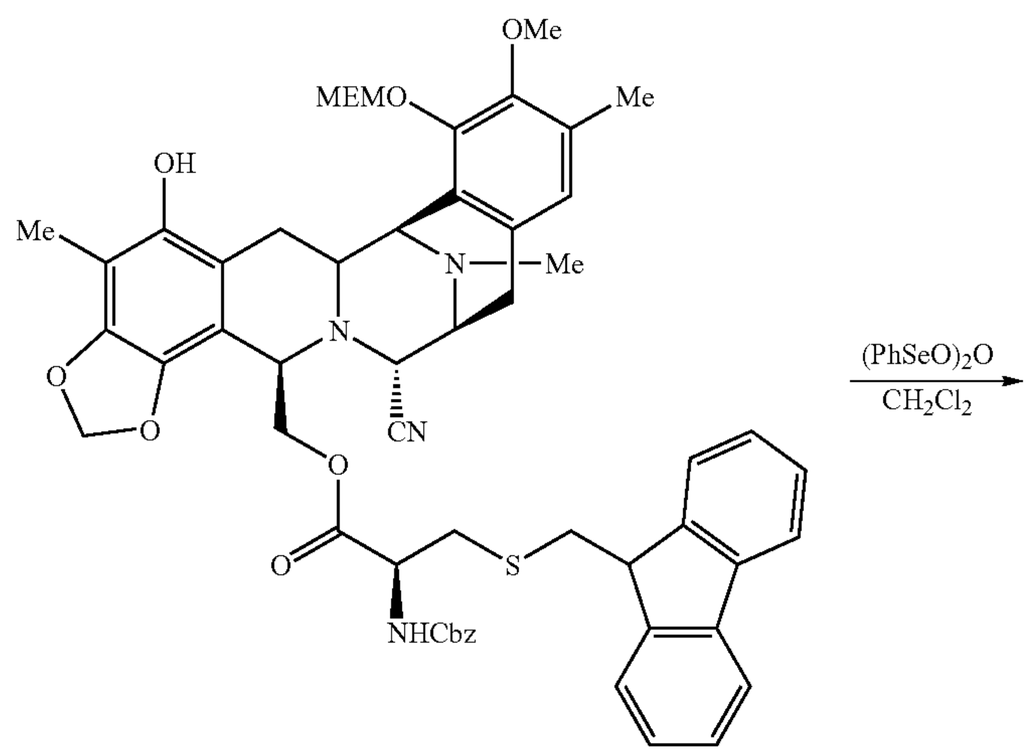

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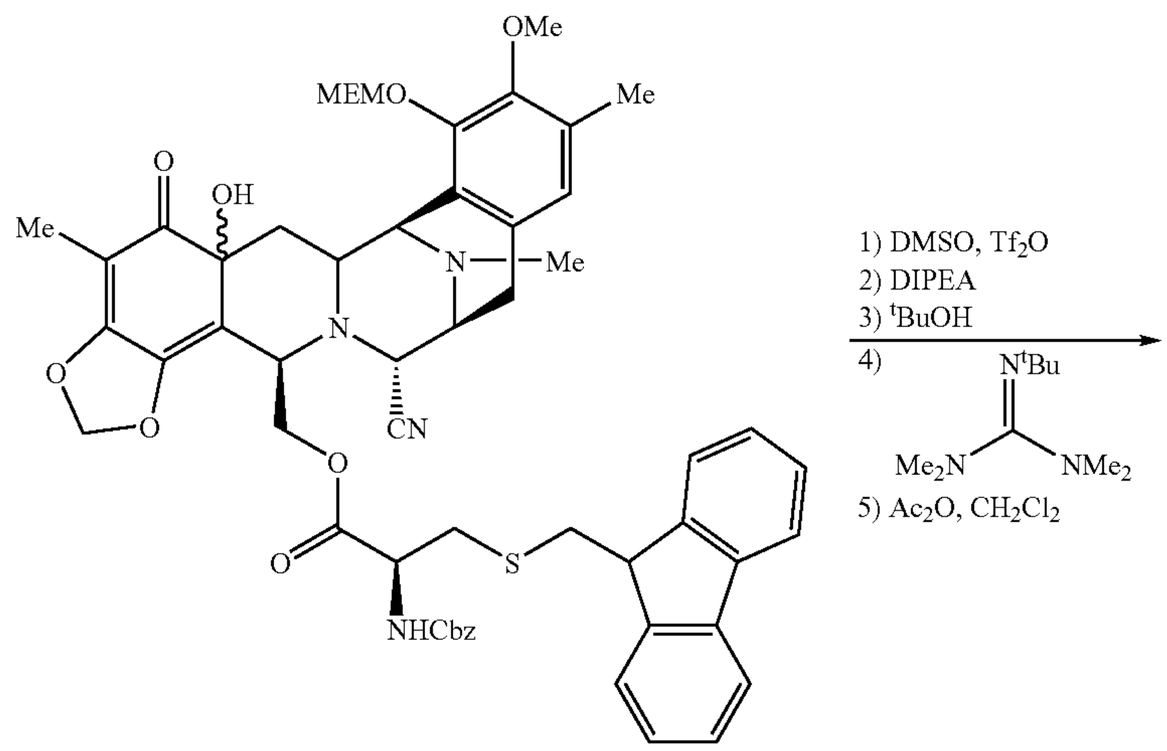

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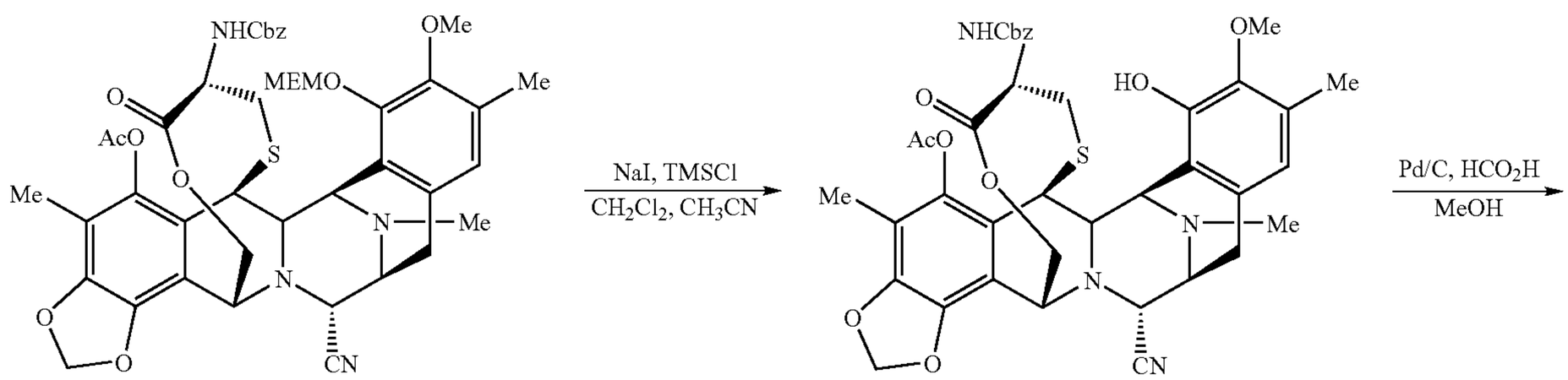

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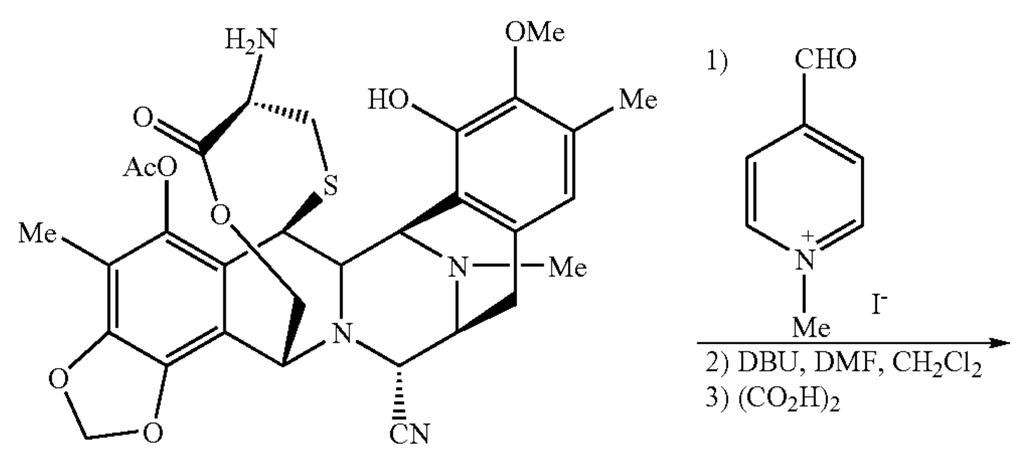

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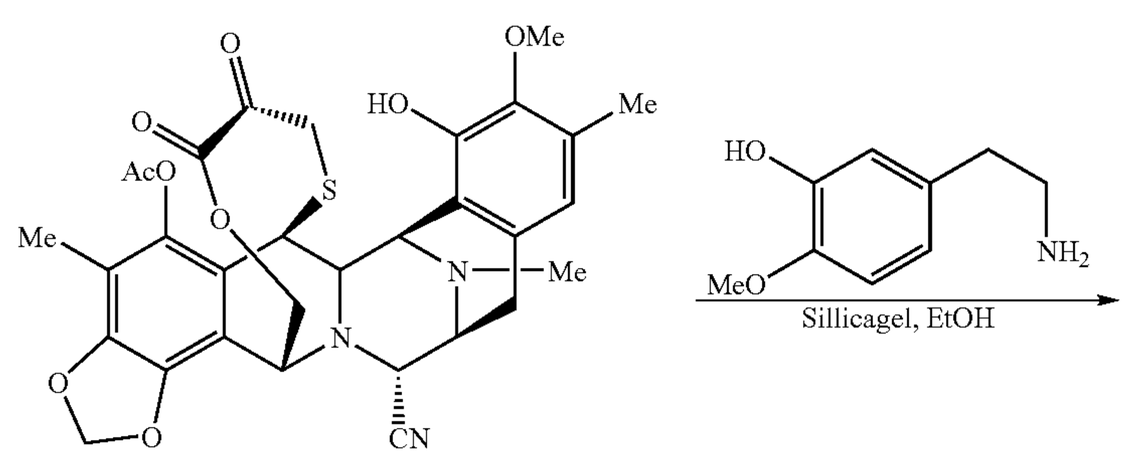

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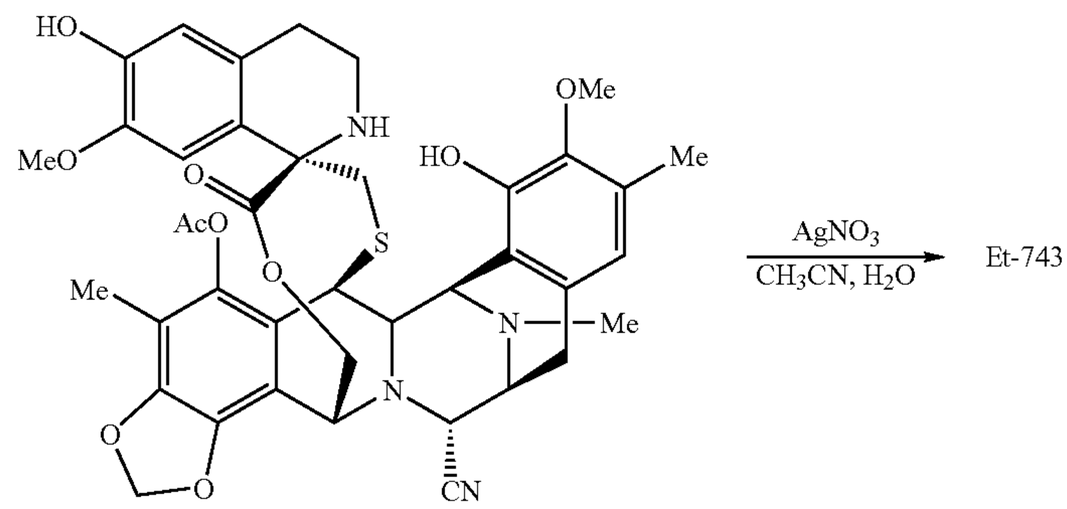

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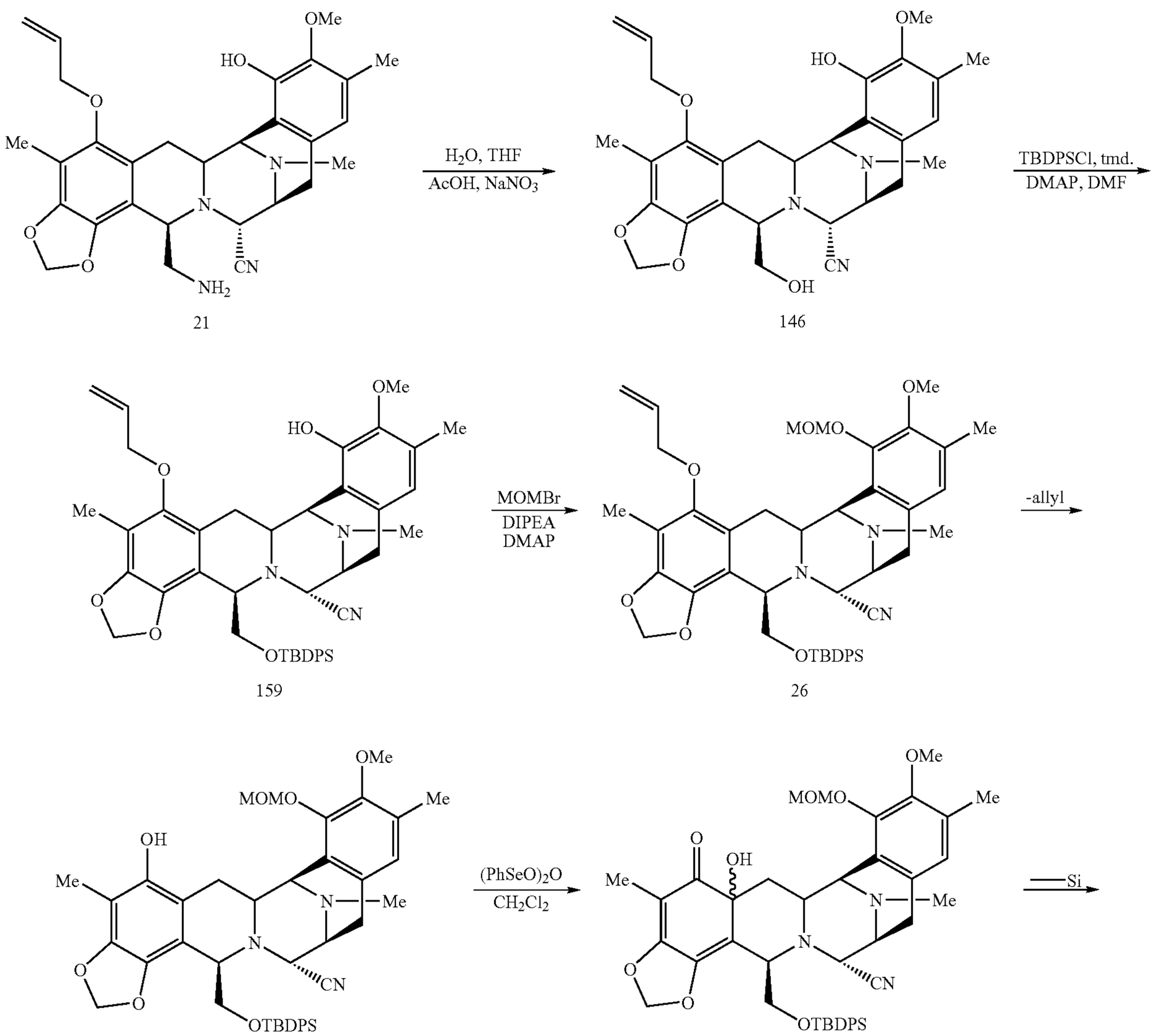
Scheme 4-ET-743: Hemisynthetic Alternative Route 4



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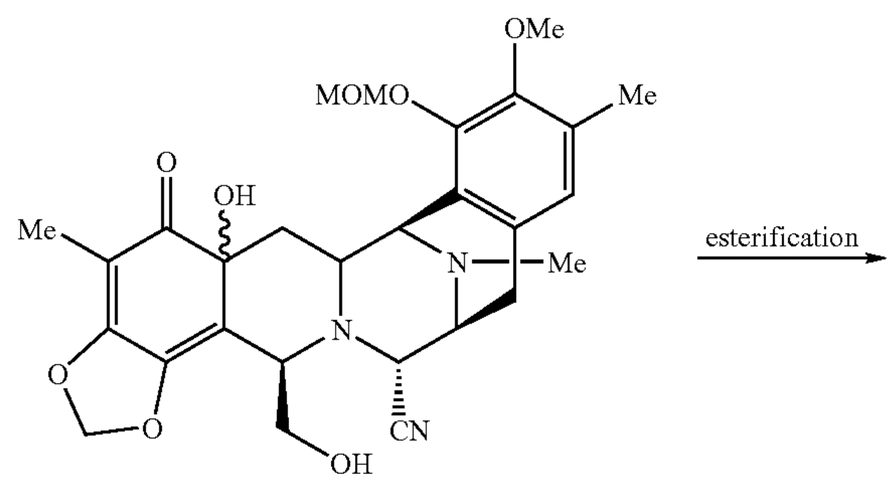

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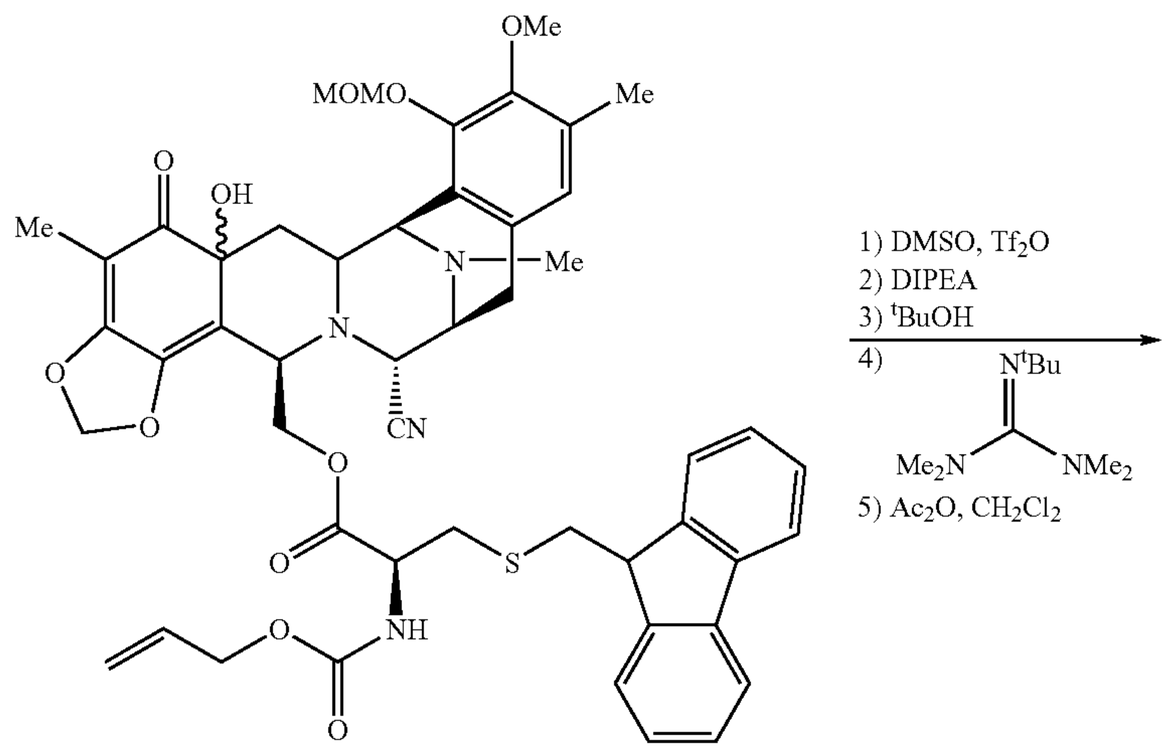

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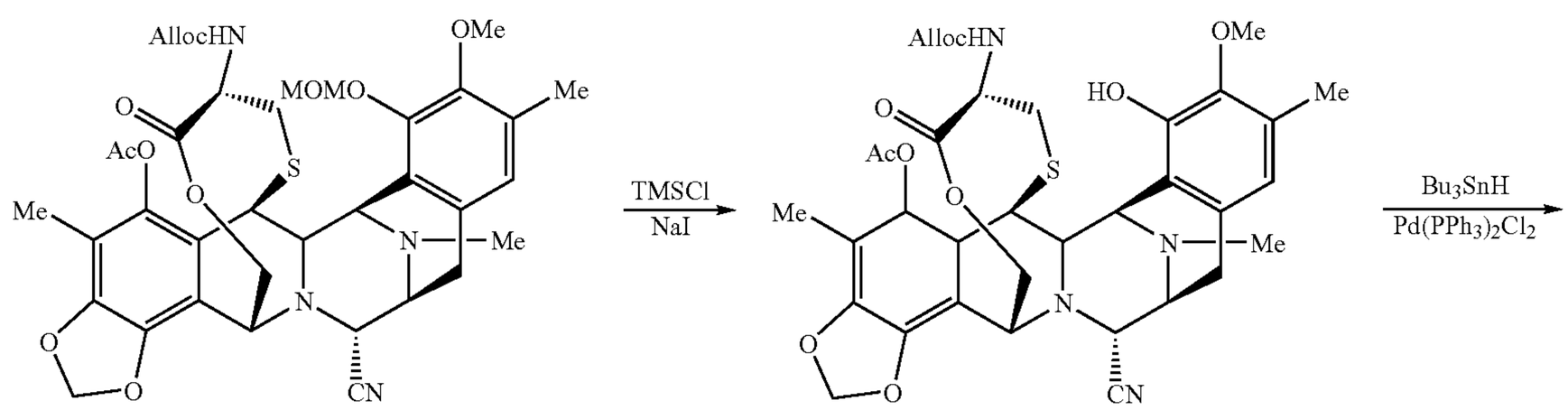

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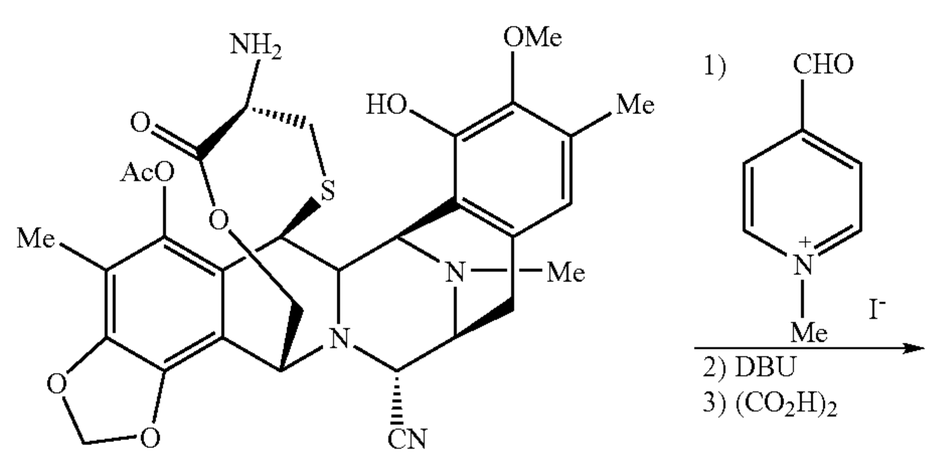

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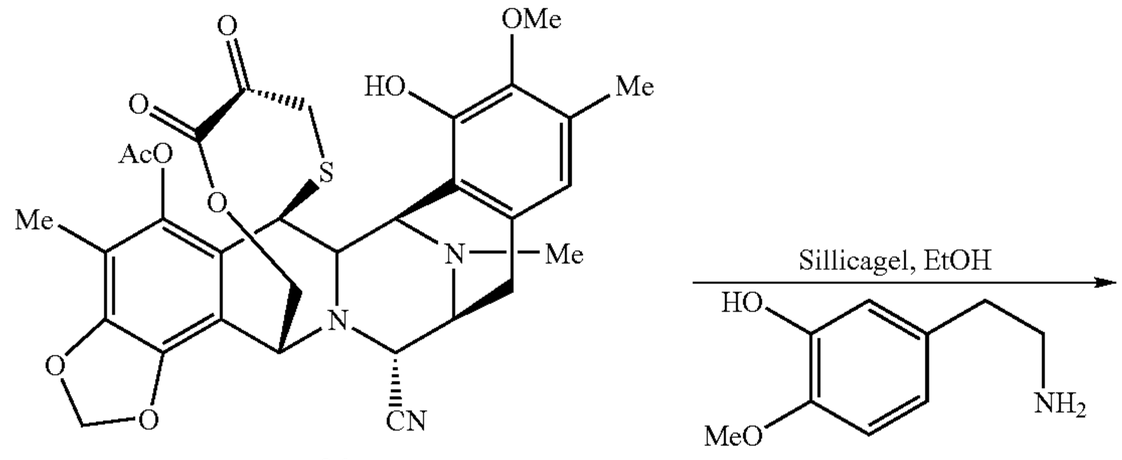

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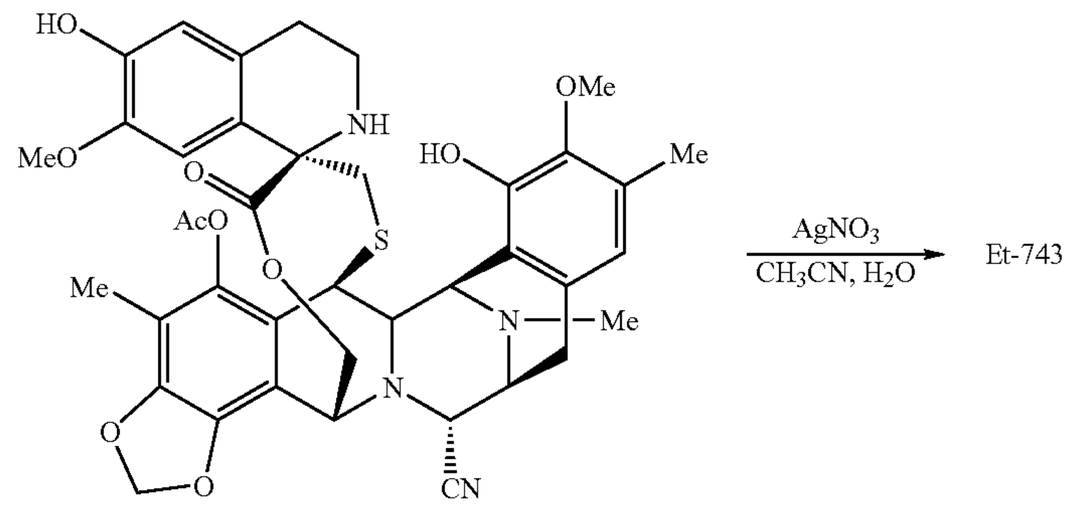

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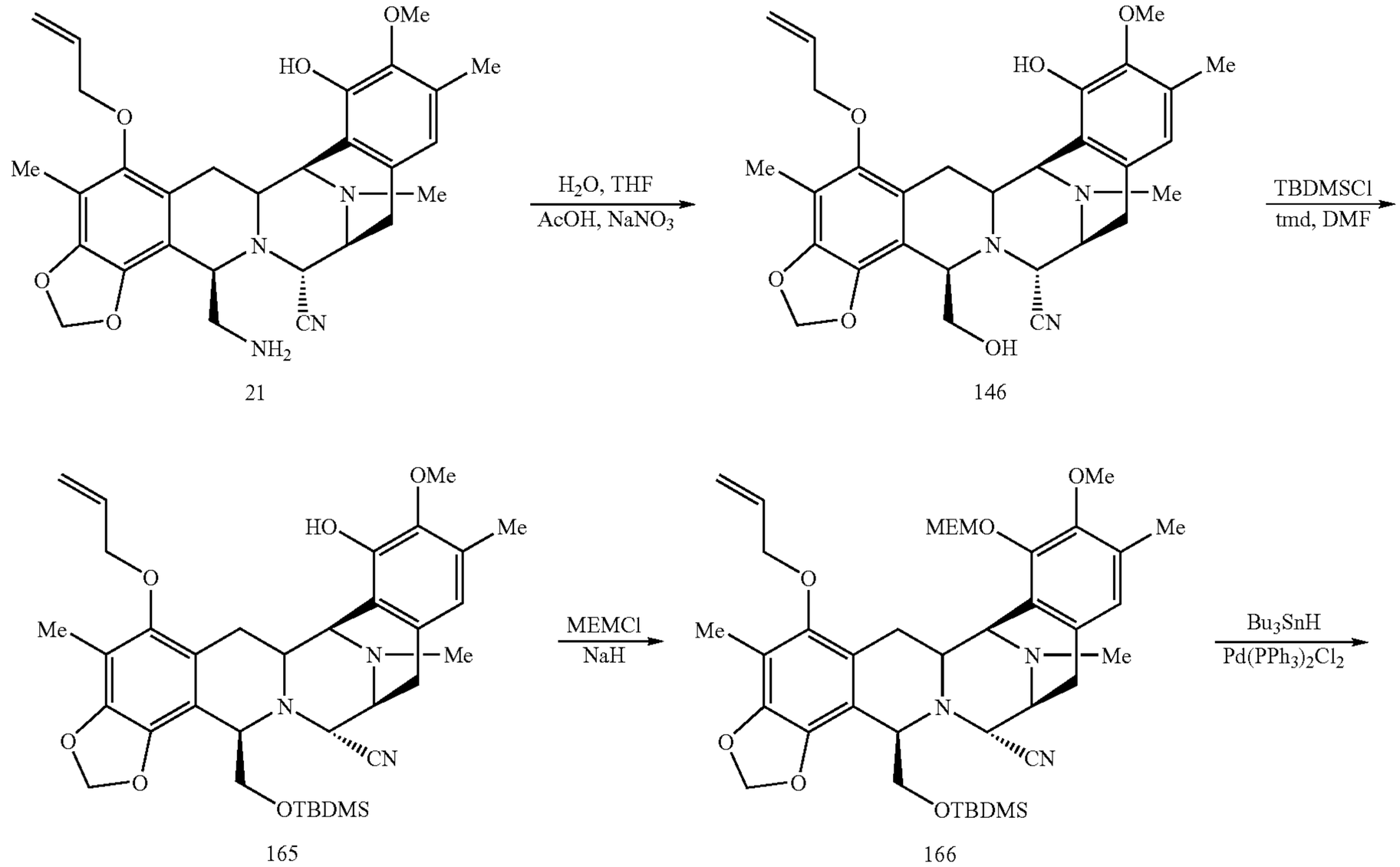
Scheme 5-ET-743: Hemisynthetic Alternative Route 5



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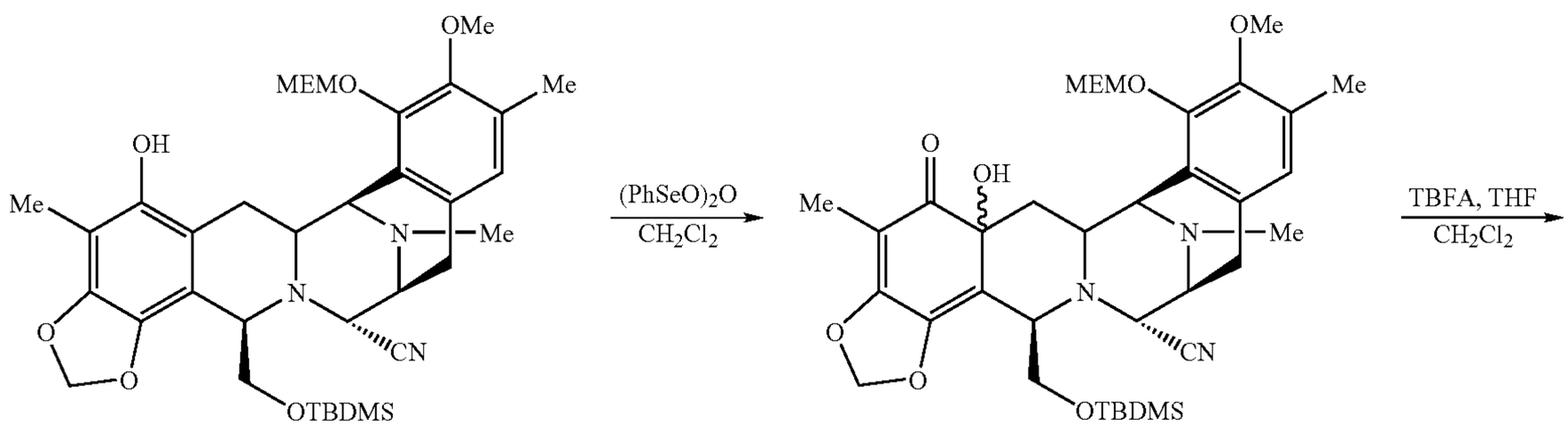

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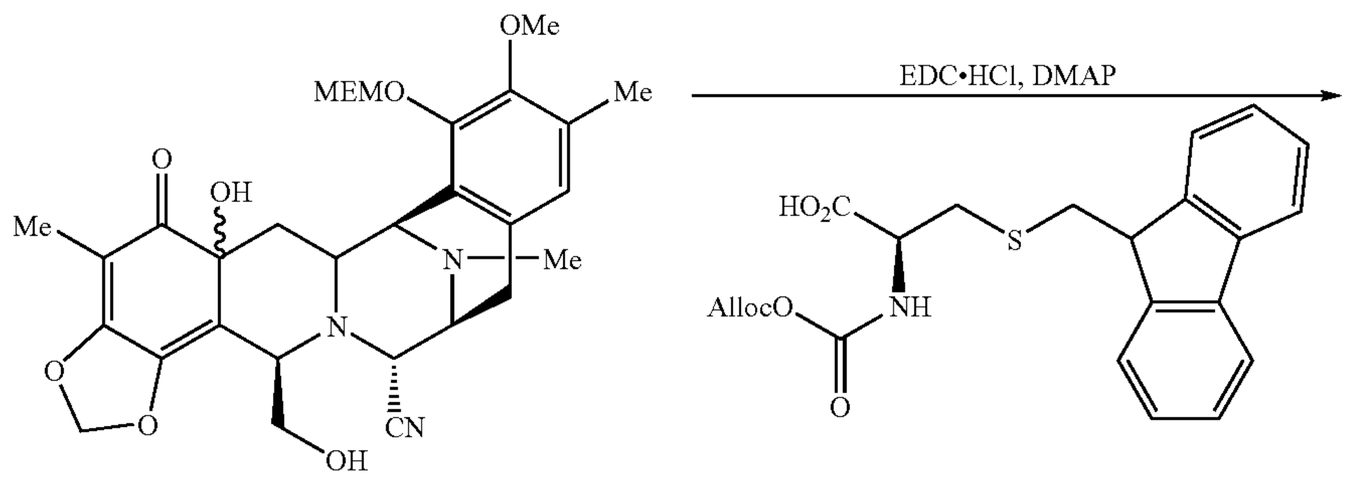

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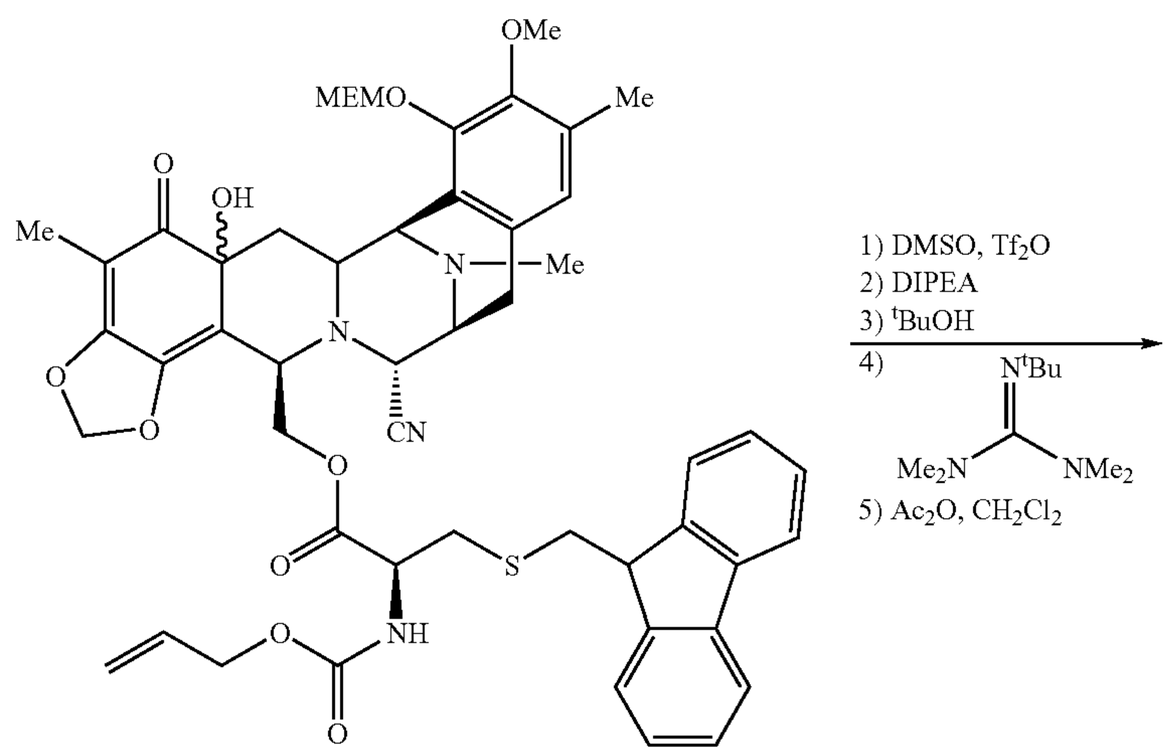

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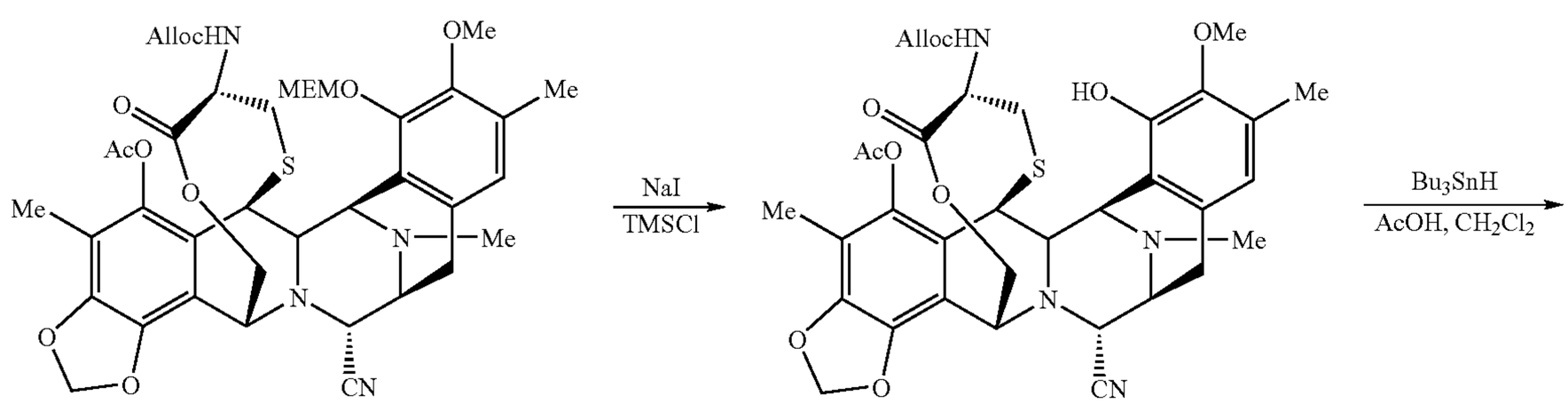

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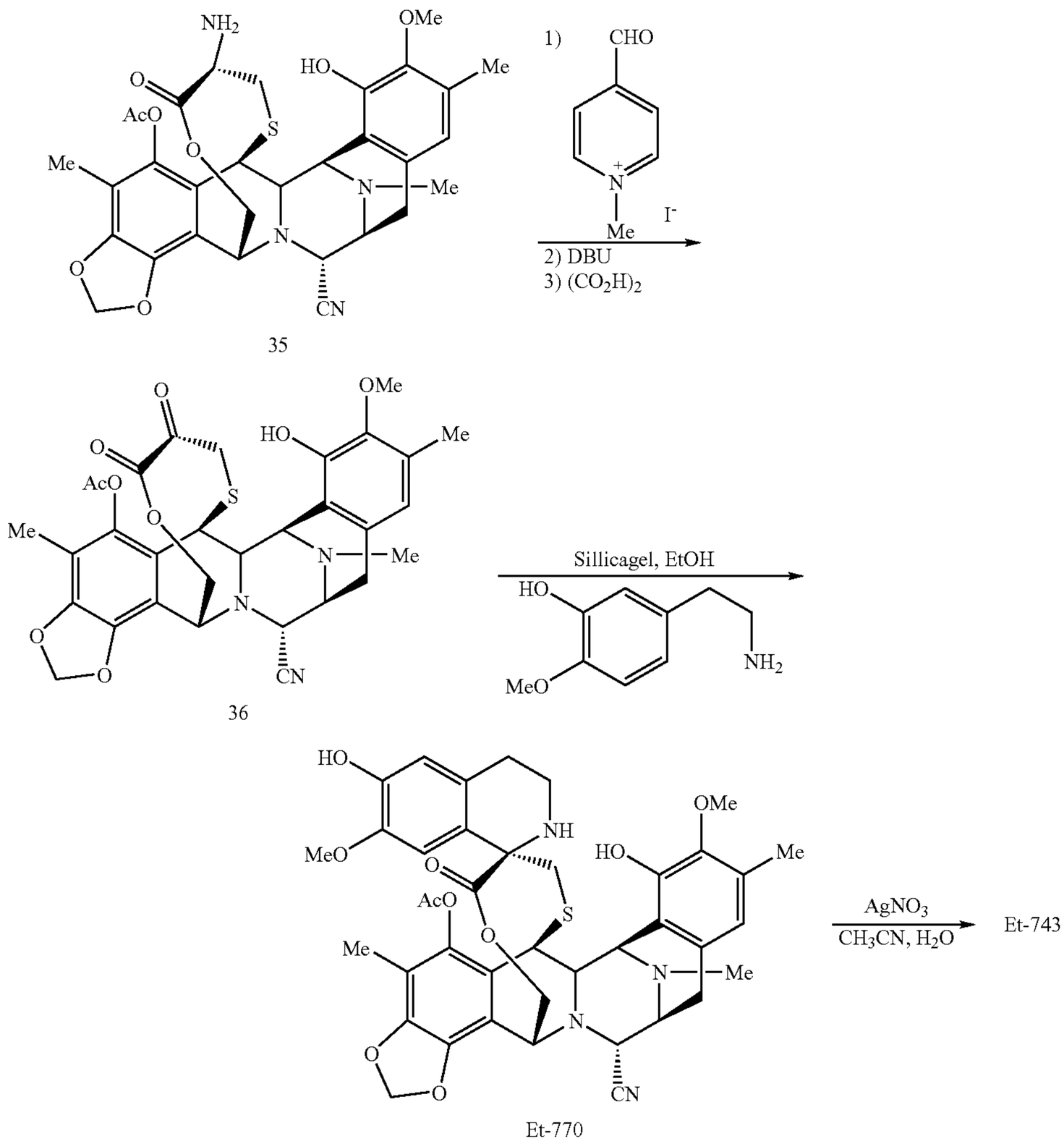
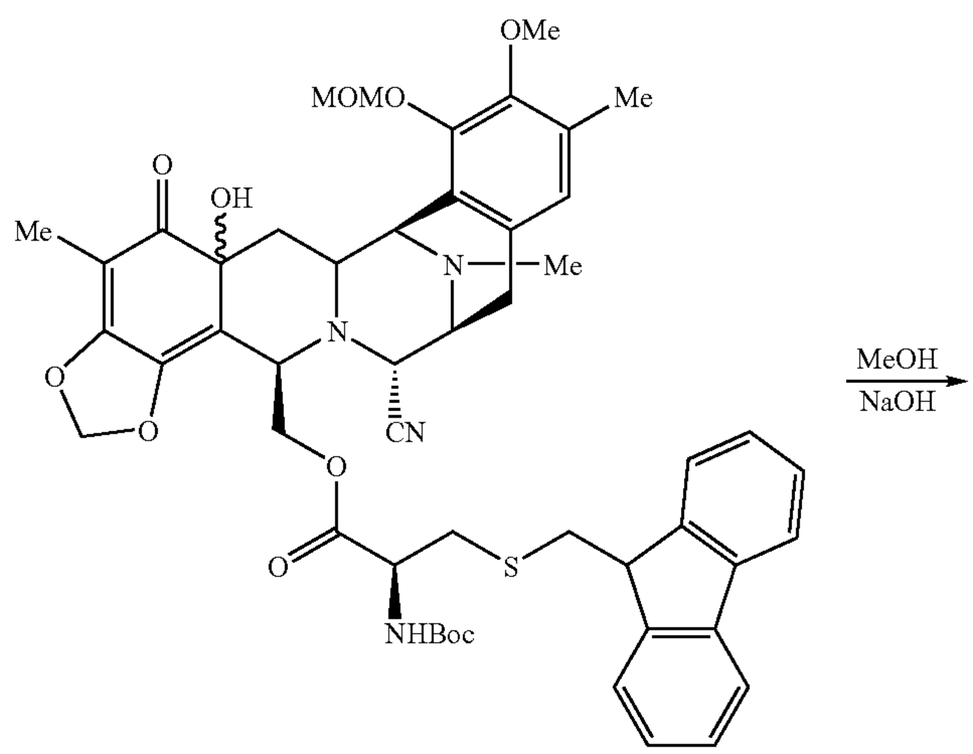


Scheme 6-ET-743 Hemisynthetic Alternative Route 6



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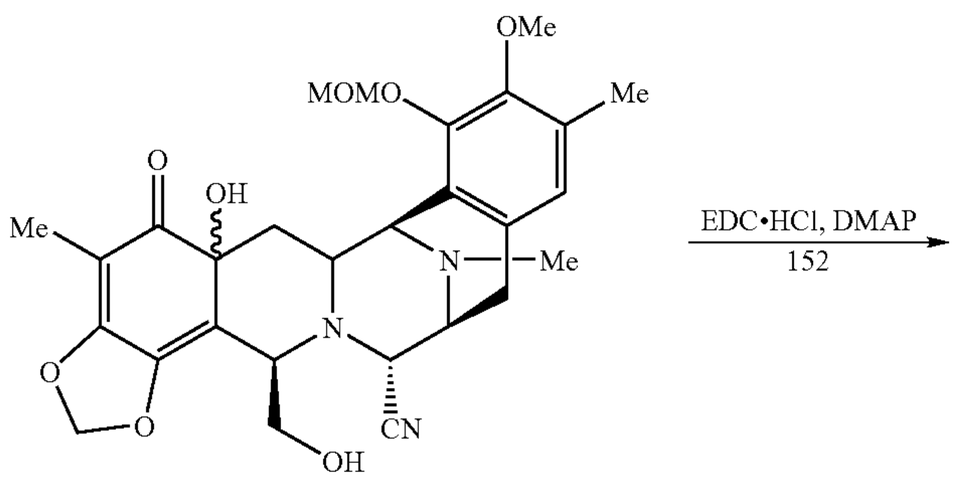

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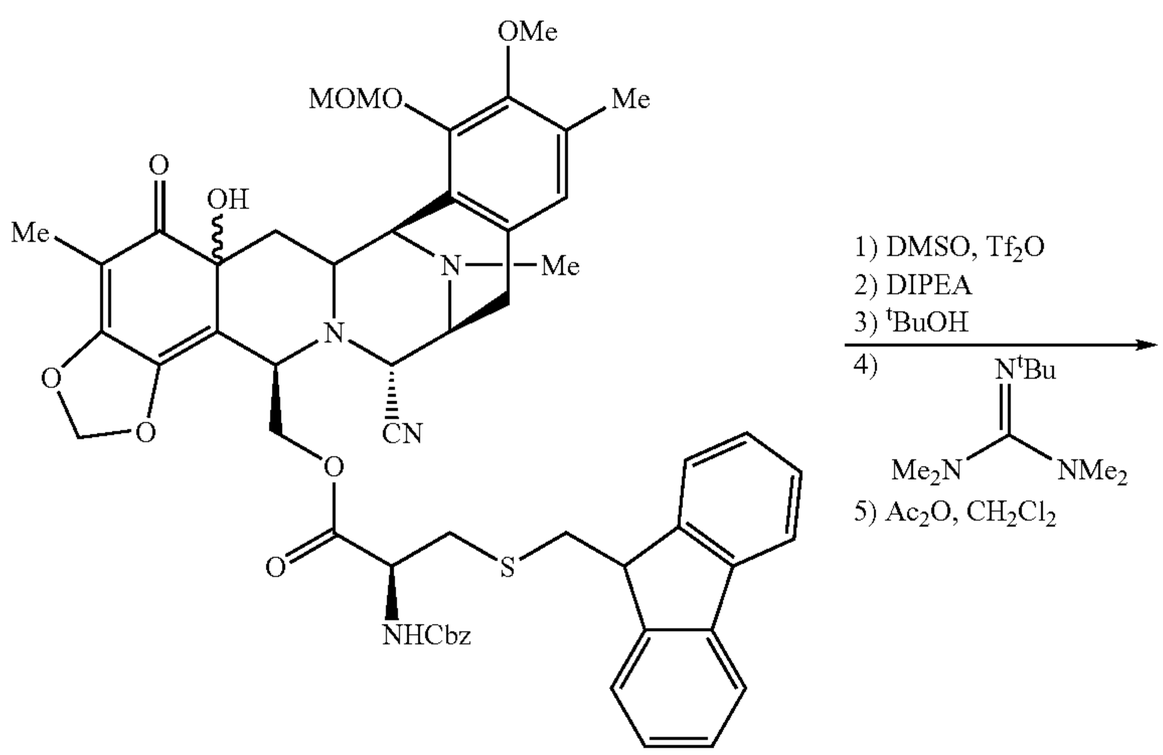

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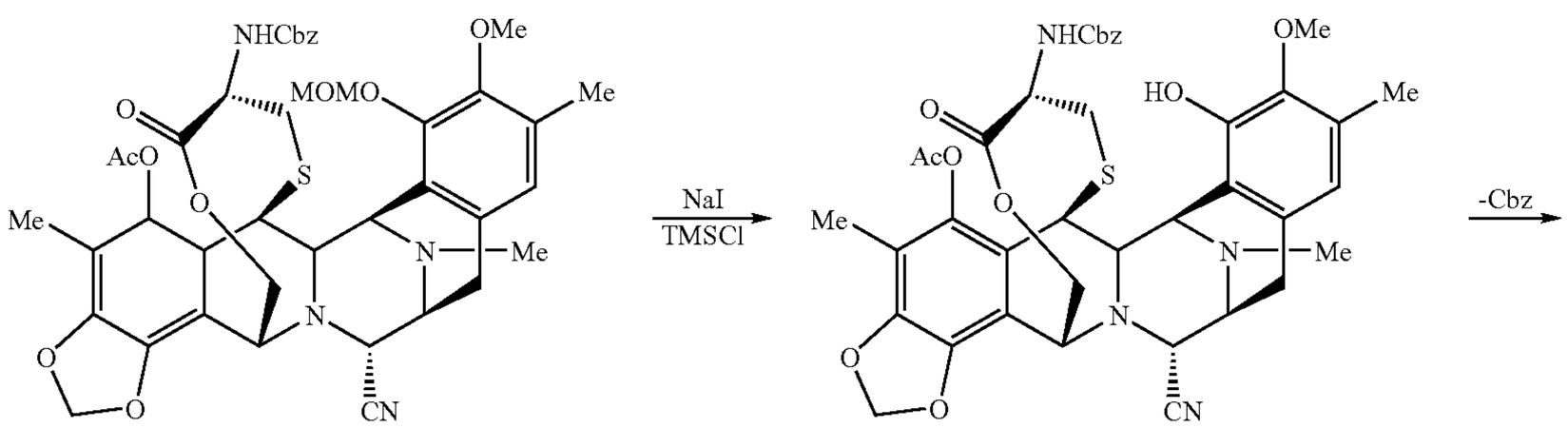

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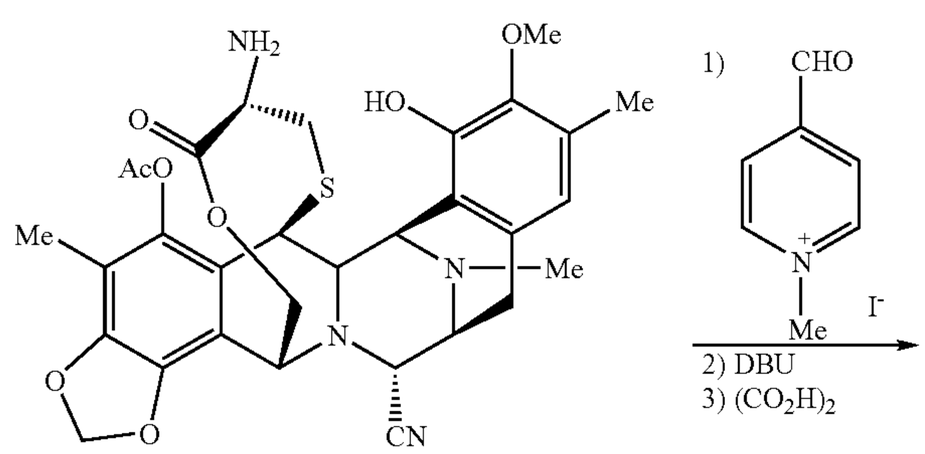

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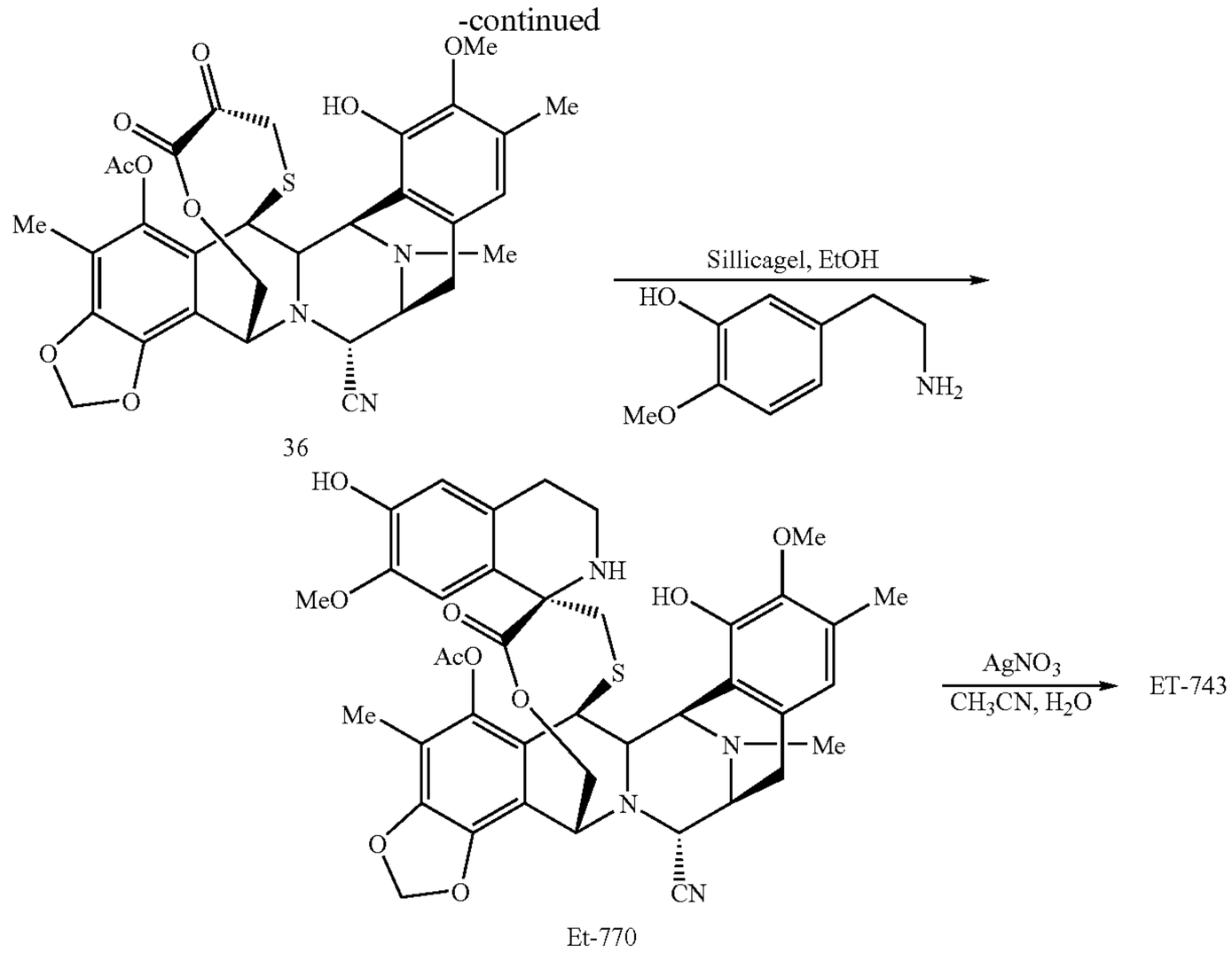


In route 1, protection of the E-ring phenol is achieved in three steps involving protection/deprotection of the amine of SF21 with Troc.

For routes 1 and 2, protection of the cysteine sidechain with Boc allows the phenol and cysteine groups to be deprotected in a single step rather than as two separate steps. For the rest of the routes, an additional deprotection step is required.

For route 2, Intermediate 25 is avoided through the use of the direct esterification methodology and the subsequent protection of the phenol with the MEM group.

In routes 2 and 3 protection of the E-ring phenol is delayed until after the diazotisation and esterification steps thereby allowing the phenol to be protected in a single step rather than by the three step sequence of route 1.

For routes 1, 2 and 3, direct esterification of the primary alcohol with the cysteine derivative eliminates the unproductive protection/deprotection steps of the primary alcohol with a silyl group (routes 4 and 5) thereby shortening the sequence by two steps.

Route 6 only contemplates herein the last steps from intermediate 161, which can be easily obtained from intermediate 21.

In routes 4 and 5 the primary alcohol produced by the initial diazotisation step is protected with silicon to allow selective protection of the E-ring phenol and avoiding intermediate 25. Following modification of the A-ring (deprotection/oxidation), the silicon group is removed and the primary alcohol esterified with the cysteine derivative.

These changes are a direct consequence of the problems that were found in the scale up of the route given in WO 0069862. As a result of these changes the overall route 2 is three steps shorter and potentially therefore more suitable and/or cheaper for routine manufacture.

Process Overview

Thus, in view of the routes 1 to 6, the present invention extends to a process for preparing an ecteinascidin product with a spiroamine-1,4-bridge, the process involving forming a 1,4 bridge using a 1-labile, 10-hydroxy, 18-protected hydroxy, di-6,8-en-5-one fused ring compound, wherein C-18 protection is removed before spiroamine introduction.

In one version of the process, the ecteinascidin product has a 21-hydroxy group, and the process includes converting a 21-cyano group to the 21-hydroxy group.

Typically the spiroamine is a spiroquinoline, especially the spiroquinoline of ecteinascidin 743.

In a preferred process the 18-protected group of the 1-labile, 10-hydroxy, 18-protected hydroxy, di-6,8-en-5-one fused ring compound is protected with: MOM, methoxymethyl; or MEM, methoxyethoxymethyl group.

Suitably the 1-labile group is an N-protected cysteinyloxymethylene group of the formula

$$—CH_2—O—CO—CNHProt^1—CH_2—S—H.$$

In this formula $Prot^1$ is typically: Boc, t-butyloxycarbonyl; Troc, 2,2,2-trichloroethyloxycarbonyl; Cbz, benzyloxycarbonyl; or Alloc, allyloxycarbonyl.

With some embodiments of the process, $Prot^1$ is removed in the same step as C-18 protection.

The 1-labile group can be generated from a 1-substituent of the formula:

$$—CH_2—O—CO—CNHProt^1—CH_2—S\text{-}Prot^2.$$

In this formula, $Prot^2$ is typically Fm, 9-fluorenylmethyl.

A 1-substituent of the formula:

$$—CH_2—O—CO—CNHProt^1—CH_2—S\text{-}Prot^2$$

can be formed by esterification of a $—CH_2—O—H$ substituent.

The esterification can be carried out before or after formation of the 10-hydroxy, di-6,8-en-5-one structure.

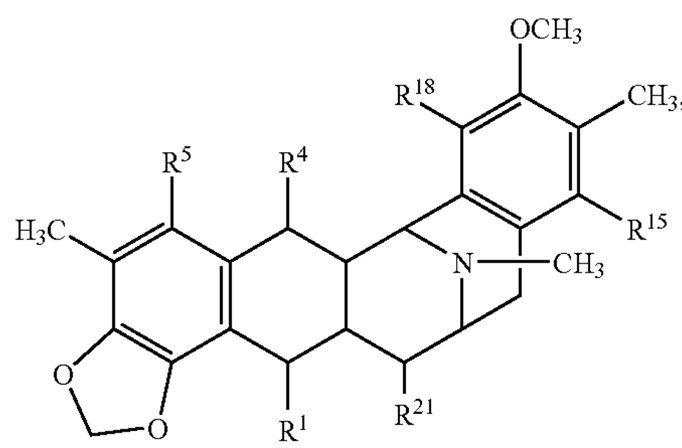
In one version, the claimed process starts from a 1-aminomethylene, 5-protected hydroxy, 7,8-dioxymethylene, 18-hydroxy, 21-cyano fused ring compound

The 1-aminomethylene group can be temporarily protected to allow protection at the 18-hydroxy group, and the temporary protection removed.

Alternatively, the C-18 hydroxy group can be protected after formation of a 1-ester function.

In another variation, the 1-aminomethylene group is converted to a 1-hydroxymethylene group and the 1-hydroxymethylne group is temporarily protected, to allow protection at the 18-hydroxy group, and the temporary protection is removed.

The fused ring structure is suitably of the formula:



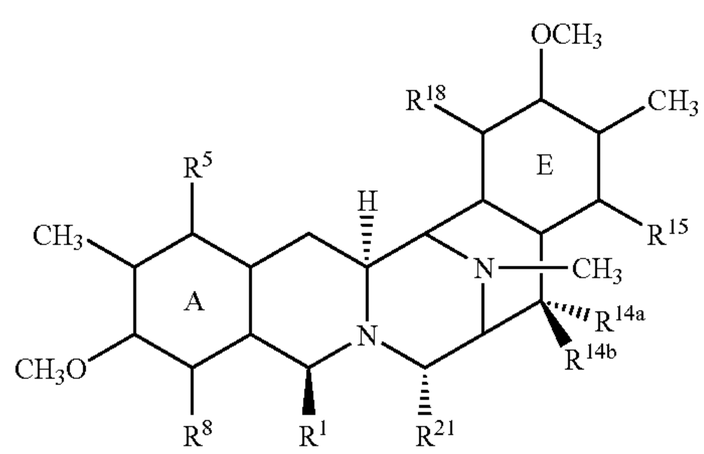
especially where R^{15} is H. One or more or all of the remaining subsitituents can be as in ecteinascidin 743.

Hemisythesis

The invention permits the use of a known compound, safracin B, also referred to as quinonamine, in hemisynthetic synthesis.

More generally, the invention relates to a hemisynthetic process for the formation of intermediates, derivatives and related structures of ecteinascidin or other tetrahydroisoquinolinephenol compounds starting from natural bis(tetrahydroisoquinoline) alkaloids. Suitable starting materials for the hemi-synthetic process include the classes of saframycin and safracin antibiotics available from different culture broths, and also the classes of reineramicin and xestomycin compounds available from marine sponges.

A general formula (XV) for the starting compounds is as follows:



where:

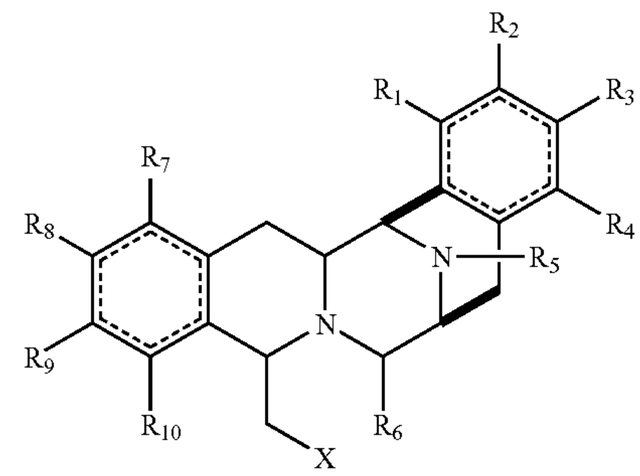
R^1 is an amidomethylene group such as —CH^2—NH—CO—$CR^{25a}R^{25b}R^{25c}$ where R^{25a} and R^{25b} form a keto group or one is —OH, —NH_2 or —$OCOCH_3$ and the other is —CH_2COCH_3, —H, —OH or —$OCOCH_3$, provided that when R^{25a} is —OH or —NH_2 then R^{25b} is not —OH, and R^{25c} is —H, —CH_3 or —CH_2CH_3, or R^1 is an acyloxymethylene group such as —CH_2—O—CO—R, where R is —$C(CH_3)$=CH—CH_3 or —CH_3;

R^5 and R^8 are independently chosen from —H, —OH or —$OCOCH_2OH$, or R^5 and R^8 are both keto and the ring A is a p-benzoquinone ring; R^{14a} and R^{14b} are both —H or one is —H and the other is —OH, —OCH_3 or —OCH_2CH_3, or R^{14a} and R^{14b} together form a keto group;

R^{15} and R^{18} are independently chosen from —H or —OH, or R^5 and R^8 are both keto and the ring A is a p-benzoquinone ring; and

R^{21} is —OH or —CN.

A more general formula for these class of compounds is provided below:



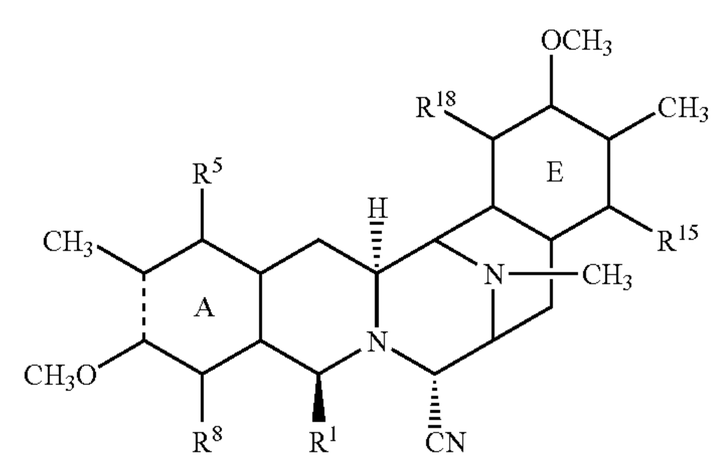
wherein the substituent groups defined by R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, R_9, R_{10} are each independently selected from the group consisting of H, OH, OCH_3, CN, =O, CH_3;

wherein X are the different amide or ester functionalities contained in the mentioned natural products;

wherein each dotted circle represents one, two or three optional double bonds.

Thus, according to the present invention, we now provide hemisynthetic routes for the production of intermediates including Intermediates 11 or 21 and thus for the production of the ecteinascidin compounds as well as phthalascidin and additional compounds. The hemisynthetic routes of the invention each comprise a number of transformation steps to arrive at the desired product. Each step in itself is a process in accordance with this invention. The invention is not limited to the routes that are exemplified, and alternative routes may be provided by, for example, changing the order of the transformation steps, as appropriate or by a change to the protecting groups used.

In particular, this invention involves the provision of a 21-cyano starting material of general formula (XVI):



where R^1, R^5, R^8, R^{14a}, R^{14b}, R^{15} and R^{18} are as defined.

Other compounds of formula (XVI) with different substituents at the 21-position may also represent possible starting materials. In general, any derivative capable of production by nucleophilic displacement of the 21-hydroxy group of

compounds of formula (XV) wherein R^{21} is a hydroxy group cis a candidate. Examples of suitable 21-substituents include but are not limited to:

a mercapto group;

an alkylthio group (the alkyl group having from 1 to 6 carbon atoms);

an arylthio group (the aryl group having from 6 to 10 carbon atoms and being unsubstituted or substituted by from 1 to 5 substituents selected from, for example, alkyl group having from 1 to 6 carbon atoms, alkoxy groups having from 1 to 6 carbon atoms, halogen atoms, mercapto groups and nitro groups);

an amino group;

a mono-or dialkylamino (the or each alkyl group having from 1 to 6 carbon atoms);

a mono-or diarylamino group (the or each aryl group being as defined above in relation to arylthio groups);

an α-carbonylalkyl group of formula —C(R^a)(R^b)—C(=O) R^c, where R^a and R^b are selected from hydrogen atoms, alkyl groups having from 1 to 20 carbon atoms, aryl groups (as defined above in relation to arylthio groups) and aralkyl groups (in which an alkyl group having from 1 to 4 carbon atoms is substituted by an aryl group a defined above in relation to arylthio groups), with the proviso that one of R^a and R^b is a hydrogen atom;

R^c is selected from a hydrogen atom, an alkyl group having from 1 to 20 carbon atoms, aryl groups (as defined above in relation to arylthio groups), an aralkyl group (in which an allyl group having from 1 to 4 carbon atoms is substituted by an aryl group a defined above in relation to arylthio groups), an alkoxy group having from 1 to 6 carbon atoms, an amino group or a mono- or dialkylamino group as defined above.

Thus, in a more general aspect, the present invention relates to processes where the first step is to form a 21-derivative using a nucleophilic reagent. We refer to such compounds as 21-Nuc compounds.

The presence of the 21-cyano group is required for some of the end-products, notably ecteinascidin 770 and phthalascidin, while for other end-products it acts as a protecting group which can readily be converted to another substituent, such as the 21-hydroxy group of ecteinascidin 743 or of 21-hydroxyphthalascidin. The adoption of the 21-cyano compound as the starting material effectively stabilises the molecule during the ensuing synthetic steps, until it is optionally removed. Other 21-Nuc compounds can offer this and other advantages.

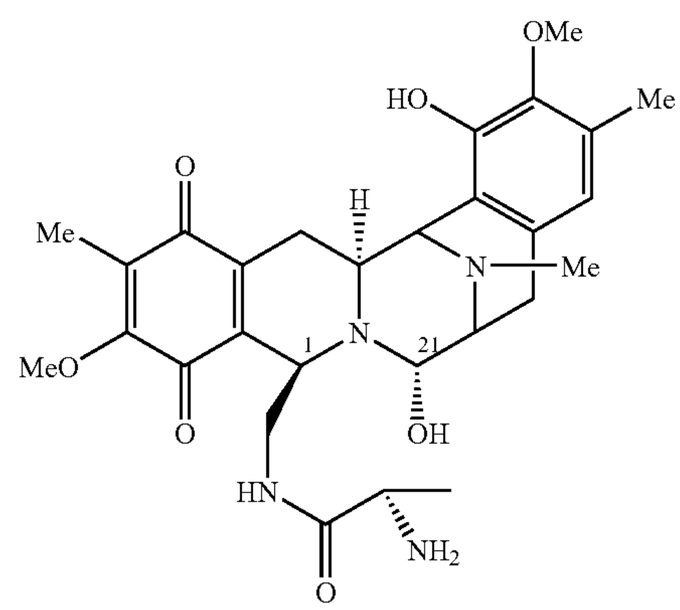
In one important aspect, the present invention consists in the use of a 21-cyano compound of the general formula (XVI) in the preparation of a bis- or tris-(tetrahydroisoquinolinephenol) compounds. Products which may be prepared include intermediates such as Intermediate 11 or 21, and the ecteinascidins, as well as new and known compounds of related structure.

Preferred starting materials include those compounds of formula (XV) or (XVI) where R^{14a} and R^{14b} are both hydrogen. Preferred starting materials also include compounds of formula (XV) or (XVI) where R^{15} is hydrogen. Furthermore, the preferred starting materials include compounds of formula (XV) or (XVI) where ring E is a phenolic ring. Preferred starting materials further include compounds of formula (XV) or (XVI) where at least one, better at least two or three of R^5, R^8, R^{15} and R^{18} is not hydrogen.

Examples of suitable starting materials for this invention include saframycin A, saframycin B, saframycin C, saframycin G, saframycin H, saframycin S, saframycin Y_3, saframycin Yd_1, saframycin Ad_1, saframycin Yd_2, saframycin AH_2, saframycin AH_2Ac, saframycin AH_1, saframycin AH_1Ac, saframycin AR_3, renieramycin A, renieramycin B, renieramycin C, renieramycin D, renieramycin E, renieramycin F, xestomycin, saframycin D, saframycin F, saframycin Mx-1, saframycin Mx-2, safracin A, safracin B and saframycin R. Preferred starting materials have a cyano group in position 21, for the group R^{21}.

In a particularly preferred aspect, the invention involves a hemisynthetic process wherein the transformation steps are applied to safracin B:

SAFRACIN B



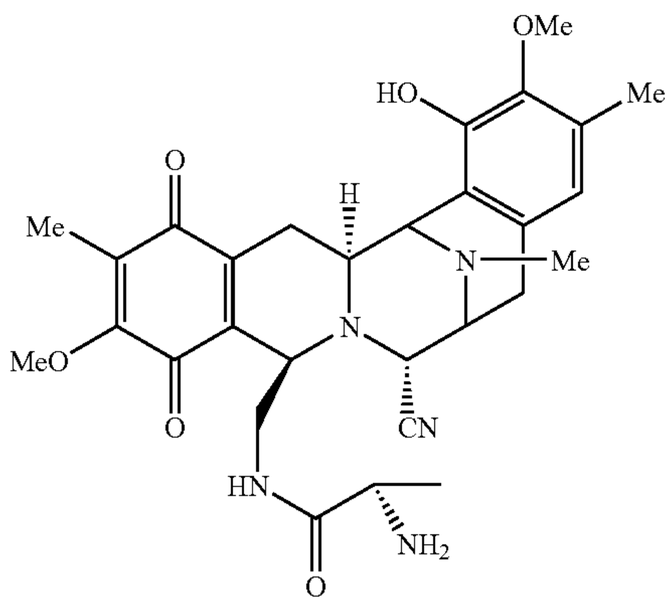
Safracin B presents a ring system closely related to the ecteinascidins. This compound has the same pentacycle structure and the same substitution pattern in the right-hand aromatic ring, ring E. Also, safracin B presents very close similarities to some of the synthetic intermediates in the total synthesis of ET-743, particularly to the intermediates 11 or 21. Such intermediate can be transformed into Et-743 using a well established method. Synthetic conversion of safracin B into intermediates 11 or 21 will therefore provide an hemisynthetic method to obtain ET-743.

Thus, we provide Intermediates 11 or 21 made from this compound safracin B, and compounds derived from Intermediate 11 or 21, particularly ecteinascidin compounds. We further provide phthalascidin made from safracin B. The invention also relates to use of safracin B in the production of Intermediates 11 or 21, ecteinascidin compounds and the other intermediates of the invention. The invention also relates to compounds described herein derived from the other suggested starting materials, and use of those compounds in the production of such compounds.

The more preferred starting materials of this invention have a 21-cyano group. The currently most preferred compound of the present invention is the compound of Formula 2. This

compound is obtained directly from safracin B and is considered a key intermediate in the hemisynthetic process.

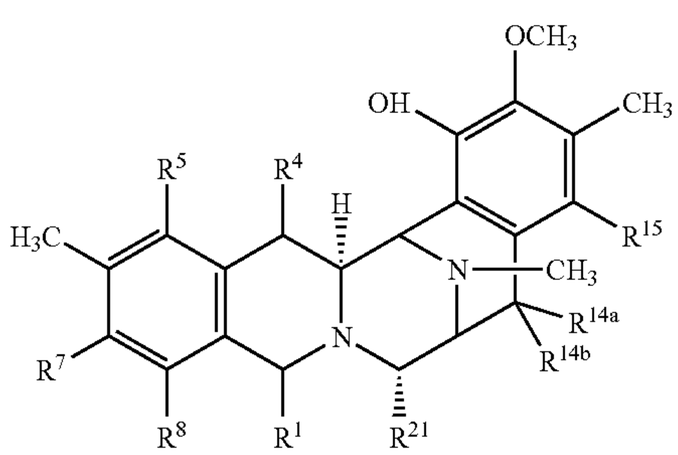
compound 2



In a related aspect, we provide cyanosafracin B by fermentation of a safracin B-producing strain of *Pseudomonas fluorescens*, and working up the cultured broth using cyanide ion. The preferred strain of *Pseudomonas fluorescens* is strain A2-2, FERM BP-14, which is employed in the procedure of EP 055,299. A suitable source of cyanide ion is potassium cyanide. In a typical work-up, the broth is filtered and excess cyanide ion is added. After an appropriate interval of agitation, such as 1 hour, the pH is rendered alkaline, say pH 9.5, and an organic extraction gives a crude extract which can be further purified to give the cyanosafracin B.

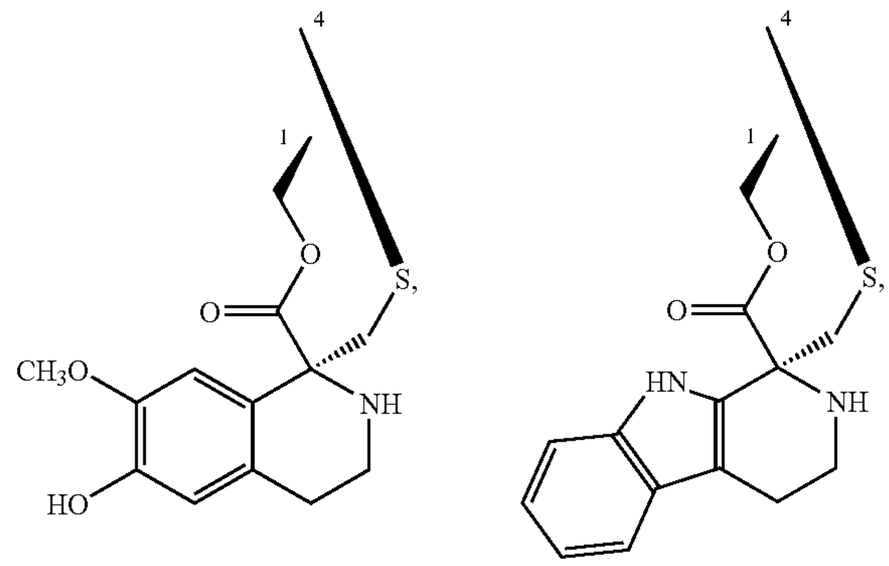
For the avoidance of doubt, the stereochemistries indicated in this patent specification are based on our understanding of the correct stereochemistry of the natural products. To the extent that an error is discovered in the assigned stereochemistry, then the appropriate correction needs to be made in the formulae given throughout in this patent specification. Furthermore, to the extent that the syntheses are capable of modification, this invention extends to stereoisomers.

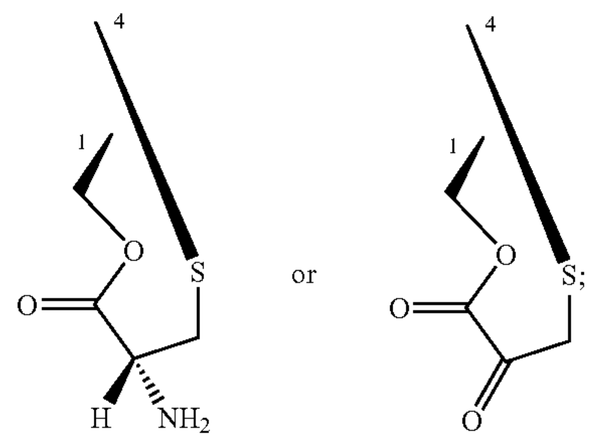
The products of this invention are typically of the formula (XVIIb):



where

R^1 and R^4 together form a group of formula (IV), (V) (VI) or (VII):





R^5 is —H or —OH;

R^7 and R^8 together form a group —O—CH_2—O—;

R^{14a} and R^{14b} are both —H or one is —H and the other is —OH, —OCH_3 or —OCH_2CH_3, or R^{14a} and R^{14b} together form a keto group; and

R^{15} is —H or —OH;

R^{21} is —H, —OH or —CN;

and derivatives including acyl derivatives thereof especially where R^5 is acetyloxy or other acyloxy group of up to 4 carbon atoms.

In the formula (XVIIb), R^1 typically with R^4 forms a group (IV) or (V). The group R^{18} is usually protected. Usually R^{21} is cyano.

Preferably R^{14a} and R^{14b} are hydrogen. Preferably R^{15} is hydrogen. The O-acyl derivatives are suitably aliphatic O-acyl derivatives, especially acyl derivatives of 1 to 4 carbon atoms, and typically an O-acetyl group, notably at the 5-position.

Suitable protecting groups for phenols and hydroxy groups include ethers and esters, such as alkyl, alkoxyalkyl, aryloxyalkyl, alkoxyalkoxyalkyl, alkylsilylalkoxyalkyl, alkylthioalkyl, arylthioalkyl, azidoalkyl, cyanoalkyl, chloroalkyl, heterocyclic, arylacyl, haloarylacyl, cycloalkylalkyl, alkenyl, cycloalkyl, alyklarylalkyl, alkoxyarylalkyl, nitroarylalkyl, haloarylalkyl, alkylaminocarbonylarylalkyl, alkylsulfinylarylalky, alkylsilyl and other ethers, and arylacyl, aryl alkyl carbonate, aliphatic carbonate, alkylsulfinylarlyalkyl carbonate, alkyl carbonate, aryl haloalkyl carbonate, aryl alkenyl carbonate, aryl carbamate, alkyl phosphinyl, alkylphosphinothioyl, aryl phosphinothioyl, aryl alkyl sulphonate and other esters. Such groups may optionally be substituted with the previously mentioned groups in R^1.

Suitable protecting groups for amines include carbamates, amides, and other protecting groups, such as alkyl, arylalkyl, sulpho- or halo-arylalkyl, haloalkyl, alkylsilylalkyl, arylalkyl, cycloalkylalkyl, alkylarylalkyl, heterocyclylalkyl, nitroarylalkyl, acylaminoalkyl, nitroaryldithioarylalkyl, dicycloalkylcarboxamidoalkyl, cycloalkyl, alkenyl, arylalkenyl, nitroarylalkenyl, heterocyclylalkenyl, heterocyclyl, hydroxyheterocyclyl, alkyldithio, alkoxy- or halo- or alkylsulphinyl arylalkyl, hetercyclylacyl, and other carbamates, and alkanoyl, haloalkanoyl, arylalkanoyl, alkenoyl, heterocyclylacyl, aroyl, arylaroyl, haloaroyl, nitroaroyl, and other amides, as well as alkyl, alkenyl, alkylsilylalkoxyalkyl, alkoxyalkyl, cyanoalkyl, heterocyclyl, alkoxyarylalkyl, cycloalkyl, nitroaryl, arylalkyl, alkoxy- or hydroxy-arylalkyl, and many other groups. Such groups may optionally be substituted with the previously mentioned groups in R^1.

Examples of such protecting groups are given in the following tables.

	abbreviation
protection for —OH group	
ethers	
methyl	
methoxymethyl	MOM
benzyloxymethyl	BOM
methoxyethoxymethyl	MEM
2-(trimethylsilyl)ethoxymethyl	SEM
methylthiomethyl	MTM
phenylthiomethyl	PTM
azidomethyl	
cyanomethyl	
2,2-dichloro-1,1-difluoroethyl	
2-chloroethyl	
2-bromoethyl	
tetrahydropyranyl	THP
1-ethoxyethyl	EE
phenacyl	
4-bromophenacyl	
cyclopropylmethyl	
allyl	
propargyl	
isopropyl	
cyclohexyl	
t-butyl	
benzyl	
2,6-dimethylbenzyl	
4-methoxybenzyl	MPM or PMB
o-nitrobenzyl	
2,6-dichlorobenzyl	
3,4-dichlorobenzyl	
4-(dimethylamino)carbonylbenzyl	
4-methylsuflinylbenzyl	Msib
9-anthrylmethyl	
4-picolyl	
heptafluoro-p-tolyl	
tetrafluoro-4-pyridyl	
trimethylsilyl	TMS
t-butyldimethylsilyl	TBDMS
t-butyldiphenylsilyl	TBDPS
triisopropylsilyl	TIPS
esters	
aryl formate	
aryl acetate	
aryl levulinate	
aryl pivaloate	ArOPv
aryl benzoate	
aryl 9-fluorocarboxylate	
aryl methyl carbonate	
1-adamantyl carbonate	
t-butyl carbonate	BOC-OAr
4-methylsulfinylbenzyl carbonate	Msz-Oar

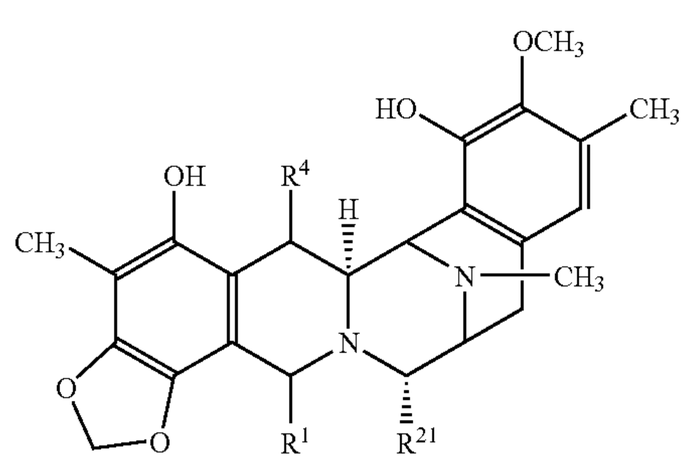
-continued

	abbreviation
2,4-dimethylpent-3-yl carbonate	Doc-Oar
aryl 2,2,2-trichloroethyl carbonate	
aryl vinyl carbonate	
aryl benzyl carbonate	
aryl carbamate	
dimethylphosphinyl	Dmp-OAr
dimethylphosphinothioyl	Mpt-OAr
diphenylphosphinothioyl	Dpt-Oar
aryl methanesulfonate	
aryl toluenesulfonate	
aryl 2-formylbenzenesulfonate	
protection for the —NH_2 group	
carbamates	
methyl	
ethyl	
9-fluorenylmethyl	Fmoc
9-(2-sulfo)fluroenylmethyl	
9-(2,7-dibromo)fluorenylmethyl	
17-tetrabenzo[a,c,g,i]fluorenylmethyl	Tbfmoc
2-chloro-3-indenylmethyl	Climoc
benz[f]inden-3-ylmethyl	Bimoc
2,7-di-t-butyl[9-(10,10-dioxo-10,10,10,10-tetrahydrothioxanthyl)]methyl	DBD-Tmoc
2,2,2-trichloroethyl	Troc
2-trimethylsilylethyl	Teoc
2-phenylethyl	hZ
1-(1-adamantyl)-1-methylethyl	Adpoc
2-chlooethyl	
1,1-dimethyl-2-chloroethyl	
1,1-dimethyl-2-bromoethyl	
1,1-dimethyl-2,2-dibromoethyl	DB-t-BOC
1,1-dimethyl-2,2,2-trichloroethyl	TCBOC
1-methyl-1-(4-biphenyl)ethyl	Bpoc
1-(3,5-di-t-butylphenyl)-1-1-methylethyl	t-Burmeoc
2-(2'-and 4'-pyridyl)ethyl	Pyoc
2,2-bis(4'-nitrophenyl)ethyl	Bnpeoc
n-(2-pivaloylamino)-1,1-dimethylethyl	
2-[(2-nitrophenyl)dithio]-1-phenylethyl	NpSSPeoc
2-(n,n-dicyclohexylcarboxamido)ethyl	
t-butyl	BOC
1-adamantyl	1-Adoc
2-adamantyl	2-Adoc
vinyl	Voc
allyl	Aloc or Alloc
1-isopropylallyl	Ipaoc
cinnamyl	Coc
4-nitrocinnamyl	Noc
3-(3'-pyridyl)prop-2-enyl	Paloc
8-quinolyl	
n-hydroxypiperidinyl	
alkyldithio	
benzyl	Cbz or Z
p-methoxybenzyl	Moz
p-nitrobenzyl	PNZ
p-bromobenzyl	
p-chlorobenzyl	
2,4-dichlorobenzyl	
4-methylsulfinylbenzyl	Msz
9-anthrylmethyl	
diphenylmethyl	
phenothiazinyl-(10)-carbonyl	
n'-p-toluenesulfonylaminocarbonyl	
n'-phenylaminothiocarbonyl	
amides	
formamide	
acetamide	
chloroacetamide	
trifluoroacetamide	TFA
phenylacetamide	
3-phenylpropanamide	
pent-4-enamide	
picolinamide	
3-pyridylcarboxamide	
benzamide	

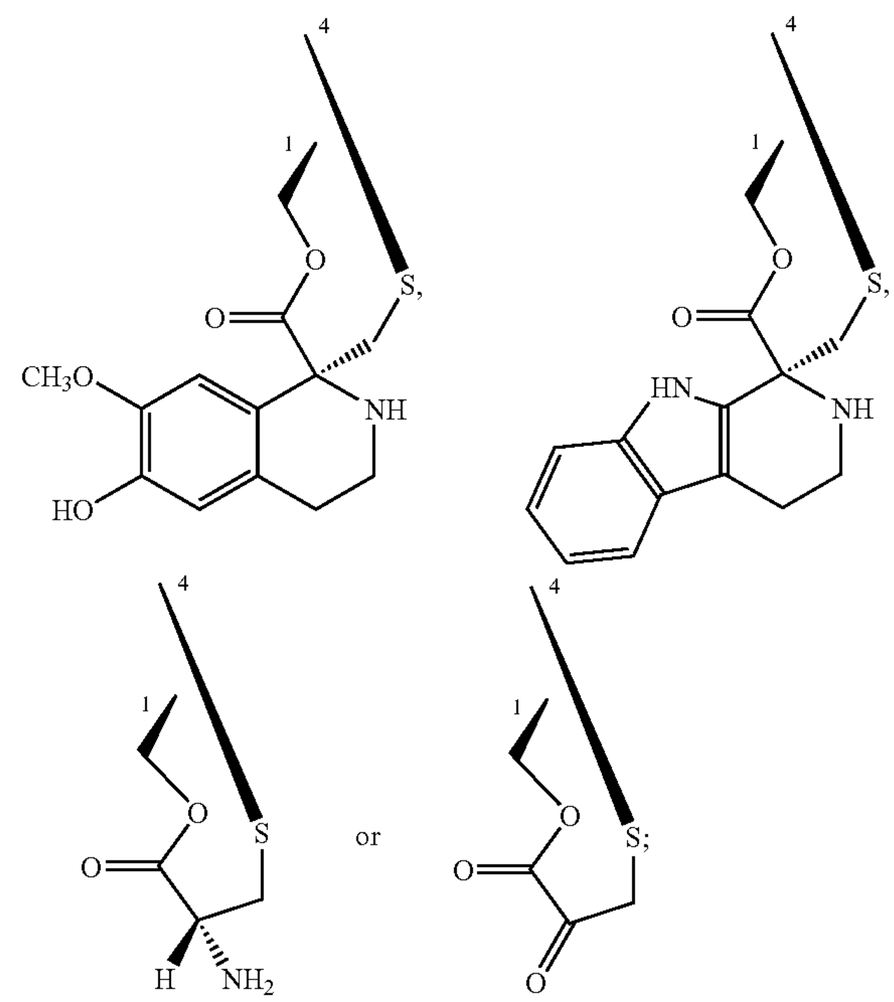
-continued

	abbreviation
p-phenylbenzamide	
n-phthalimide	
n-tetrachlorophthalimide	TCP
4-nitro-n-phthalimide	
n-dithiasuccinimide	Dts
n-2,3-diphenylmaleimide	
n-2,5-dimethylpyrrole	
n-2,5-bis(triisopropylsiloxyl)pyrrole	BIPSOP
n-1,1,4,4-tetramethyldisiliazacyclopentante adduct	STABASE
1,1,3,3-tetramethyl-1,3-disilaisoindoline	BSB
special —NH protective groups	
n-methylamine	
n-t-butylamine	
n-allylamine	
n-[2-trimethylsilyl)ethoxy]methylamine	SEM
n-3-acetoxypropylamine	
n-cyanomethylamine	
n-(1-isopropyl-4-nitro-2-oxo-3-pyrrolin-3-yl)amine	
n-2,4-dimethoxybenzylamine	Dmb
2-azanorbornenes	
n-2,4-dinitrophenylamine	
n-benzylamine	Bn
n-4-methoxybenzylamine	MPM
n-2,4-dimethoxybenzylamine	DMPM
n-2-hydroxybenzylamine	Hbn
n-(diphenylmethyl)amino	DPM
n-bis(4-methoxyphenyl)methylamine	
n-5-dibenzosuberylamine	DBS
n-triphenylmethylamine	Tr
n-[(4-methoxyphenyl)diphenylmethyl]amino	MMTr
n-9-phenylflurenylamine	Pf
n-ferrocenylmethylamine	Fcm
n-2-picolylamine n'-oxide	
n-1,1-dimethylthiomethyleneamine	
n-benzylideneamine	
n-p-methoxybenzylideneamine	
n-diphenylmethyleneamine	
n-(5,5-dimethyl-3-oxo-1-cyclohexenyl)amine	
n-nitroamine	
n-nitrosoamine	
diphenylphosphinamide	Dpp
dimethylthiophosphinamide	Mpt
diphenylthiophosphinamide	Ppt
dibenzyl phosphoramidate	
2-nitrobenzenesulfenamide	Nps
n-1-(2,2,2-trifluoro-1,1-diphenyl)ethylsufenamide	TDE
3-nitro-2-pyridinesulfenamide	Npys
p-toluenesulfonamide	Ts
benzenesulfonamide	

Particular ecteinascidin products of this invention include compounds of the formula (XVIII):



where R^1 and R^4 form a group of formula (IV), (V), (VI) or (VII):



more particularly a group (I or (V);

R^{21} is —H, —OH or —CN, more particularly —OH or —CN;

and acyl derivatives thereof, more particularly 5-acyl derivatives including the 5-acetyl derivative.

In general, the conversion of the 21-cyano starting compound to an ecteinascidin product of, for example, formula (XVIII) involves:

a) conversion if necessary of a quinone system for the ring E into the phenol system

b) conversion if necessary of a quinone system for the ring A into the phenol system;

c) conversion of the phenol system for the ring A into the methylenedioxyphenol ring;

d) formation of the bridged spiro ring system of formula (IV), (VI) or (VII) across the 1-position and 4-position in ring B; and

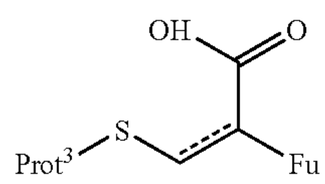
e) derivatisation as appropriate, such as acylation.

Step (a), conversion if necessary of a quinone system for the ring E into the phenol system, can be effected by conventional reduction procedures. A suitable reagent system is hydrogen with a palladium-carbon catalyst, though other reducing systems can be employed.

Step (b), conversion if necessary of a quinone system for the ring A into the phenol system is analogous to step (a), and more detail is not needed.

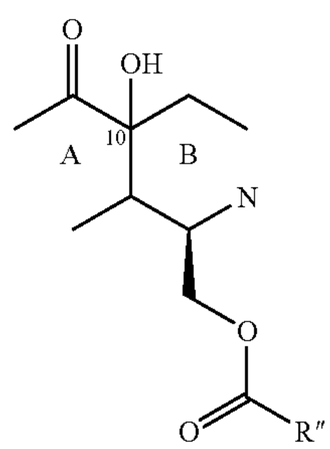
Step (c), conversion of the phenol system for the ring A into the methylenedioxyphenol ring, can be effected in several ways, possibly along with step (b). For example, a quinone ring A can be demethylated in the methoxy substituent at the 7-position and reduced to a dihydroquinone and trapped with a suitable electrophilic reagent such as CH_2Br_2, $BrCH_2Cl$, or a similar divalent reagent directly yielding the methylenedioxy ring system, or with a divalent reagent such as thiocarbonyldiimidazol which yields a substituted methylenedioxy ring system which can be converted to the desired ring.

Step (d) is typically effected by appropriate substitution at the 1-position with a bridging reagent that can assist formation of the desired bridge, forming an exendo quinone methide at the 4-position and allowing the methide to react with the 1-substituent to bring about the bridged structure. Preferred bridging reagents are of formula (XIX)

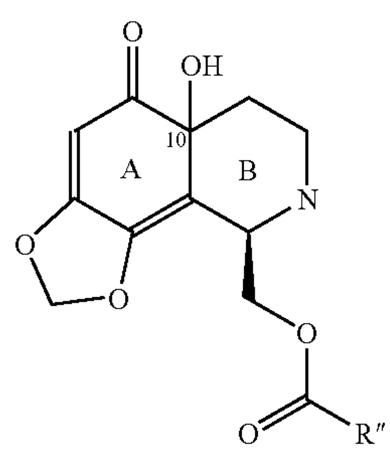


where Fu indicates a protected functional group, such as a group —NHProt^{4a}, Prot^3 is a protecting group, and the dotted line shows an optional double bond.

Suitably the methide is formed by first introducing a hydroxy group at the 10-position at the junction of rings A and B to give a partial structure of formula (XX):



or more preferably a partial structure of formula (XXI):



where the group R" is chosen for the desired group of formula (IV), (V), (VI) or, (VII). For the first two such groups, the group R" typically takes the form —CHFu-CH$_2$—SProt^3. The protecting groups can then be removed and modified as appropriate to give the desired compound.

A typical procedure for step (d) is provided in U.S. Pat. No. 5,721,362 incorporated by reference. Particular reference is made to the passage at column 8, step (l) and Example 33 of the US patent, and related passages.

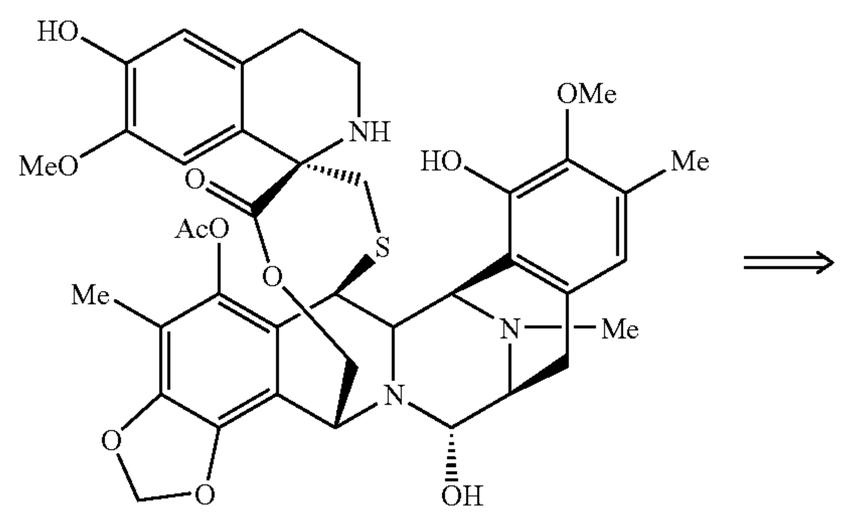
Derivatisation in step (e) can include acylation, for instance with a group R^a—CO—, where R^a can be various groups such as alkyl, alkoxy, alkylene, arylalkyl, arylalkylene, amino acid acyl, or heterocyclyl, each optionally substituted with halo, cyano, nitro, carboxyalkyl, alkoxy, aryl, aryloxy, heterocyclyl, heterocyclyloxy, alkyl, amino or substituted amino. Other acylating agents include isothiocyanates, such as aryl isothiocyanates, notably phenyl isocyanate. The alkyl, alkoxy or alkylene groups of R^a suitably have 1 to 6 or 12 carbon atoms, and can be linear, branched or cyclic. Aryl groups are typically phenyl, biphenyl or naphthyl. Heterocyclyl groups can be aromatic or partially or completely unsaturated and suitably have 4 to 8 ring atoms, more preferably 5 or 6 ring atoms, with one or more heteroatoms selected from nitrogen, sulphur and oxygen.

Without being exhaustive, typical R^a groups include alkyl, haloalkyl, alkoxyalkyl, haloalkoxyalkyl, arylalkylene, haloalkylarylakylene, acyl, haloacyl, arlyalkyl, alkenyl and amino acid. For example, R^a—CO— can be acetyl, trifluoroacetyl, 2,2,2-trichloroethoxycarbonyl, isovalerylcarbonyl, trans-3-(trifluoromethyl)cinnamoylcarbonyl, heptafluorobutyrylcarbonyl, decanoylcarbonyl, trans-cinnamoylcarbonyl, butyrylcarbonyl, 3-chloropropyonylcarbonyl, cinnamoylcarbonyl, 4-methylcinnamoylcarbonyl, hydrocinnamoylcarbonyl, or transhexenoylcarbonyl, or alanyl, arginyl, aspartyl, asparagyl, cystyl, glutamyl, glutaminyl, glycyl, histidyl, hydroxyprolyl, isoleucyl, leucyl, lysyl, methionyl, phenylalanyl, prolyl, seryl, threonyl, thyronyl, tryptophyl, tyrosyl, valyl, as well as other less common amino acid acyl groups, as well as phthalimido and other cyclic amides. Other examples may be found among the listed protecting groups. Compounds wherein —CO—R^a is derived from an amino acid and include an amino group can themselves form acyl derivatives. Suitable N-acyl commands include dipeptides which in turn can form N-acyl derivatives.

By way of illustration, it is now feasible to transform cyanosafracin B compound of formula 2 into ET-743 resulting in a shorter and more straightforward way to make ET-743 than methods previously described.

The retrosynthetic analysis to make ET-743 using compound 29 is depicted in scheme I.

Scheme I



ET-743

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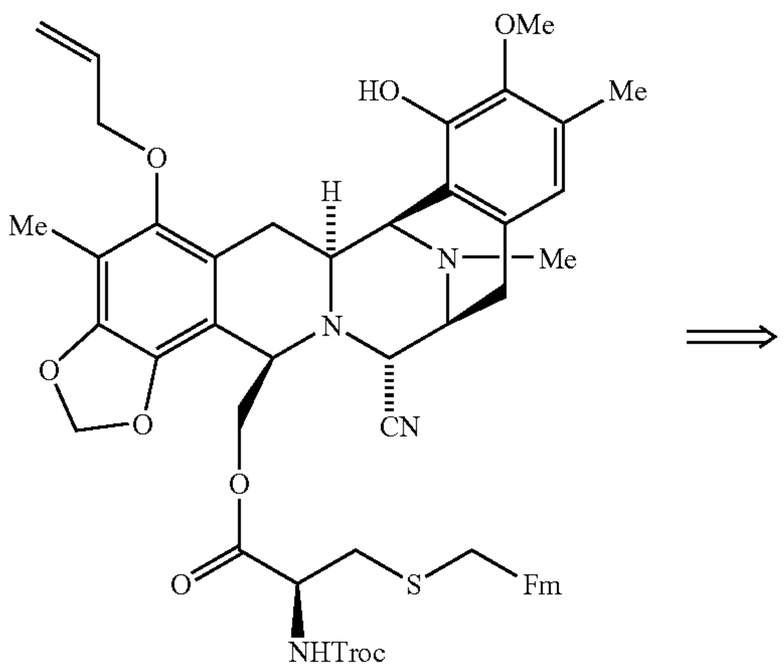

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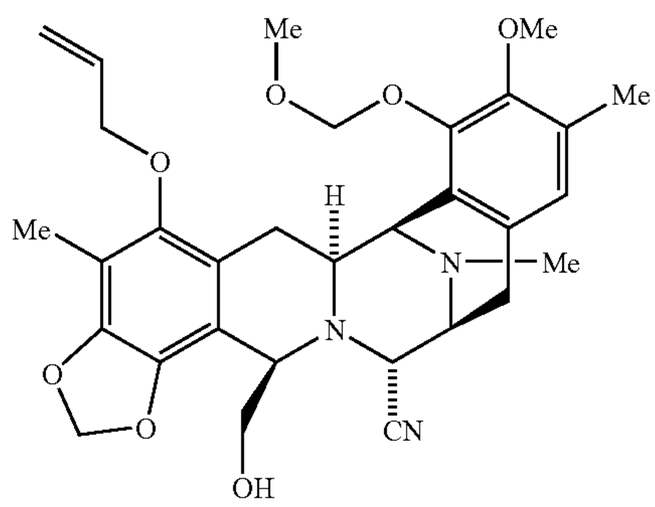

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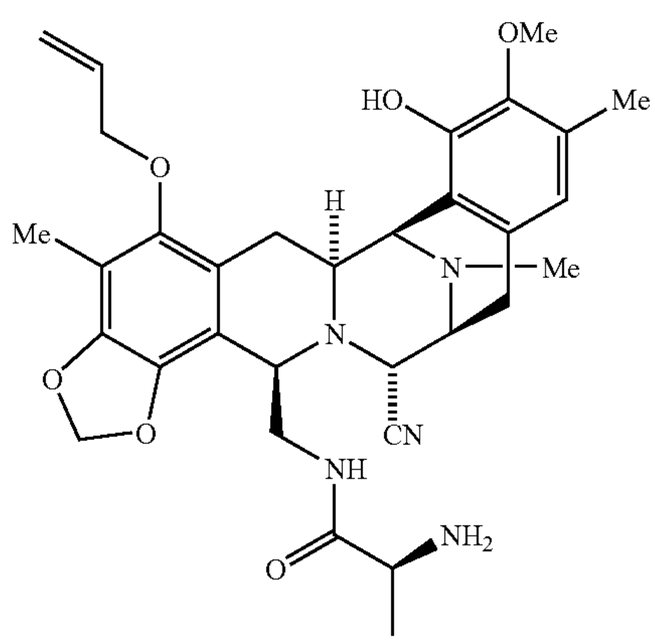


SAFRACIN-B

Following the above scheme I it is possible to obtain ET-743 in 21 linear steps. This method transforms cyanosafracin B into intermediate 25 through a sequence of reactions that involves essentially (1) removal of methoxy group placed in ring A, (2) reduction of ring A and formation of methylenedioxy group in one pot, (3) hydrolysis of amide function placed over carbon 1, (4) transformation of the resulting amine group into hydroxyl group. Furthermore the method avoids protection and de-protection of the primary alcohol function at the position 1 in ring B of compound 25 using directly a cysteine residue 29 to form intermediate 27. Cysteine derivative 29 is protected in the amino group with β-β-β-trichloroethoxycarbonyl protecting group in order to have compatibility with the existing allyl and MOM groups. Intermediate 27 is directly oxidized and cycled. These circumstances, together with a different de-protecting strategy in the later stages of the synthesis makes the route novel and more amenable to industrial development than the process of U.S. Pat. No. 5,721,362.

The conversion of the 2-cyano compound into Intermediate 25 usually involves the following steps (see scheme II):

formation of the protected compound of Formula 14 by reacting 2 with tert-butoxycarbonyl anhydride;

converting of 14 into the di-protected compound of Formula 15 by reacting with bromomethylmethyl ether and diisopropylethylamine in acetonitrile;

selectively elimination of the methoxy group of the quinone system in 15 to obtain the compound of Formula 16 by reacting with a methanolic solution of sodium hydroxide;

transforming of 16 into the methylene-dioxy compound of Formula 18 by employing the next preferred sequence: (1) quinone group of compound 16 is reduced with 10% Pd/C under hydrogen atmosphere; (2) the hydroquinone intermediate is converted into the methylenedioxy compound of Formula 17 by reacting with bromochloromethane and caesium carbonate under hydrogen atmosphere; (3) 17 is transformed into the compound of Formula 18 by protecting the free hydroxyl group as a OCH_2R group. This reaction is carried out with $BrCH_2R$ and caesium carbonate, where R can be aryl, $CH{=}CH_2$, OR' etc.

elimination of the tert-butoxycarbonyl and the methyloxymethyl protecting groups of 18 to afford the compound of Formula 19 by reacting with a solution of HCl in dioxane. Also this reaction is achieved by mixing 18 with a solution of trifluoroacetic acid in dichloromethane;

formation of the thiourea compound of Formula 20 by reacting 19 with phenylisothiocyanate;

converting compound of Formula 20 into the amine compound of Formula 21 by reacting with a solution of hydrogen chloride in dioxane;

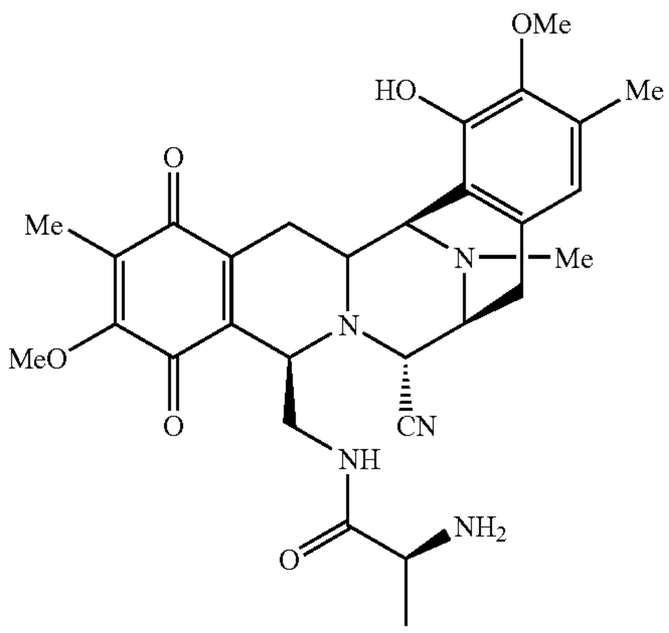
transforming compound of Formula 21 into the N-Troc derivative 22 by reacting with trichloroethyl chloroformate and pyridine;

formation of the protected hydroxy compound of Formula 23 by reacting 22 with bromomethylmethyl ether and diisopropylethylamine;

transforming compound of Formula 23 into the N—H derivative 24 by reacting with acetic acid and zinc;

conversion of compound of Formula 24 into the hydroxy compound of Formula 25 by reaction with sodium nitrite in acetic acid. Alternatively, it can be used nitrogen tetroxide in a mixture of acetic acid and acetonitrile followed by treatment with sodium hydroxide. Also, it can be used sodium nitrite in a mixture of acetic anhydride-acetic acid, followed by treatment with sodium hydroxide.

Scheme II



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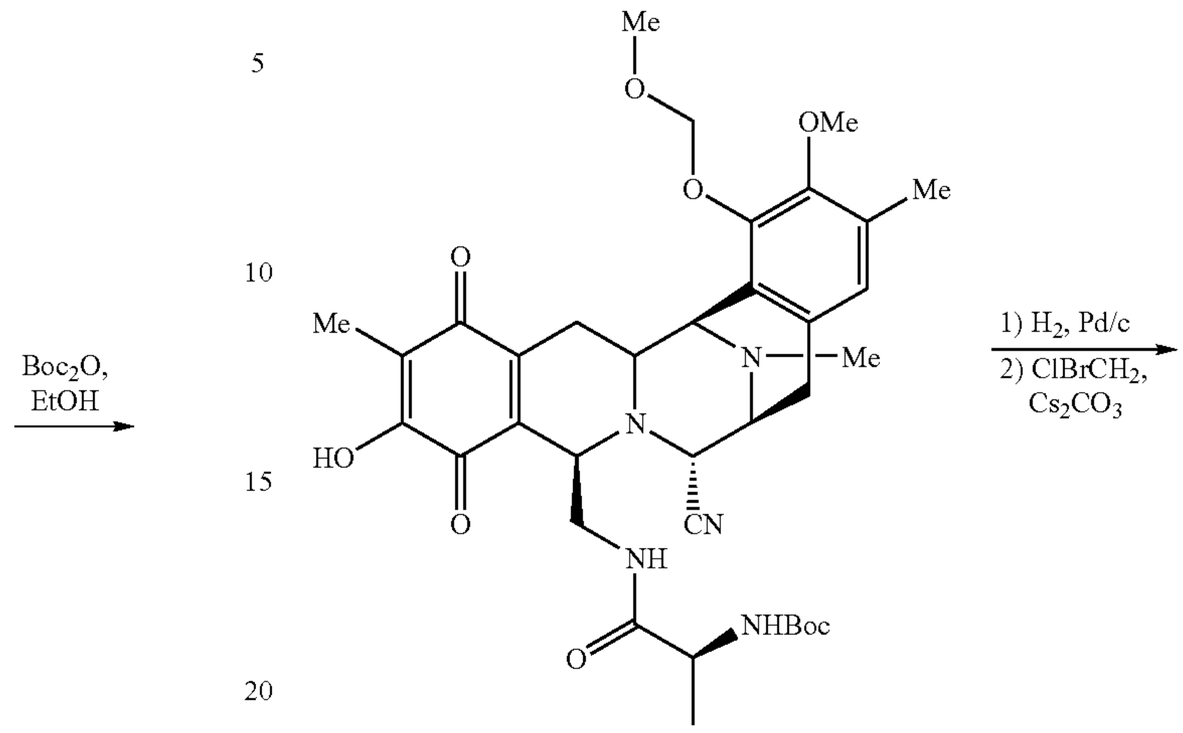

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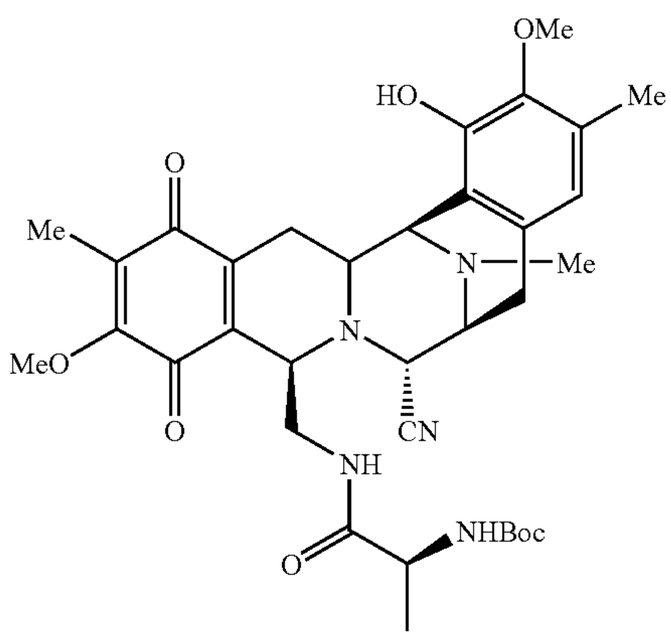

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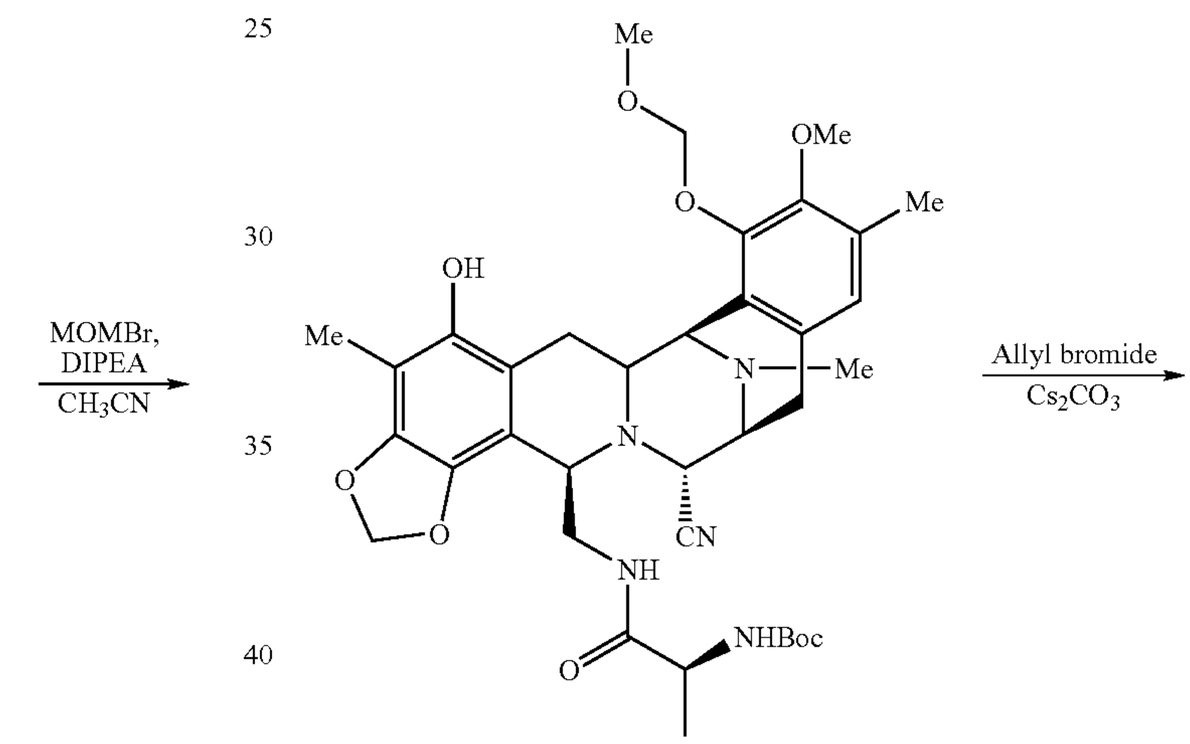

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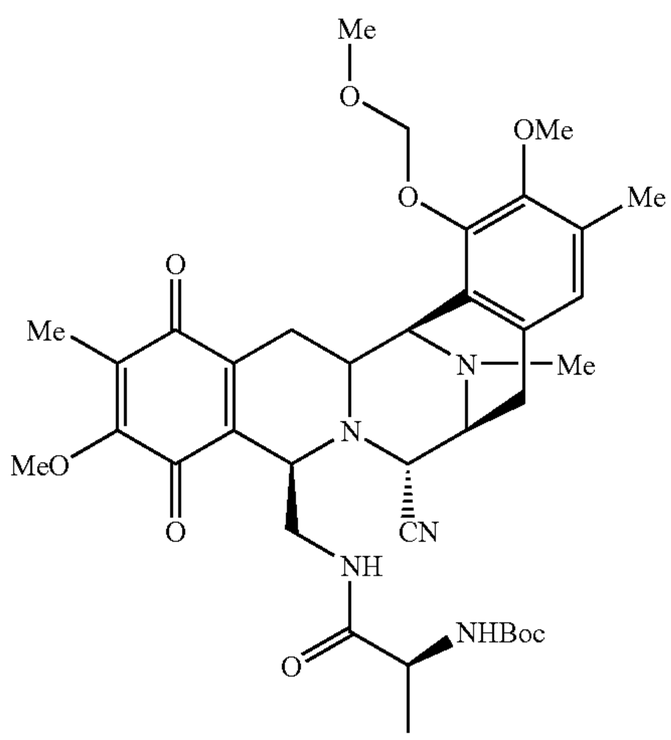

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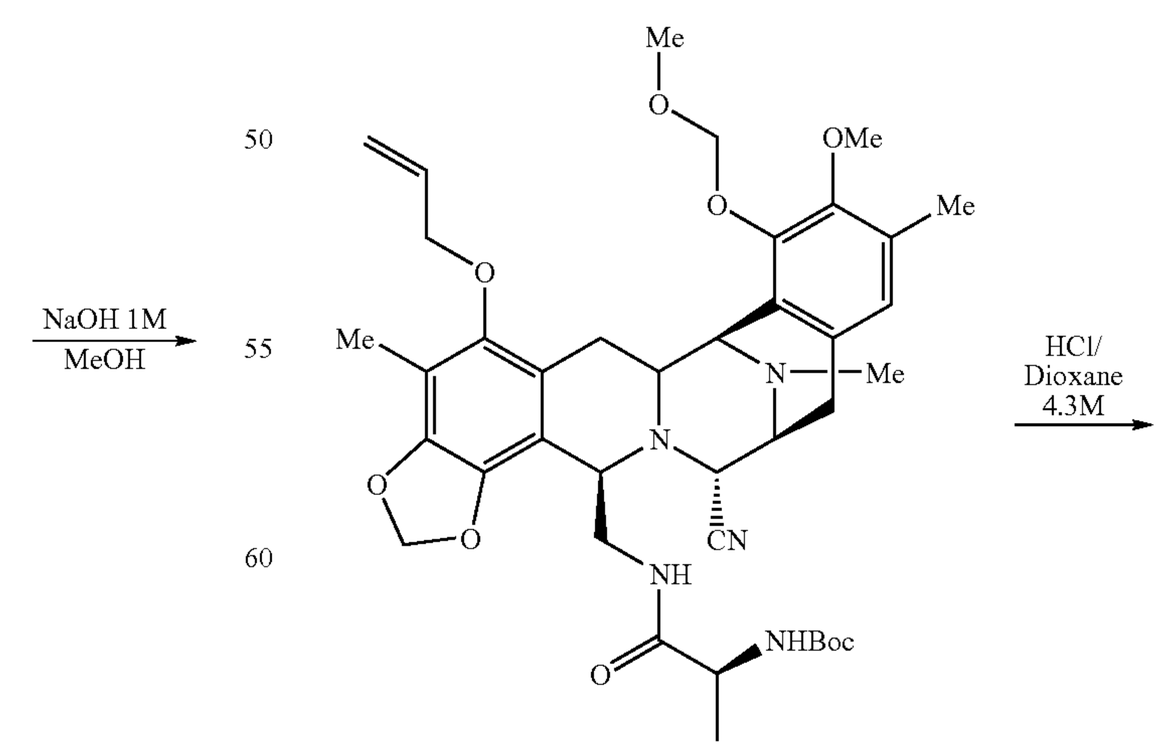

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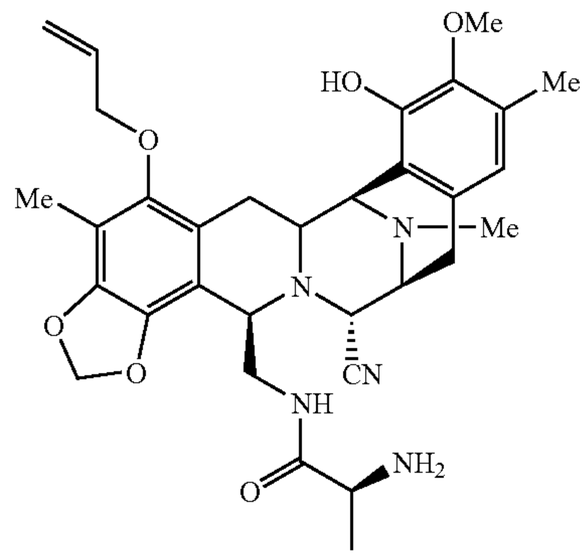

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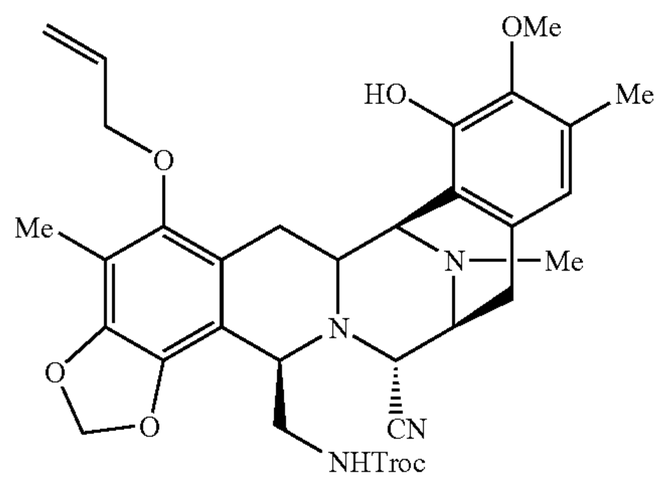

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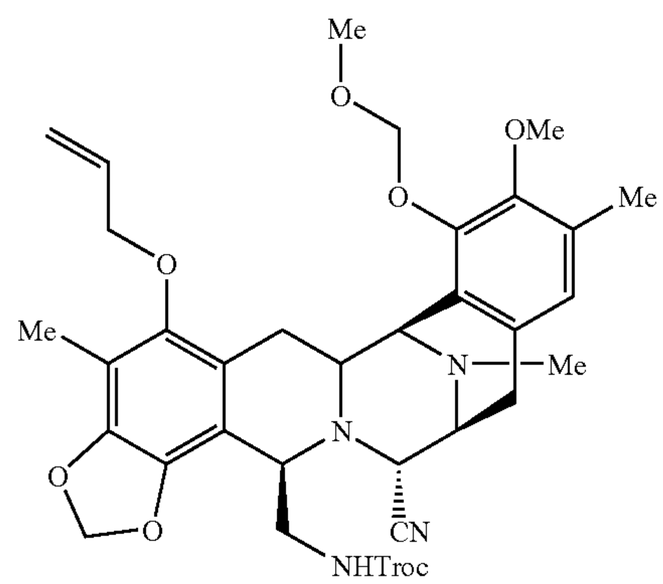

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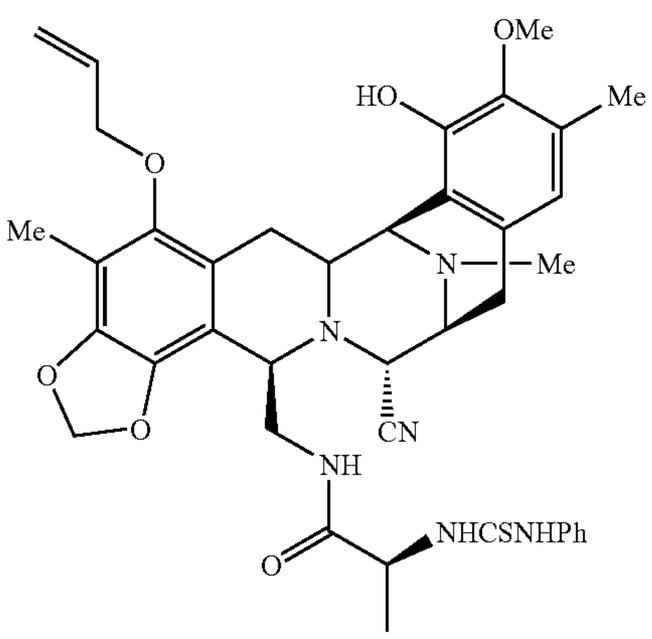

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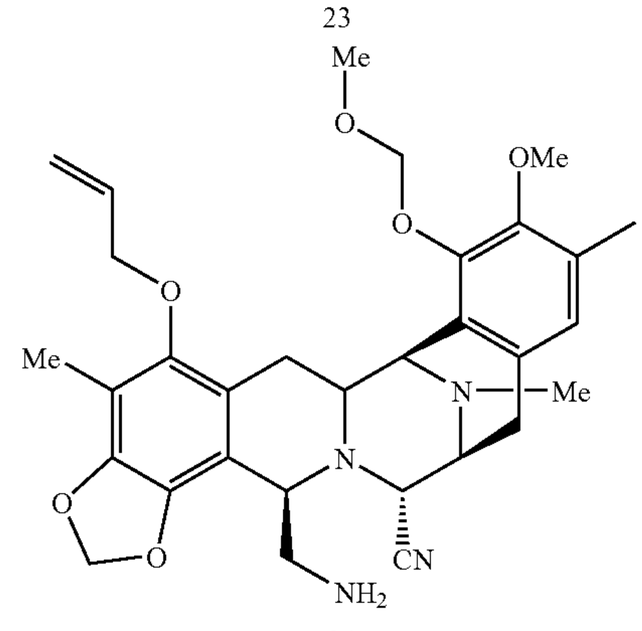

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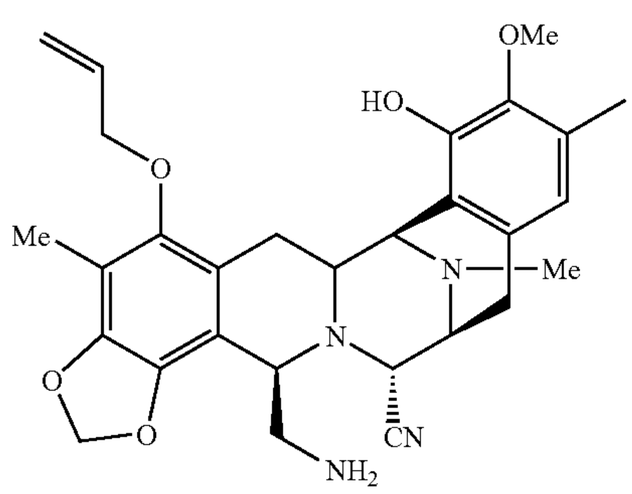

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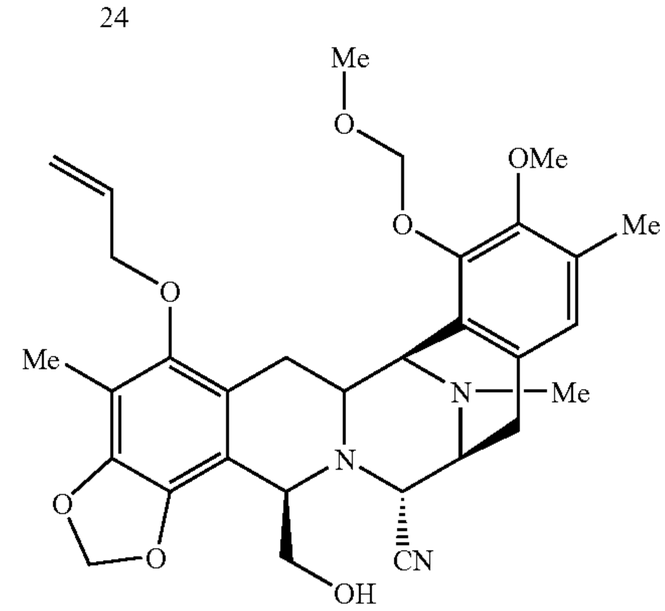

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Phenylisothiocyanate, CH_2Cl_2 → 19 → 20

HCl/ Dioxane 4.3M → 20 → 21

ClTroc, pyr., CH_2Cl_2 → 21 → 22

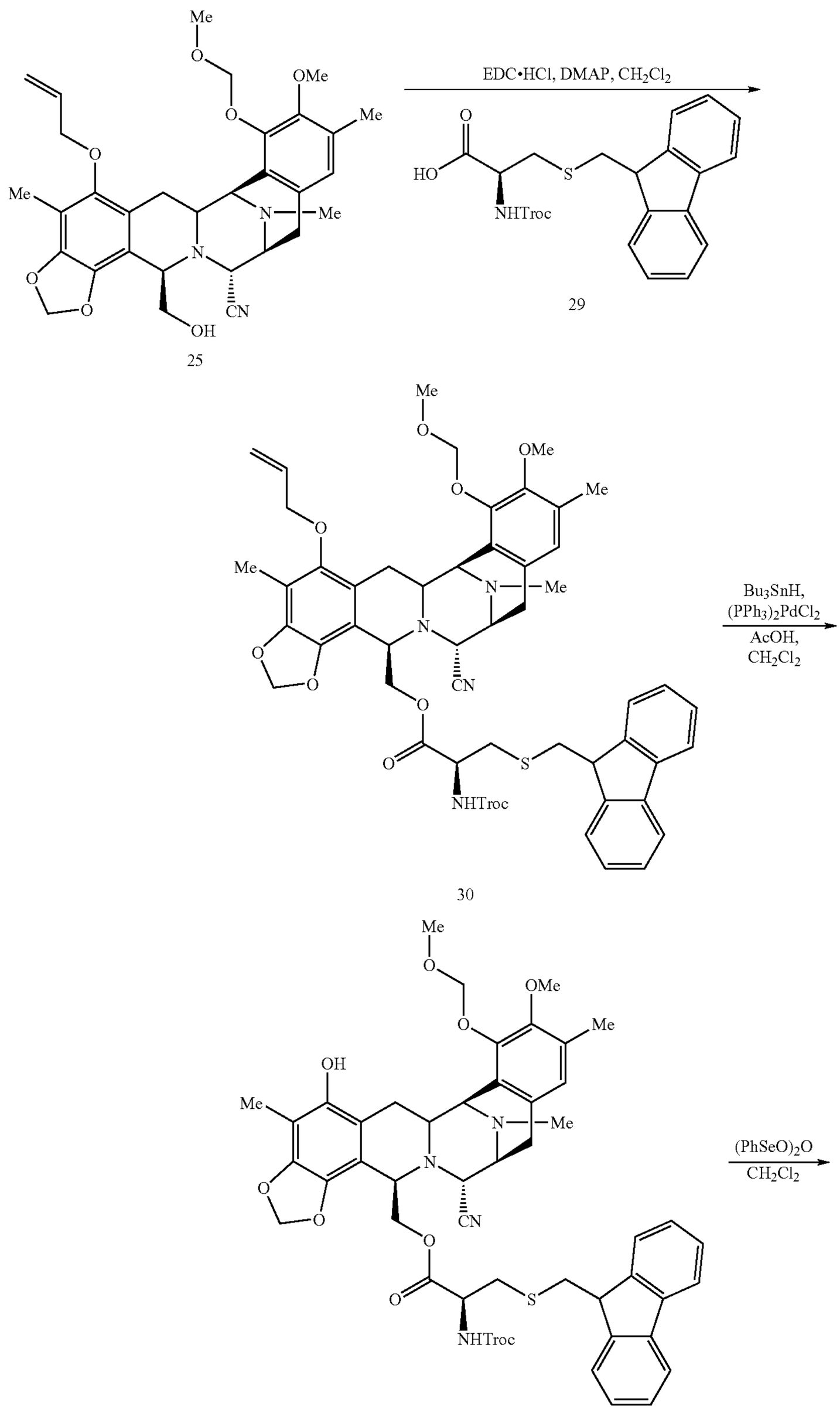
BrMOM, DIPEA, DMAP, CH_3CN → 22 → 23

AcOH aq., Zn → 23 → 24

H_2O, THF, AcOH, $NaNO_3$ → 24 → 25

The conversion of the Intermediate 25 compound into ET-743 using cysteine derivative 29 usually involves the following steps (see scheme III):

Scheme III



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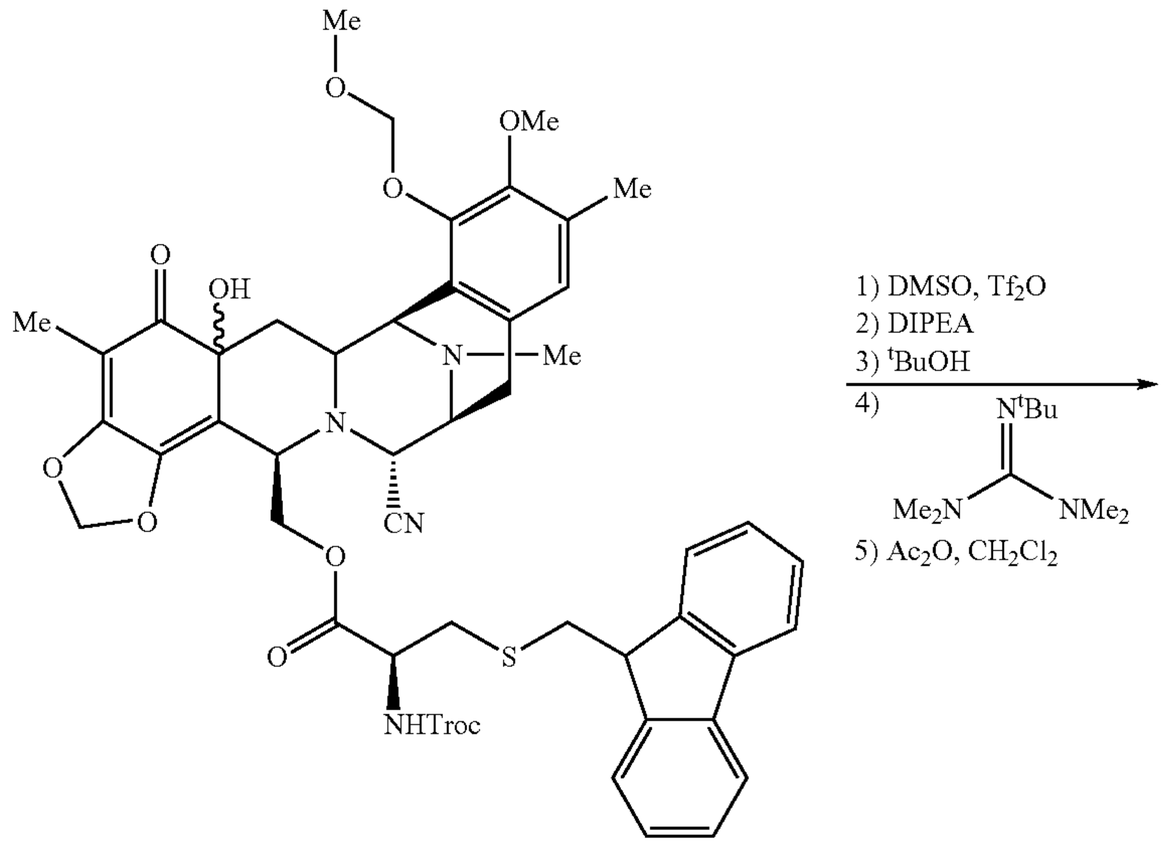

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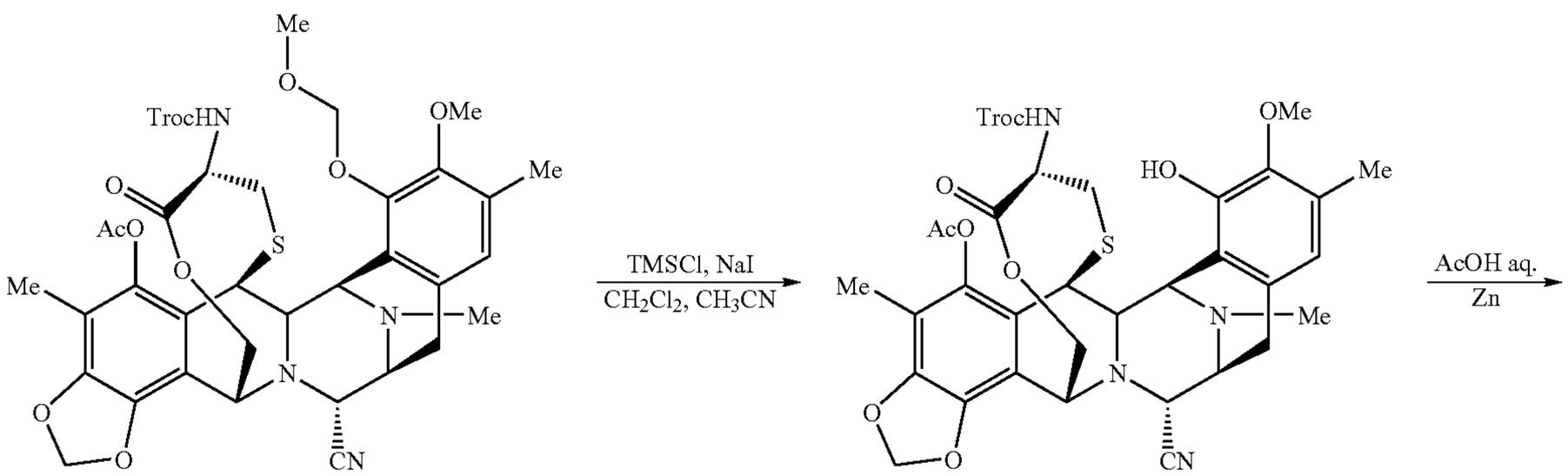
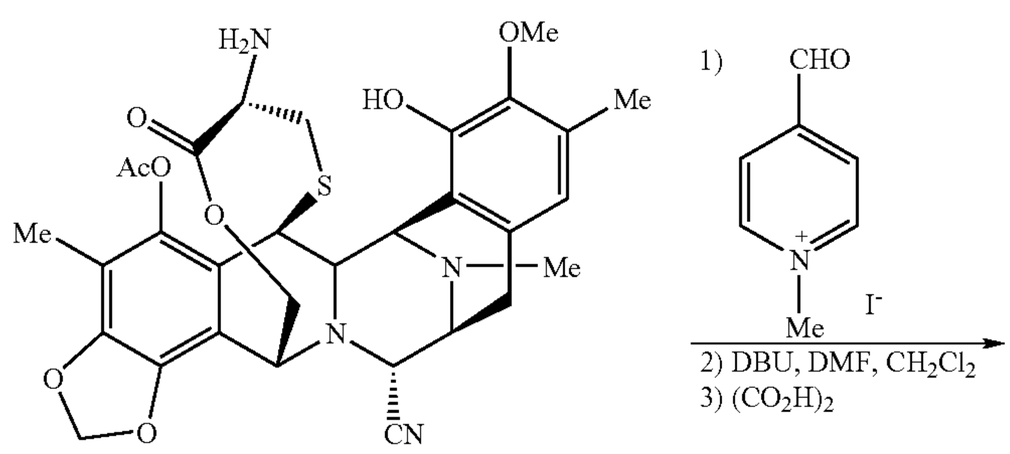


33 34



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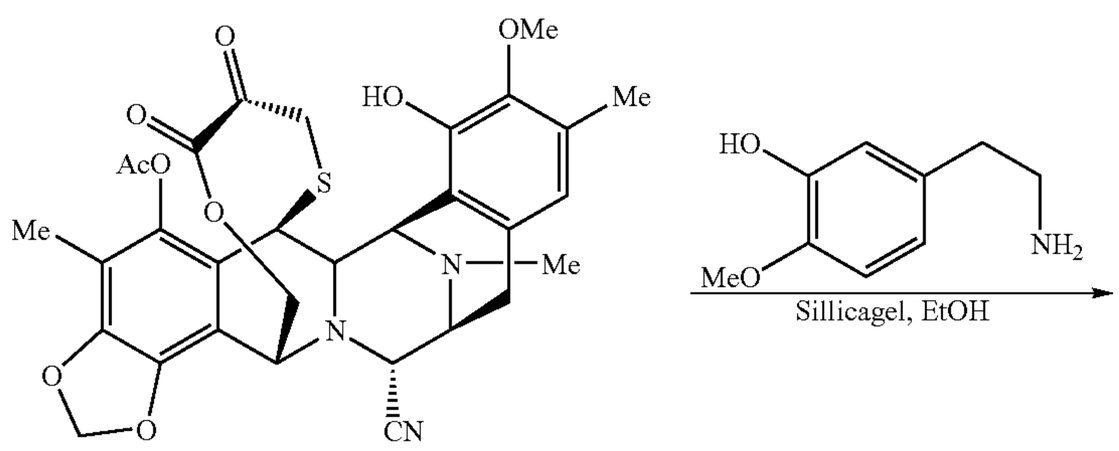

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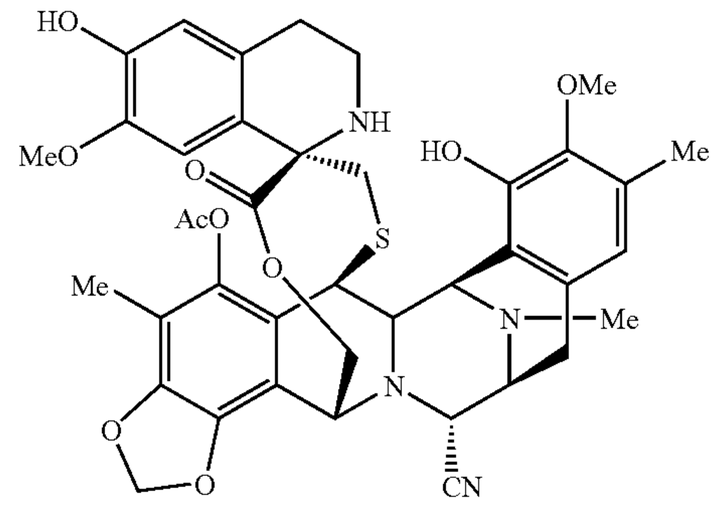
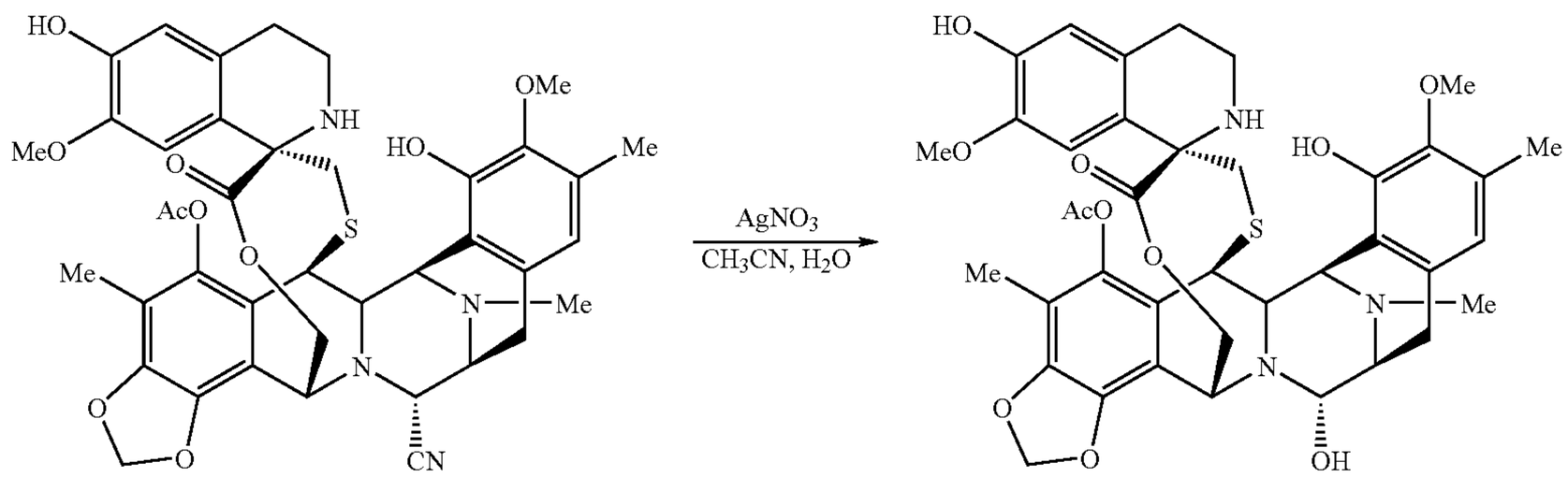


Et-770



Et-770 Et-743

transforming compound of formula 24 into the derivative 30 by protecting the primary hydroxyl function with (S)-N-2,2,2-tricloroethoxycarbonyl-S-(9H-fluoren-9-ylmethyl)cysteine 29;

converting the protected compound of formula 30 into the phenol derivative 31 by cleavage of the allyl group with tributyltin hydride and dichloropalladium-bis (triphenylphosphine);

transforming the phenol compound of Formula 31 into compound of formula 32 by oxidation with benzeneseleninic anhydride at low temperature;

transforming the hydroxy compound of formula 32 into the lactone 33 by the following sequence: (1) Reacting compound of formula 32 with 2 eq. of triflic anhydride and 5 eq. of DMSO. (2) followed by reaction with 8 eq. of diisopropylethylamine. (3) followed by reaction with 4 eq of t-butyl alcohol (4) followed by reaction with 7 eq of 2-tert-Butyl-1,1,3,3, tetramethylguanidine (5) followed by reaction with 10 eq of acetic anhydride;

transforming the lactone compound 33 into hydroxyl compound 34 by removal of MOM protecting group with TMSI;

cleaving the N-trichloroethoxycarbonyl group of the compound of formula 34 into compound 35 by reaction with Zn/AcOH;

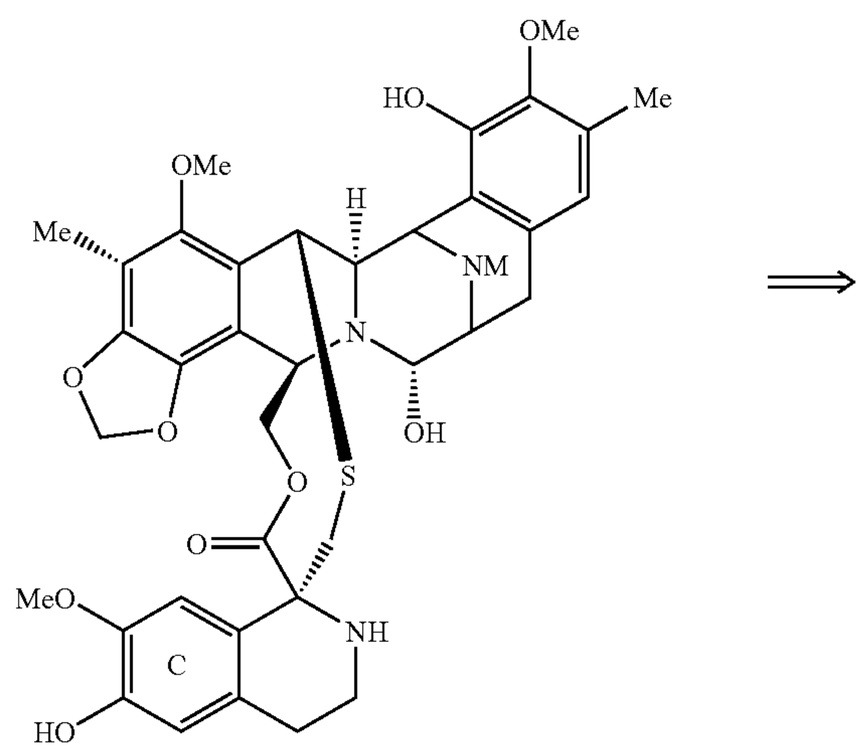
transforming the amino compound 35 into the corresponding α-keto lactone compound 36 by reaction with N-methylpyridinium carboxaldehyde chloride followed by DBU;

forming ET-770 by reacting compound of Formula 36 with 3-hydroxy-4-methoxyphenylethylamine;

transforming ET-770 into ET-743 by reaction with silver nitrate in a mixture of AcN/H_2O.

Formation of Intermediate 11 and Related Intermediates

The retrosynthetic analysis is described in the following sequence.



ET-743

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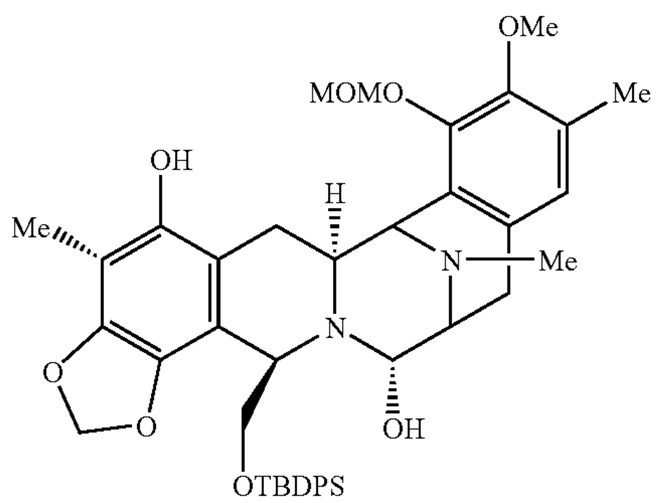
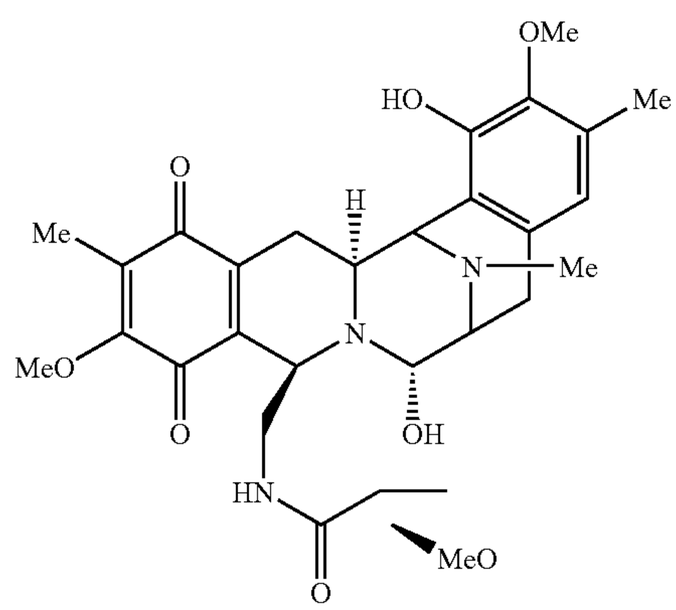
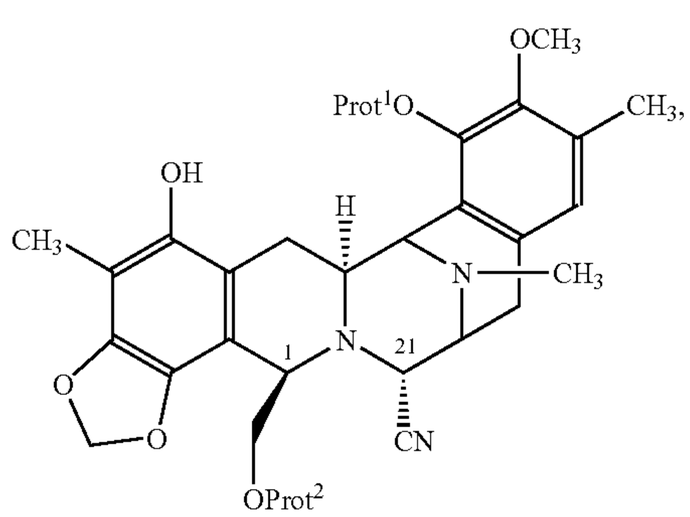


int-11



In the present invention, a key class of intermediate includes Intermediate 11 and has the general formula (XXI):



where $Prot^1$ and $Prot^2$ are hydroxy protecting groups, preferably different. For Intermediate 11 itself, the group $Prot^1$ is a methoxymethyl group, and $Prot^2$ is a t-butyldiphenylsilyl group.

The conversion of the 21-cyano compound to Intermediate 11 or a related intermediate of formula (X) usually involves the following steps:

a) conversion if necessary of a quinone system for the ring E into the phenol system
b) formation of the —$OProt^1$ group at the 18-position, in ring E;
c) formation of the —CH_2—$OProt^2$ group at the 1-position, in ring B; and
d) conversion if necessary of a quinone system for the ring A into the phenol system;
e) conversion of the phenol system for the ring A into the methylenedioxyphenol ring.

Step (b), formation of the —$OProt^1$ group at the 18-position in ring E, is a typical protection reaction for a phenol group, and no special comments need to be made. Appropriate conditions are chosen depending on the nature of the protecting group. The other steps are similar to the other reactions.

Step (b), formation of the —CH_2—$OProt^2$ group at the 1-position in ring B, is normally carried out by forming a group —CH_2NH_2 at the 1-position and then converting the amine function to a hydroxy function and protecting. Thus, where the starting material has a group R^1 which is —CH^2—NH—CO—$CR^{25a}R^{25b}R^{25c}$ then it is matter of removing the N-acyl group. Where the starting material has a group R^1 which is —CH_2—O—CO—R then no change may be needed for an ecteinascidin product where the substituent R^1 is the same. For other products, it is matter of removing the O-acyl group. Various procedures are available for such de-acylations. In one variation, the deacylation and conversion to a hydroxy function are performed in one step. Thereafter, the hydroxy group can be acylated or otherwise converted to give the appropriate R^1 group.

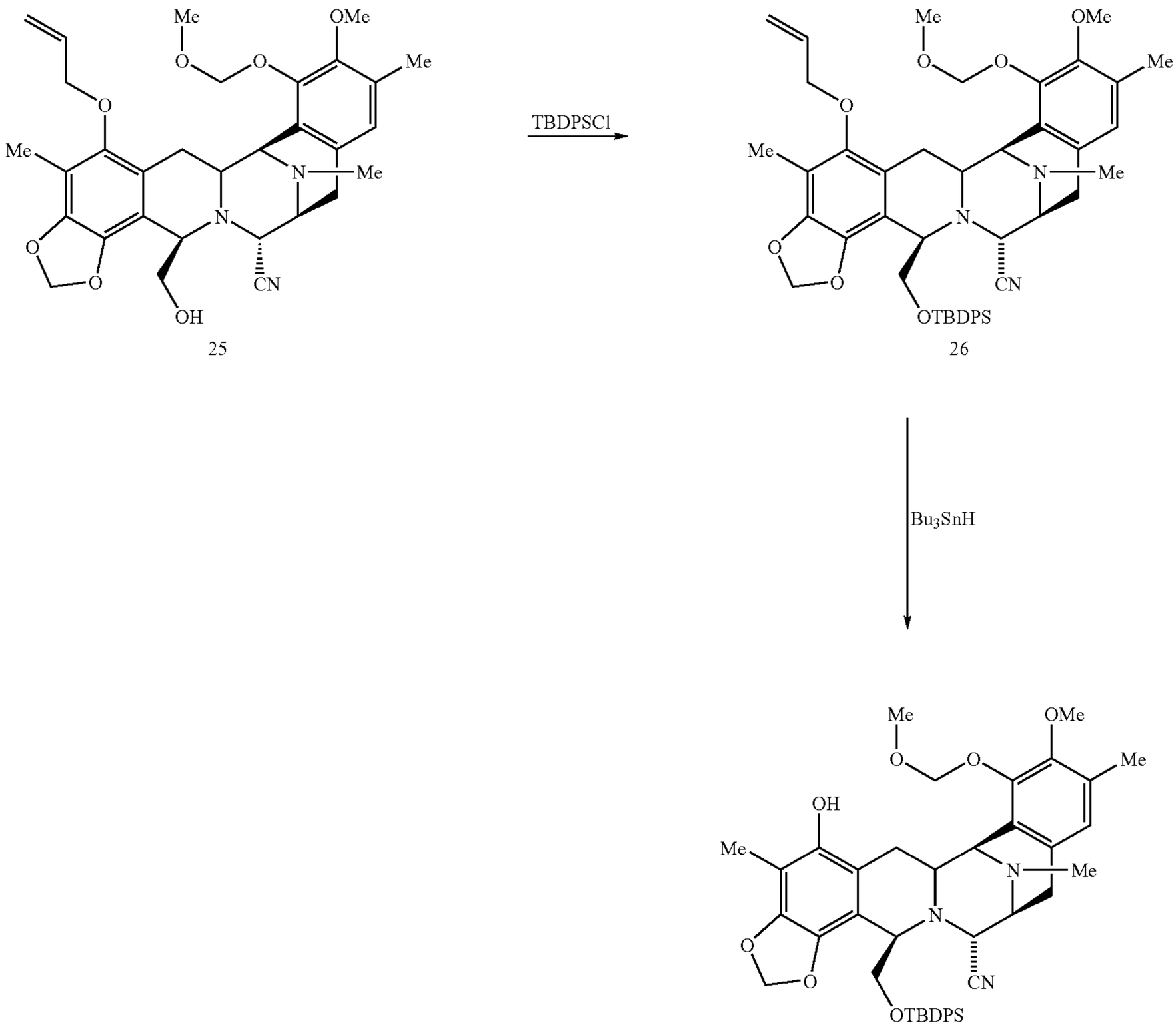
U.S. Pat. No. 5,721,362 describe synthetic methods to make ET-743 through a long multistep synthesis. One of the Intermediates of this synthesis is Intermediate 11. Using cyanosafracin B as starting material it is possible to reach Intermediate 11 providing a much shorter way to make such Intermediate and therefor improving the method to make ET-743

Cyanosafracin B can be converted into Intermediate 25 by the methods described above. From Intermediate 25 is possible to reach Intermediate 11 using the following steps, see scheme VII.

formation of the protected hydroxy compound of Formula 26 by reacting 25 with tert-butyldiphenylsilyl chloride in the presence of a base;

final cleavage of the allyl group with tributyltin hydride and dichloropalladium-bis (triphenylphosphine) in 26 that leads to the formation of the intermediate 11.

Scheme VII



One embodiment of the synthetic process of the present invention to transform safracin B into intermediate 11 is a modification and extension of Scheme VIII and comprises the sequential steps of: stereospecifically converting the compound Safracin B to the compound of Formula 2 by selective replacement of OH by CN by reacting with KCN in acid media;

forming the thiourea compound of Formula 3 by reacting compound of Formula 2 with phenyl isothiocyanate;

converting the thiourea compound of Formula 3 into the acetamide of Formula 5 by an hydrolysis in acid media followed by addition of acetic anhydride; The intermediate amine compound of Formula 4 can be isolated by quenching the hydrolysis in acid media with sodium bicarbonate, but this intermediate is highly unstable, and is transformed quickly into a five member cyclic imine, named compound 6;

forming the protected compound of Formula 7 by reacting with bromomethylmethyl ether and diisopropylethylamine in dichloromethane;

selectively de-methylating the methoxy group of the quinone system of compound of Formula 7 into the compound of Formula 8 by reacting with methanolic solution of sodium hydroxide;

transforming the compound of Formula 8 into methylenedioxy-compound of Formula 9 by the preferred following sequence: (1) quinone group of compound 8 is reduced with 10% Pd/C under hydrogen atmosphere; (2) the hydroquinone intermediate is converted into the methylenedioxy compound of Formula 9 by reacting with bromochloromethane and cesium carbonate under hydrogen atmosphere; (3) compound of Formula 9 is transformed into compound of Formula 10 by protecting the free hydroxyl group as a OCH_2R group, by reacting with $BrCH_2R$ and cesium carbonate, where R can be aryl, $CH{=}CH_2$, OR' etc.;

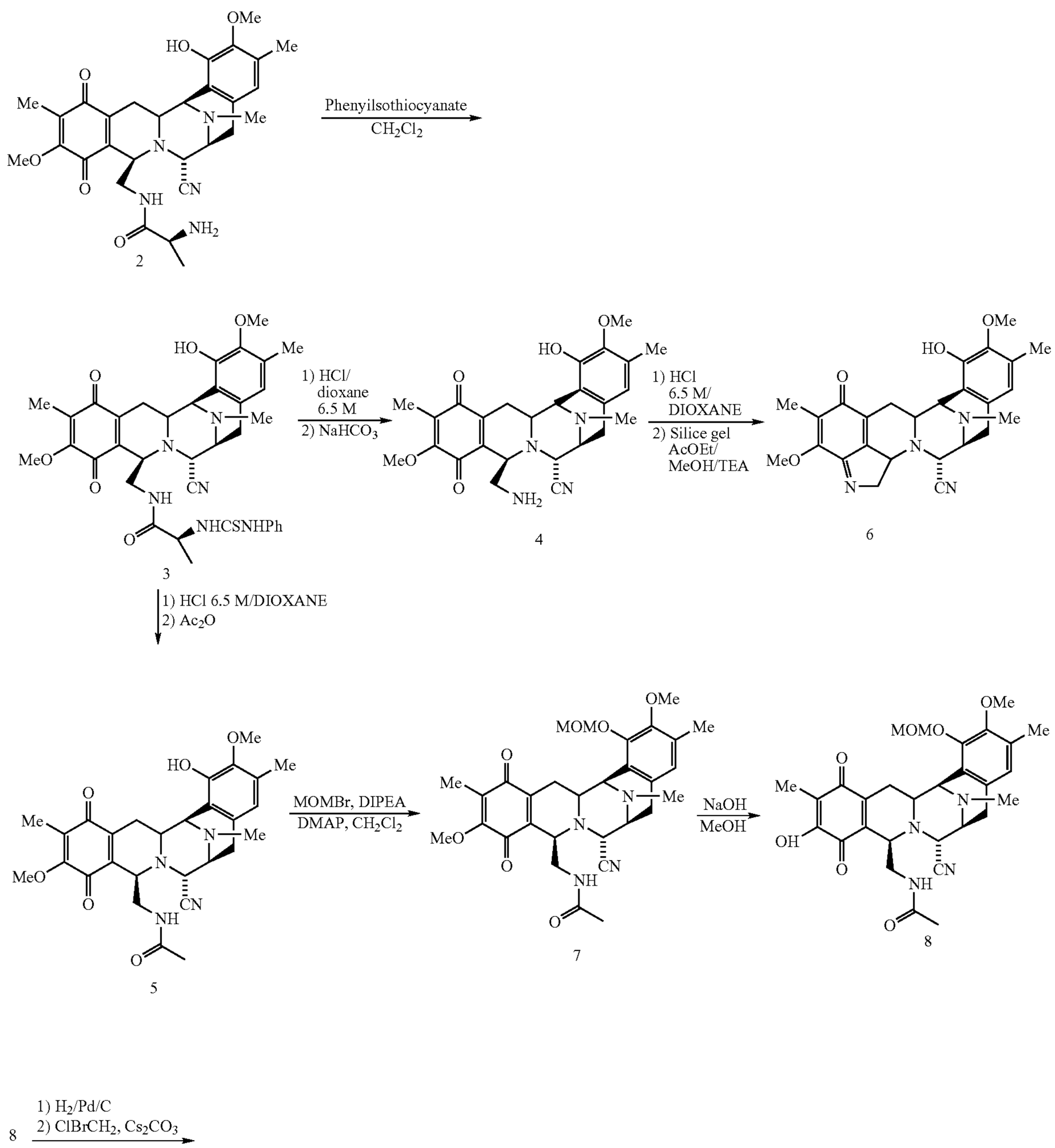
converting the acetamide group of compound of Formula 10 into the corresponding hydroxyl group of Formula 25 by reaction with nitrogen tetroxide in a mixture of acetic acid and acetic acetate followed by treatment with sodium hydroxide; alternatively can be used sodium nitrite in a mixture of acetic anhydride acetic acid, followed by treat-

ment with sodium hydroxide; alternatively the acetamide group of compound of Formula 10 can be converted into the primary amine group by reacting with hydrazine or with Boc_2O, DMAP followed by hydrazine; such primary amine can be converted into the corresponding hydroxyl group (compound of Formula 25) by an oxidative conversion of the primary amine into the corresponding aldehyde with 4-formyl-1-methylpyridinium benzenesulphonate or other pyridinium ion, followed by DBU or other base treatment and further hydrolization, and followed by the reduction of the aldehyde to the corresponding hydroxyl group with lithium aluminium hydride or other reducing agent;

forming the protected compound of Formula 26 by reacting with t-butyldiphenylsilyl chloride and dimethylaminopyridine in dichloromethane (Scheme VII);

transforming the silylated compound of Formula 26 into the intermediate 11 by deprotection of the OCH_2R protecting group, by reacting under reductive conditions or acid conditions. Typical procedures are with palladium black under hydrogen atmosphere, or aqueous TFA, or tributyltin hydride and dichlorobis (triphenylphosphine palladium).

Scheme VIII



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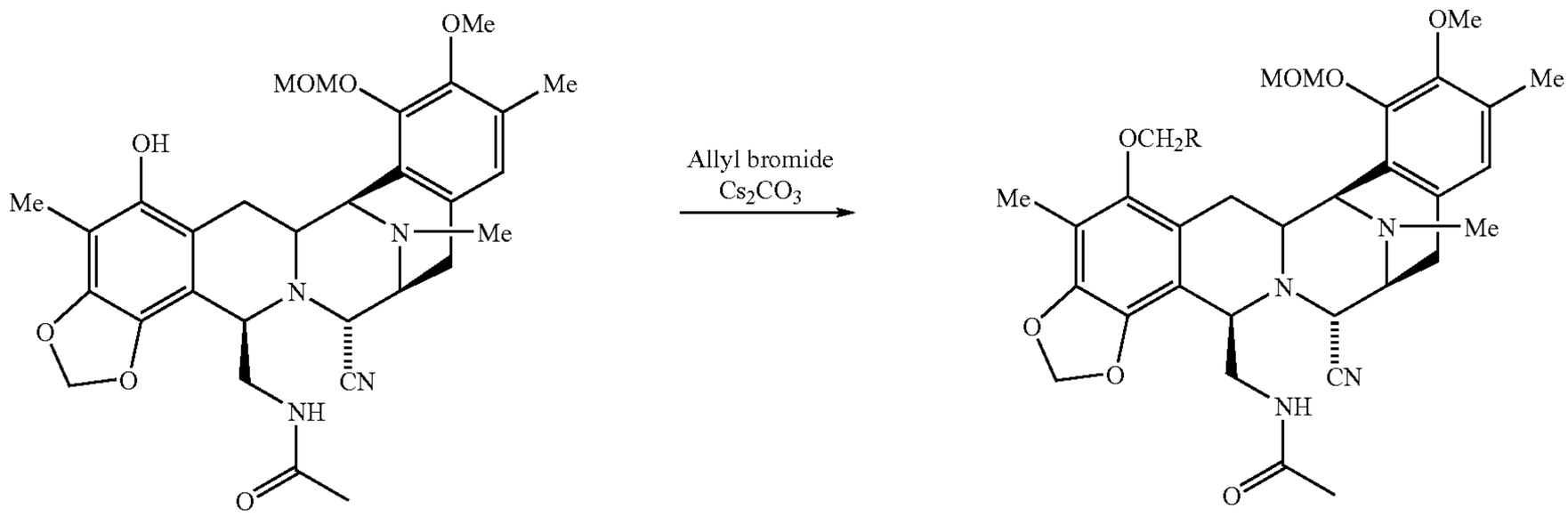
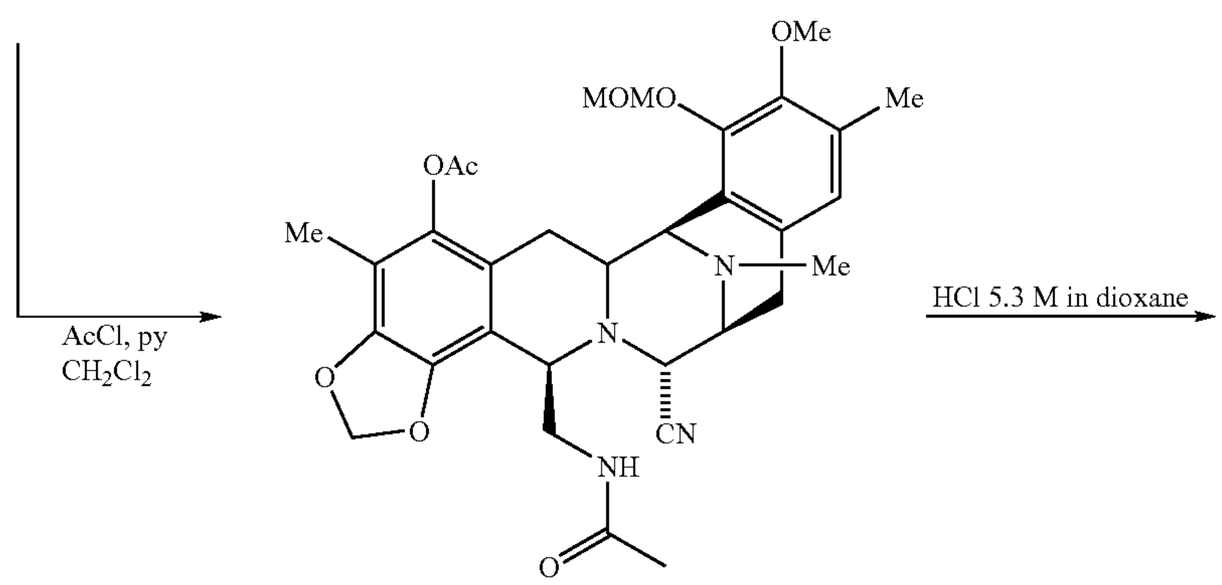
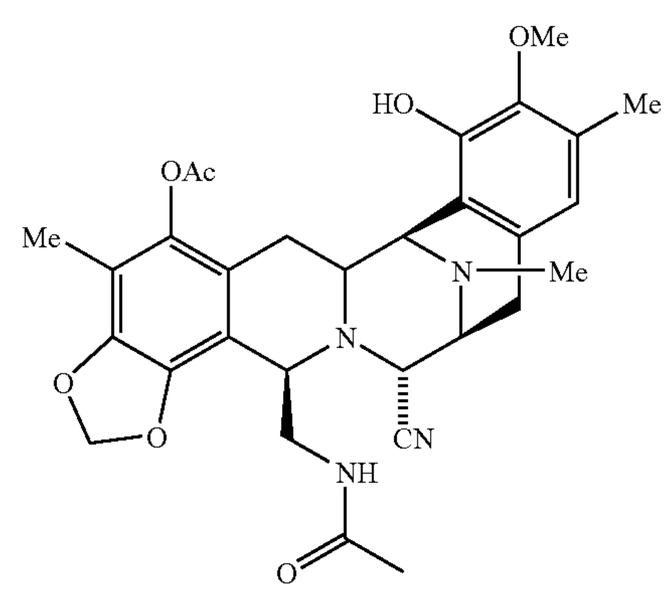


9 10



46



47

In yet another preferred modification, the cyano compound of Formula 2 can be transformed into Intermediate 11 using an extension of the scheme II, involving the further steps of.

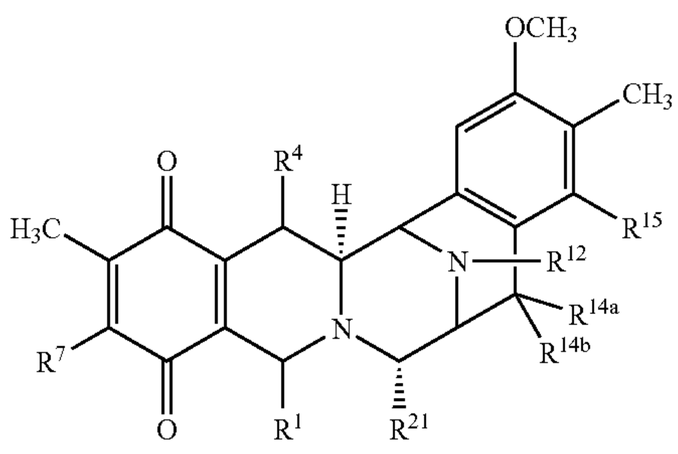
formation of the protected hydroxy compound of Formula 26 by reacting 25 with tert-butyldiphenylsilyl chloride in the presence of a base;

final cleavage of the allyl group with tributyltin hydride and dichloropalladium-bis (triphenylphosphine) in 26 that leads to the formation of the intermediate 11.

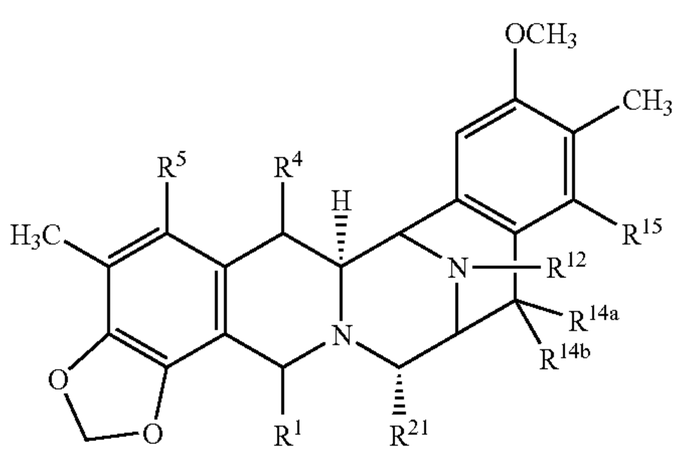
Thus, by these and other routes, it is possible to transform cyanosafracin B into a number of intermediates and derivatives with potential antitumor therapeutic activity. These intermediates can be made starting from already described compounds, or using alternative routes.

Novel Intermediate Compounds

In the light of the preceding explanations, it can be seen that the present invention provides novel intermediate compounds. Depending on ring A, the intermediates are of formula (XXIIa):



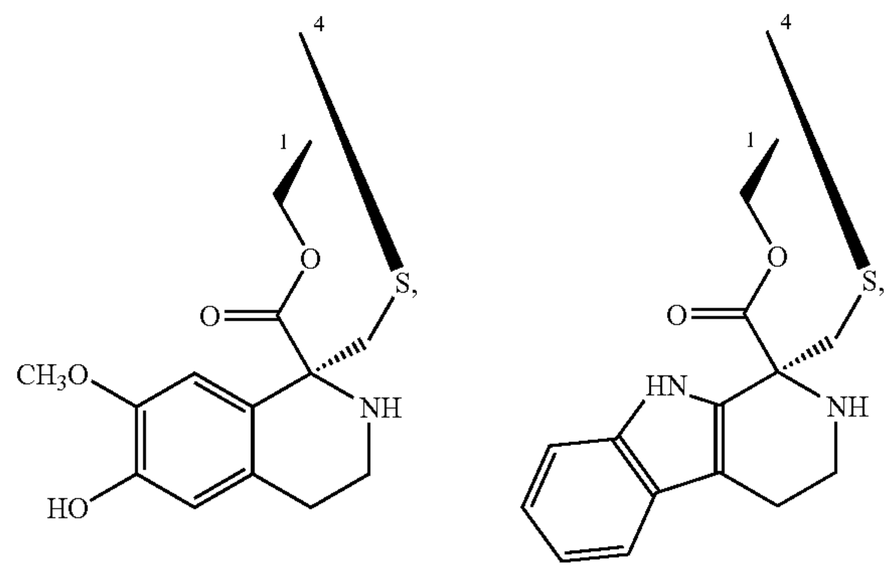
or of formula (XXIIb):

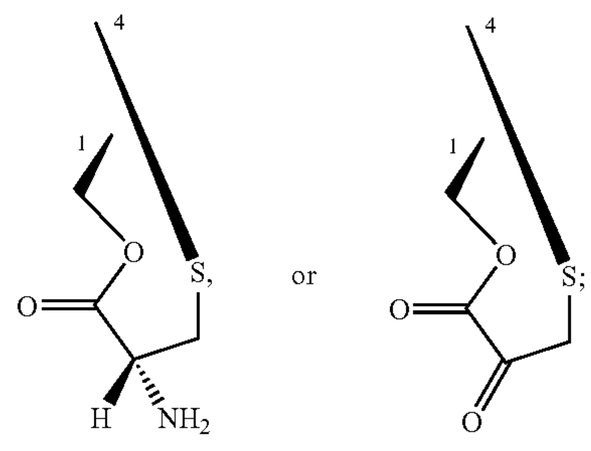


where:

R^1 is —CH_2NH_2 or —CH_2OH, or a protected or derivatised version of such a group and R^4 is —H; or

R^{1a} and R^4 together form a group of formula (IV), (V), (VI) or (VII):





R^5 is —OH or a protected or derivatised version of such a group;

R^{14a} and R^{14b} are both —H or one is —H and the other is —OH or a protected or derivatised version of such a group, —OCH_3 or —OCH_2CH_3, or R^{14a} and R^{14b} together form a keto group;

R^{12} is —H—, —CH_3— or —CH_2CH_3—;

R^{15} is —H, —OH or a protected or derivatised version of such a group; and

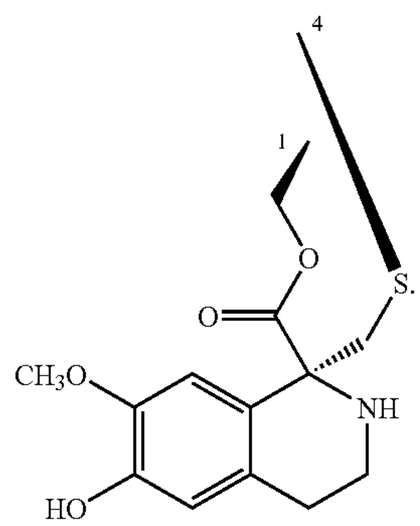
R^{18} is —OH or a protected or derivatised version of such a group.

In one embodiment, preferably at least of R^1, R^5, R^{14a}, R^{14b}, R^{15} or R^{18} is a protected or derivatised group.

In one variation of this invention, the group R^1 is not a tert-butyldiphenylsilyl substituent and/or the group R^{18} is not a methoxymethyl group.

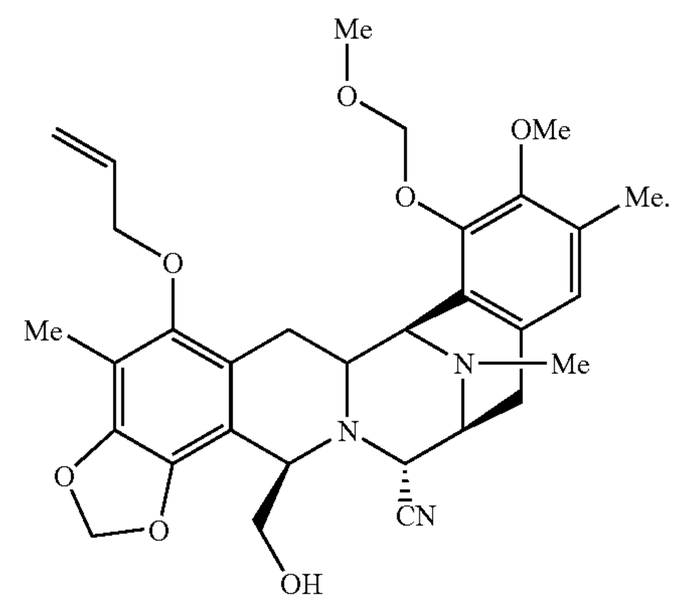
Preferably R^1 is —CH_2NH_2 or —CH_2OH, or a protected or derivatised version of such a group and R^4 is —H; or

R^{1a} and R^4 together form a group:



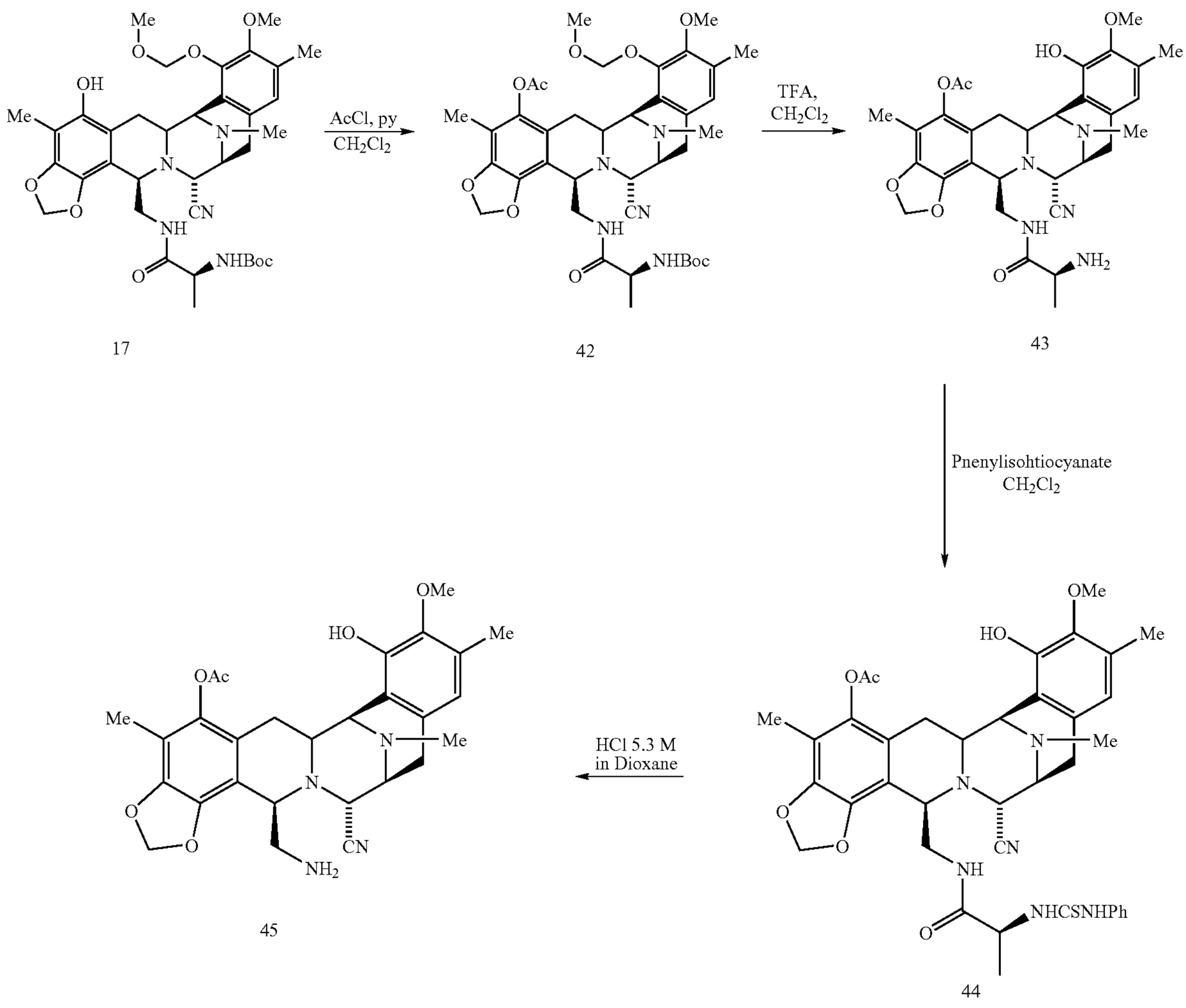
Preferably R^{14a} and R^{14b} are both —H.

One preferred class of intermediates includes the compound which we identify as compound 25, of formula:



The preferred class is thus of the general formula where the group MOM is replaced by any other protecting group.

Other preferred intermediates includes the compounds which we identify as compound 45 and 43 (Scheme IX).



Other N-acyl derivatives may readily be made from compound 45 and are an important part of this invention. Suitable acyl groups include those previously mentioned. The corresponding 21-hydroxy compounds are also useful and are among the active compounds which we have found.

Novel Active Compounds

We have additionally found that certain of the compounds of the invention which we initially prepared as intermediates have exceptional activity in the treatment of cancers, such as leukaemias, lung cancer, colon cancer, kidney cancer and melanoma.

Thus, the present invention provides a method of treating any mammal, notably a human, affected by cancer which comprises administering to the affected individual a therapeutically effective amount of a compound of the invention, or a pharmaceutical composition thereof.

The present invention also relates to pharmaceutical preparations, which contain as active ingredient a compound or compounds of the invention, as well as the processes for their preparation.

Examples of pharmaceutical compositions include any solid (tablets, pills, capsules, granules, etc.) or liquid (solutions, suspensions or emulsions) with suitable composition or oral, topical or parenteral administration, and they may contain the pure compound or in combination with any carrier or other pharmacologically active compounds. These compositions may need to be sterile when administered parenterally.

Administration of the compounds or compositions of the present invention may be by any suitable method, such as intravenous infusion, oral preparations, intraperitoneal and intravenous administration. We prefer that infusion times of up to 24 hours are used, more preferably 2-12 hours, with 2-6 hours most preferred. Short infusion times which allow treatment to be carried out without an overnight stay in hospital are especially desirable. However, infusion may be 12 to 24 hours or even longer if required. Infusion may be carried out at suitable intervals of say 2 to 4 weeks. Pharmaceutical compositions containing compounds of the invention may be delivered by liposome or nanosphere encapsulation, in sustained release formulations or by other standard delivery means.

The correct dosage of the compounds will vary according to the particular formulation, the mode of application, and the particular situs, host and tumour being treated. Other factors like age, body weight, sex, diet, time of administration, rate of excretion, condition of the host, drug combinations, reaction sensitivities and severity of the disease shall be taken into account. Administration can be carried out continuously or periodically within the maximum tolerated dose.

The compounds and compositions of this invention may be used with other drugs to provide a combination therapy. The other drugs may form part of the same composition, or be provided as a separate composition for administration at the same time or a different time. The identity of the other drug is not particularly limited, and suitable candidates include:

a) drugs with antimitotic effects, especially those which target cytoskeletal elements, including microtubule modulators such as taxane drugs (such as taxol, paclitaxel, taxotere, docetaxel), podophylotoxins or vinca alkaloids (vincristine, vinblastine);
b) antimetabolite drugs such as 5-fluorouracil, cytarabine, gemcitabine, purine analogues such as pentostatin, methotrexate);
c) alkylating agents such as nitrogen mustards (such as cyclophosphamide or ifosphamide);
d) drugs which target DNA such as the antracycline drugs adriamycin, doxorubicin, pharmorubicin or epirubicin;
e) drugs which target topoisomerases such as etoposide;
f) hormones and hormone agonists or antagonists such as estrogens, antiestrogens (tamoxifen and related compounds) and androgens, flutamide, leuprorelin, goserelin, cyprotrone or octreotide;
g) drugs which target signal transduction in tumour cells including antibody derivatives such as herceptin;
h) alkylating drugs such as platinum drugs (cis-platin, carbonplatin, oxaliplatin, paraplatin) or nitrosoureas;
i) drugs potentially affecting metastasis of tumours such as matrix metalloproteinase inhibitors;
j) gene therapy and antisense agents;
k) antibody therapeutics;
l) other bioactive compounds of marine origin, notably the didemnins such as aplidine;
m) steroid analogues, in particular dexamethasone;
n) anti-inflammatory drugs, in particular dexamethasone; and
o) anti-emetic drugs, in particular dexamethasone.

The present invention also extends to the compounds of the invention for use in a method of treatment, and to the use of the compounds in the preparation of a composition for treatment of cancer.

Cytotoxic Activity

Cell Cultures. Cells were maintained in logarithmic phase of growth in Eagle's Minimum Essential Medium, with Earle's Balanced Salts, with 2.0 mM L-glutamine, with non-essential amino acids, without sodium bicarbonate (EMEM/neaa); supplemented with 10% Fetal Calf Serum (FCS), 10^{-2} M sodium bicarbonate and 0.1 g/l penicillin-G+streptomycin sulfate.

A simple screening procedure has been carried out to determine and compare the antitumour activity of these compounds, using an adapted form of the method described by Bergeron et al (1984). The tumour cell line employed have been P-388 (suspension culture of a lymphoid neoplasm from DBA/2 mouse), A-549 (monolayer culture of a human lung carcinoma), HT-29 (monolayer culture of a human colon carcinoma) and MEL-28 (monolayer culture of a human melanoma).

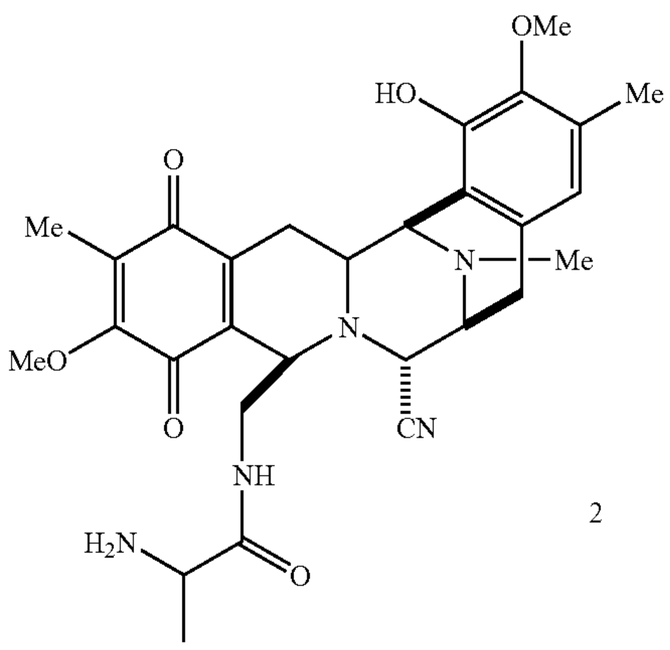
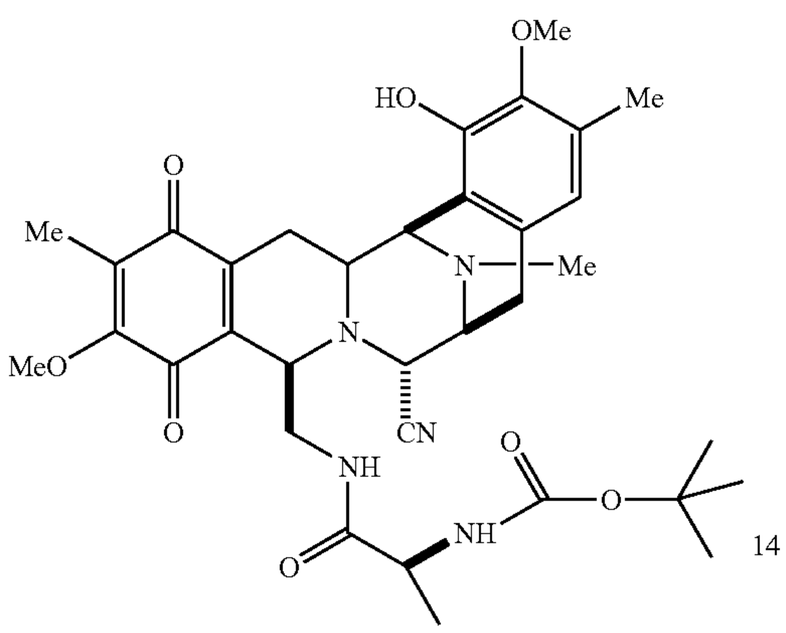
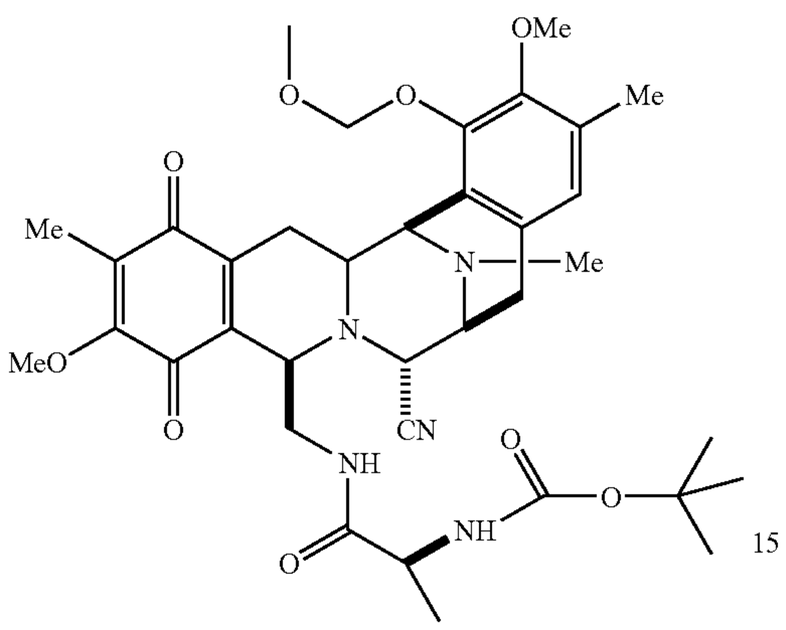
P-388 cell were seeded into 16 mm wells at 1×10^4 cells per well in 1 ml aliquots of MEM 5FCS containing the indicated concentration of drug. A separate set of cultures without drug was seeded as control growth to ensure that cells remained in exponential phase of growth. All determinations were carried out in duplicate. After three days of incubation at 37° C., 10% CO_2 in a 98% humid atmosphere, an approximately IC_{50} was determined by comparing the growth in wells with drug to the growth in wells control.

A-549, HT-29 and MEL-28 were seeded into 16 mm wells at 2×10^4 cells per well in 1 ml aliquots of MEM 10FCS containing the indicated concentration of drug. A separate set of cultures without drug was seeded as control growth to ensure that cells remained in exponential phase of growth. All determinations were carried out in duplicate. After three days of incubation at 37° C., 10% CO_2 in a 98% humid atmosphere, the wells were stained with 0.1% Crystal Violet. An approximately IC_{50} was determined by comparing the growth in wells with drug to the growth in wells control.

1. Raymond J. Bergeron, Paul F. Cavanaugh, Jr., Steven J. Kline. Robert G. Hughes, Jr., Gary T. Elliot and Carl W. Porter. Antineoplastic and antiherpetic activity of spermidine catecholamide iron chelators. *Biochem. Bioph. Res. Comm.* 1984, 121(3), 848-854.

2. Alan C. Schroeder, Robert G. Hughes, Jr. and Alexander Bloch. Effects of Acyclic Pyrimidine Nucleoside Analoges. *J. Med. Chem*. 1981, 24 1078-1083.

Cytotoxic Activity

Compound	IC_{50} (μM)					
	P-388	A-549	HT-29	MEL-28	CV-1	DU-145
 2	0.009	0.018	0.018	0.018	0.023	
 14	0.15	>0.15	0.15	>0.15		
 15	1.44	1.44	1.44	1.44		

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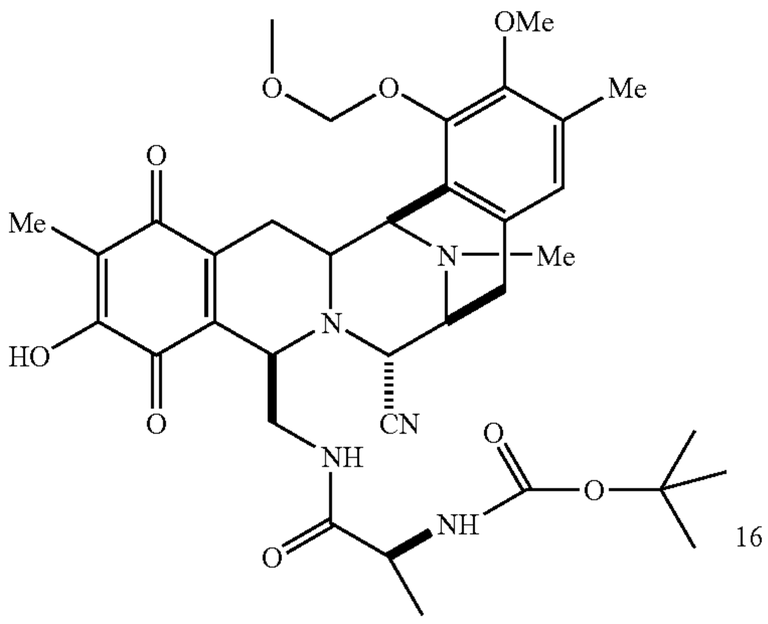
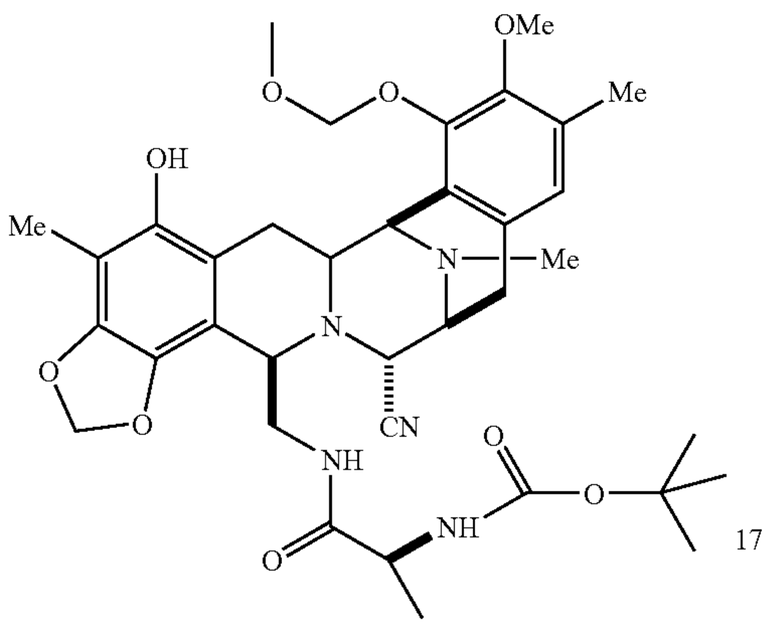
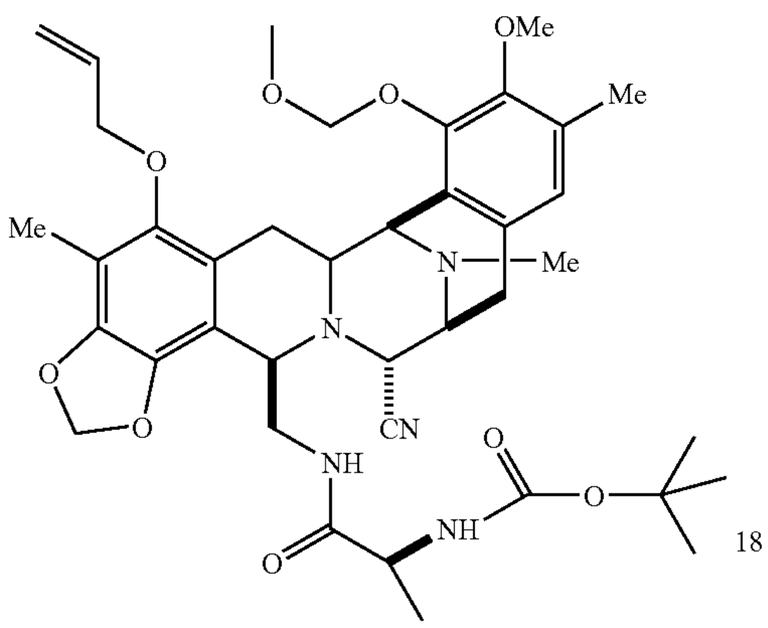
Compound	IC$_{50}$ (μM)					
	P-388	A-549	HT-29	MEL-28	CV-1	DU-145
 16	>1.5	>1.5	>1.5	>1.5		
 17	1.4	1.4	1.4	1.4		
 18	0.01	0.01	0.01	0.01		

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Compound	IC$_{50}$ (μM)					
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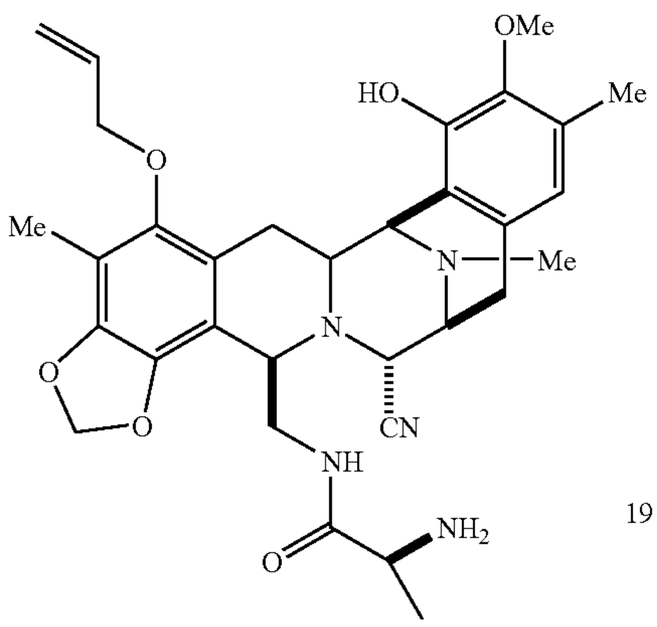
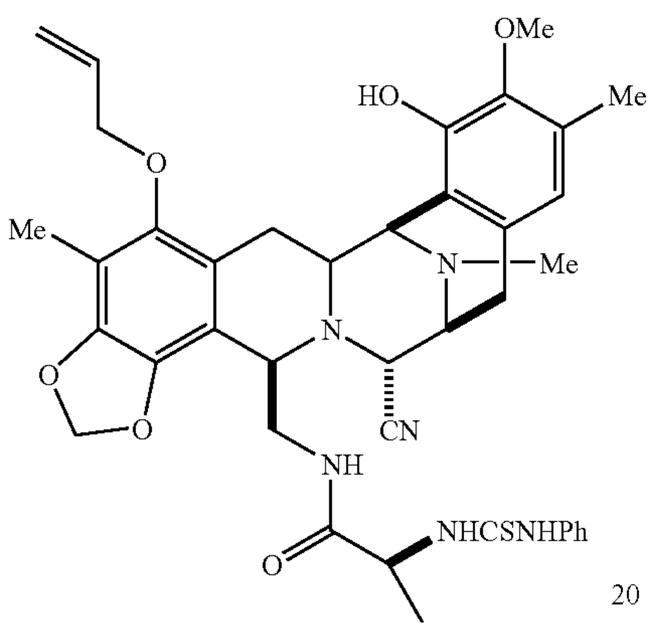
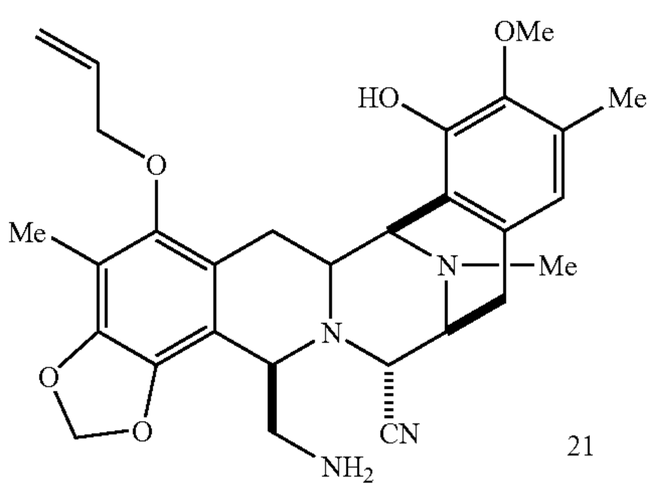
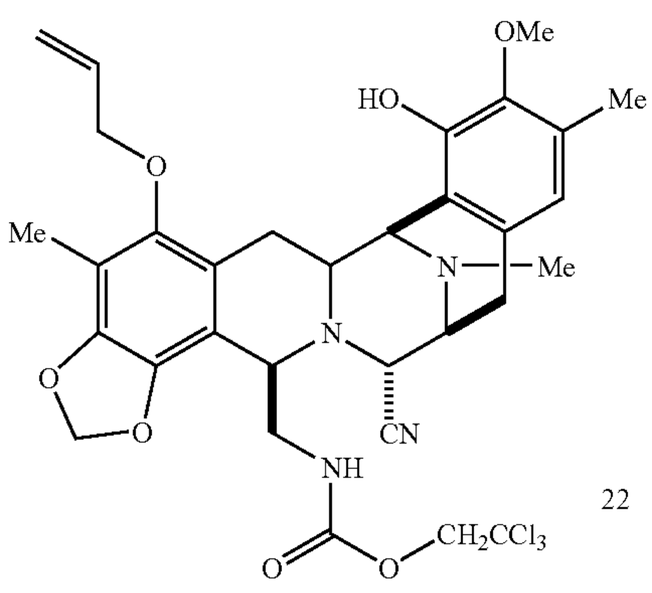
 19	0.08	0.16	0.01	0.16		
 20	0.01	0.01	0.01	0.01		
 21	0.019	0.019	0.019	0.019		
 22	0.014	0.014	0.014	0.014	0.014	0.014

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Compound	IC$_{50}$ (μM)					
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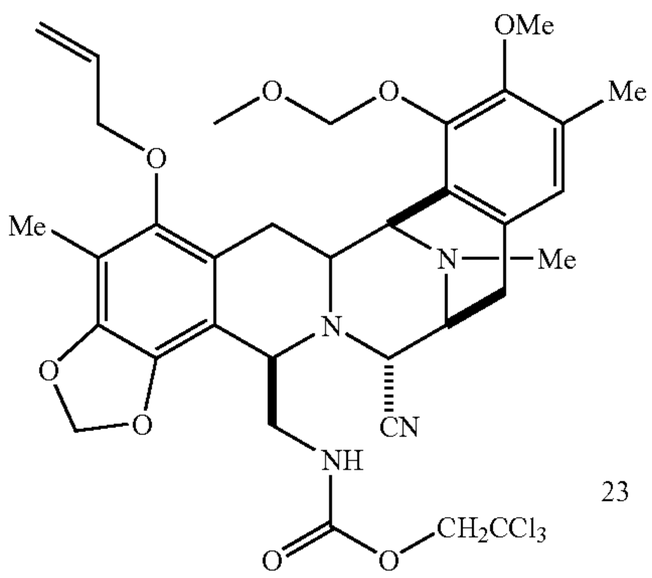
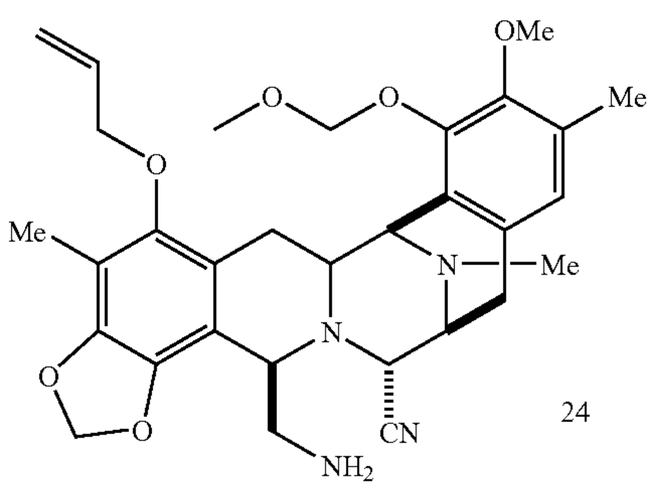
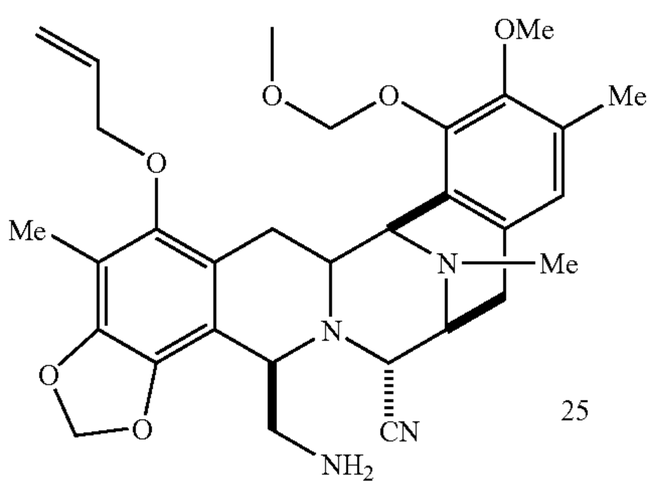
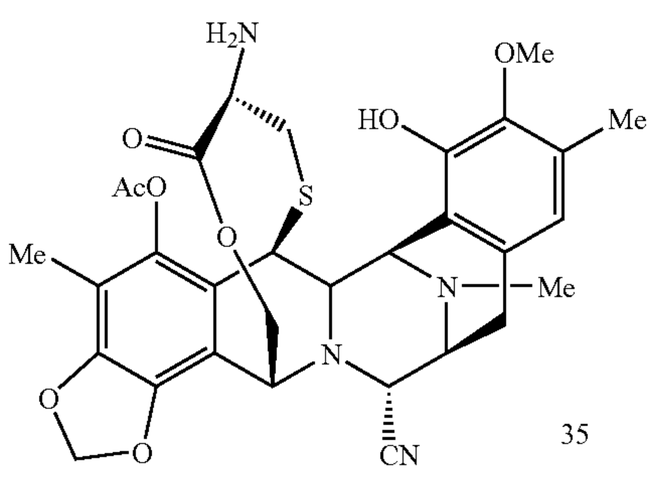
 23	0.13	0.13	0.13	0.13	0.13	0.13
 24	0.18	1.8	1.8	1.8	1.8	1.8
 25	0.2	0.2	0.2	0.2		0.2
 35	0.008	0.008	0.008	0.008		

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Compound	IC$_{50}$ (μM)					
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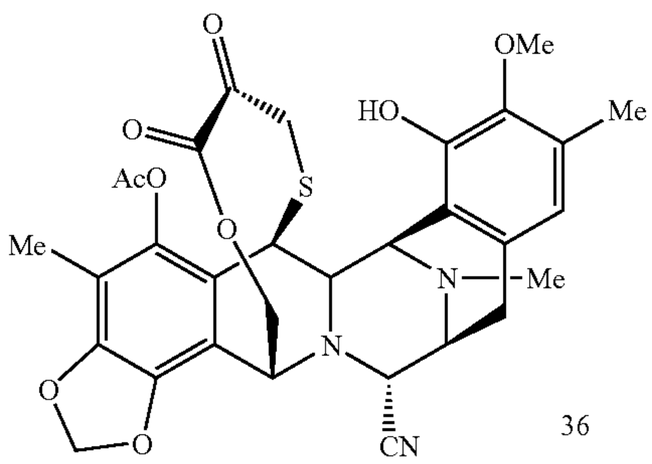
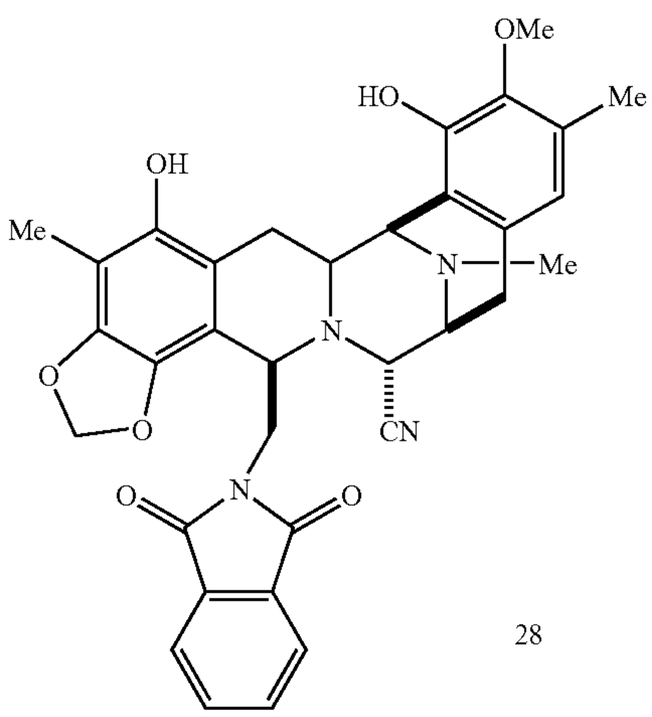
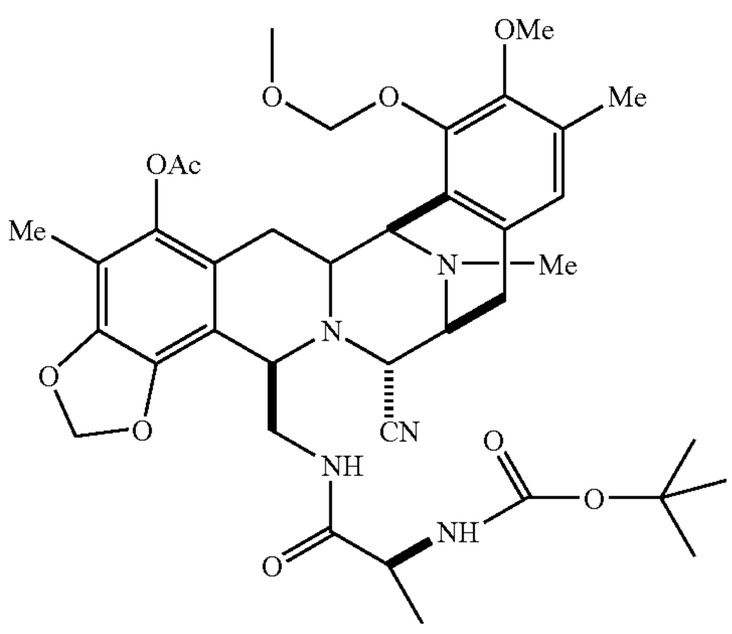
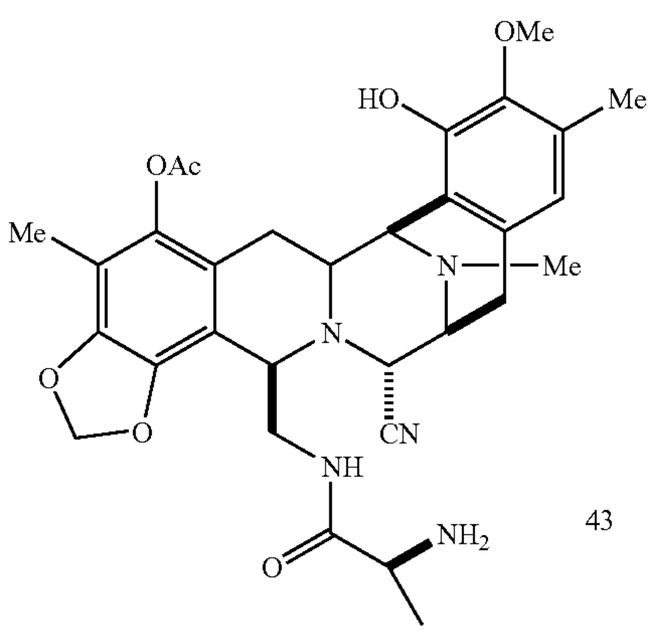
 36	0.01	0.01	0.01	0.01		
 28	0.001	0.001	0.001	0.001	0.001	0.001
 42	0.13	0.13	0.13	0.13		0.13
 43	0.008	0.016	0.008	0.008		0.016

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Compound	IC$_{50}$ (μM)					
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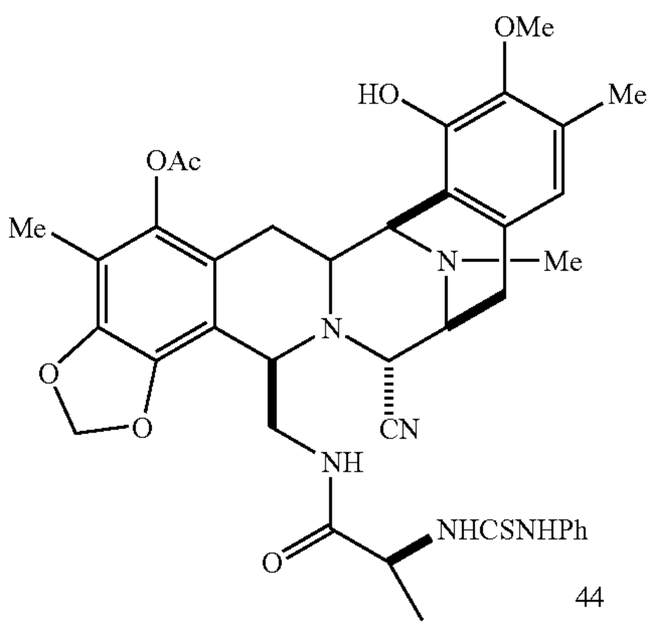
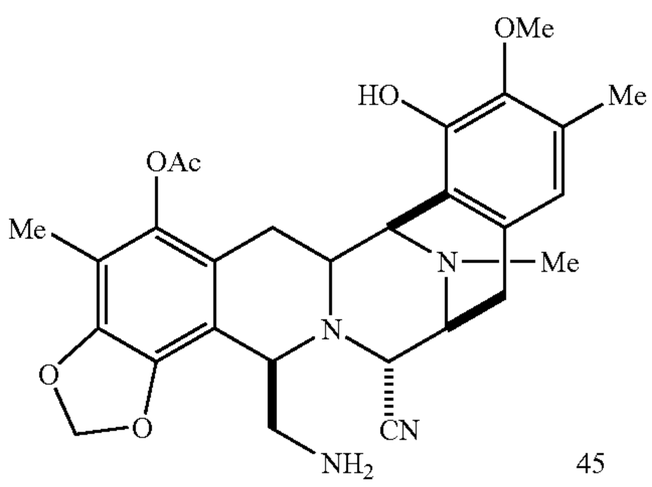
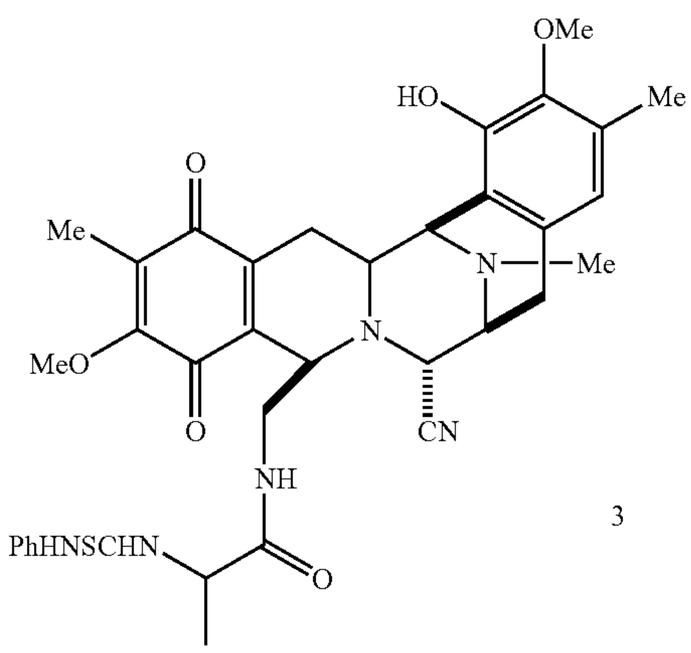
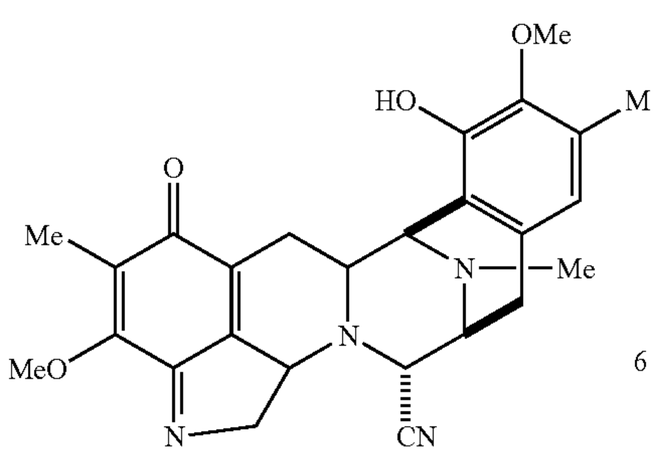
 44	0.001	0.001	0.001	0.001		0.001
 45	0.01	0.01	0.01	0.01		0.01
 3	0.015	0.015	0.015	0.015	0.018	
 6	2.171	2.171	2.171	2.171	2.171	

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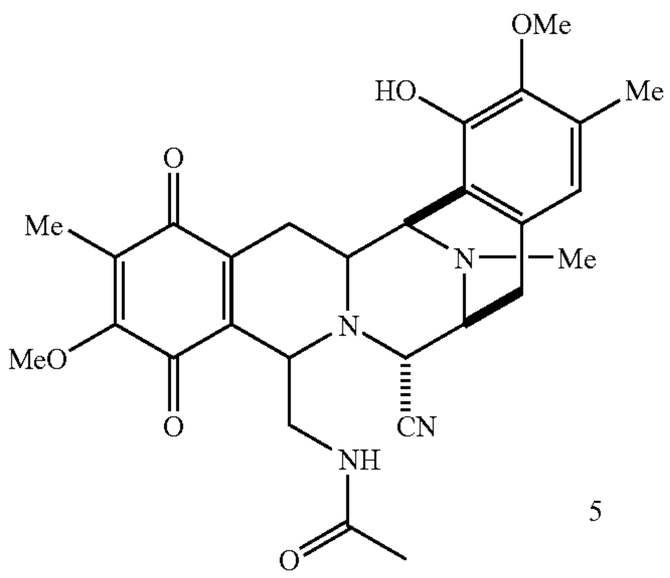
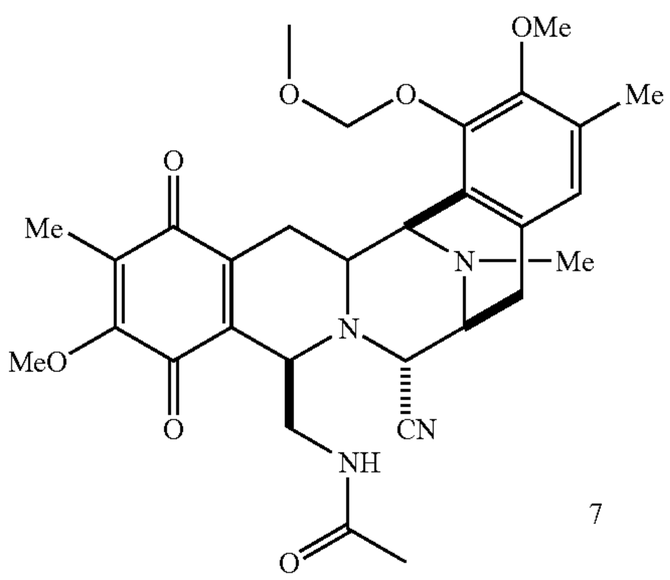
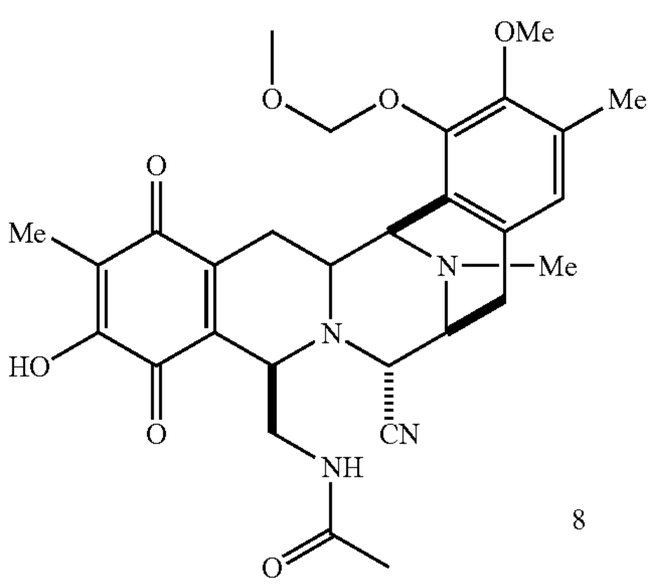
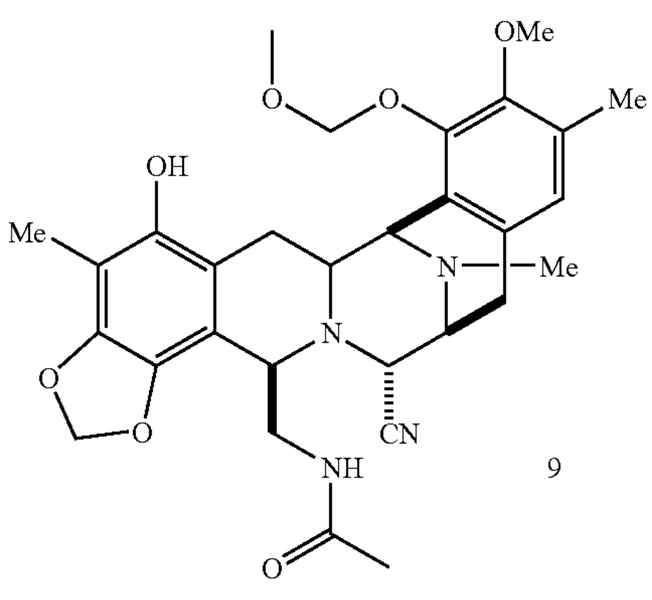
Compound	IC$_{50}$ (μM)					
	P-388	A-549	HT-29	MEL-28	CV-1	DU-145
 5	0.005	0.005	0.005	0.005		
 7	0.22	0.22	0.22	0.22	0.22	
 8	>9	>18.1	>18.1	>18.1	>18.1	
 9	>1.77	>1.77	>1.77	>1.77		>1.77

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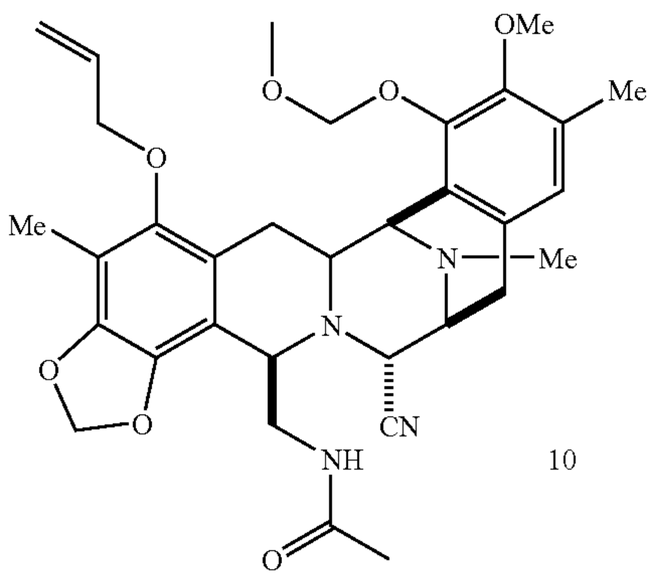
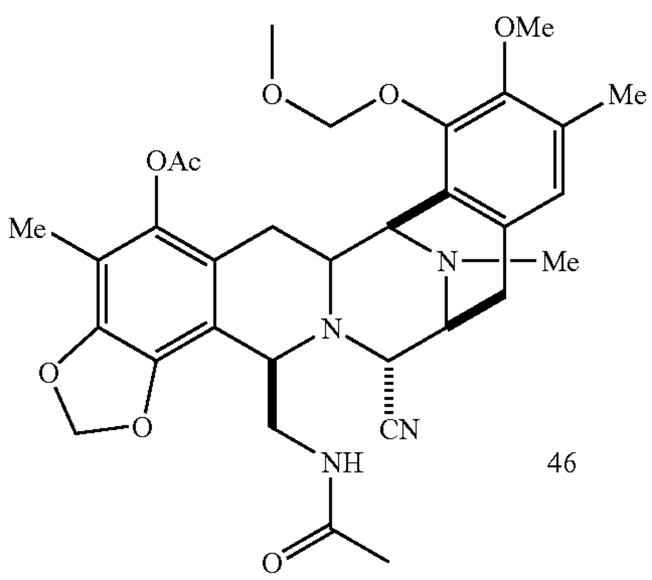
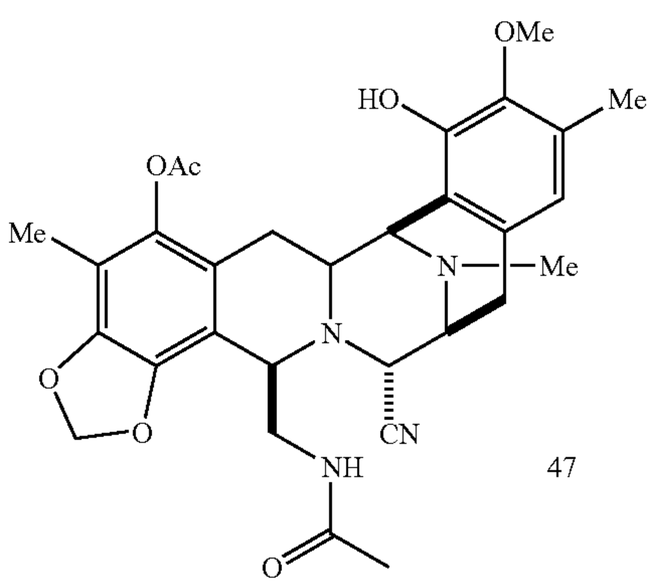
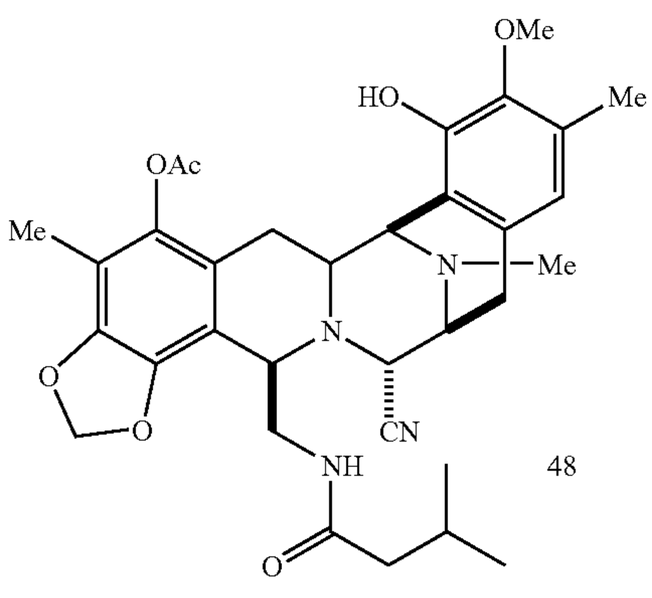
Compound	IC_{50} (μM)					
	P-388	A-549	HT-29	MEL-28	CV-1	DU-145
 10	>1.65	>1.65	>1.65	>1.65		>1.65
 46	0.016	0.016	0.016	0.016		0.016
 47	0.001	0.001	0.001	0.001		0.001
 48	0.0008	0.001	0.0008	0.0008		0.001

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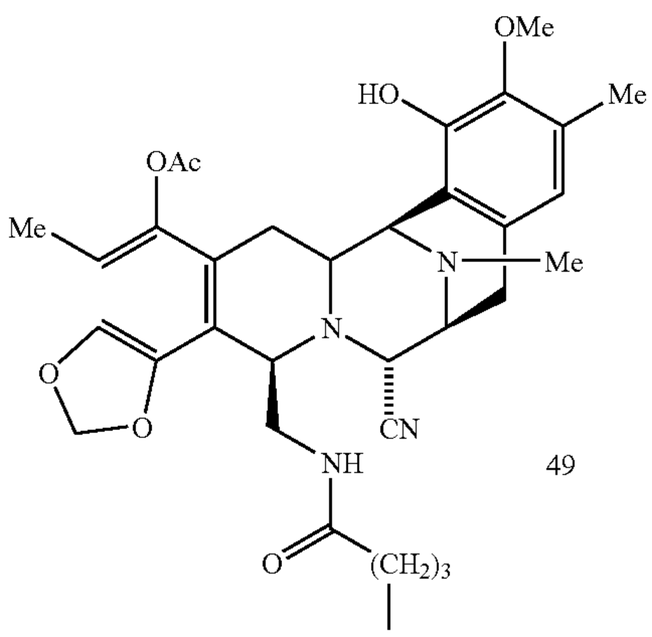
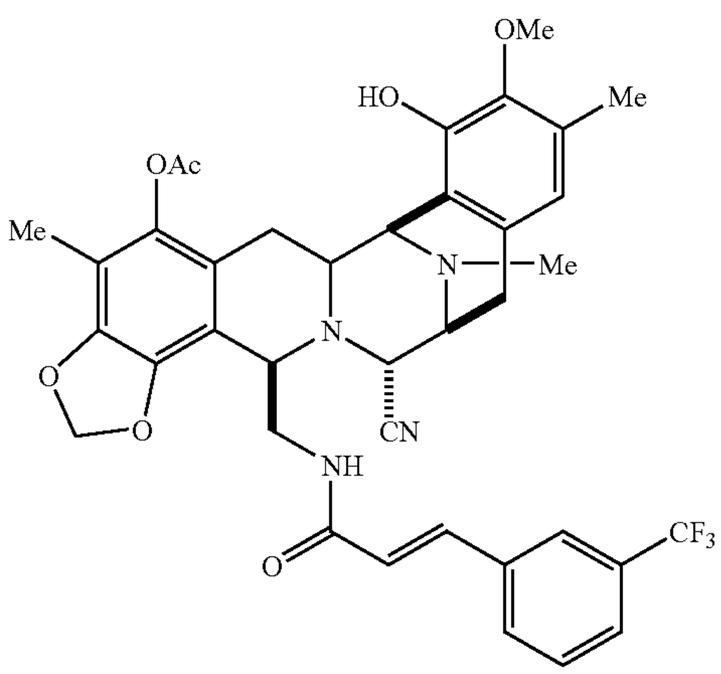
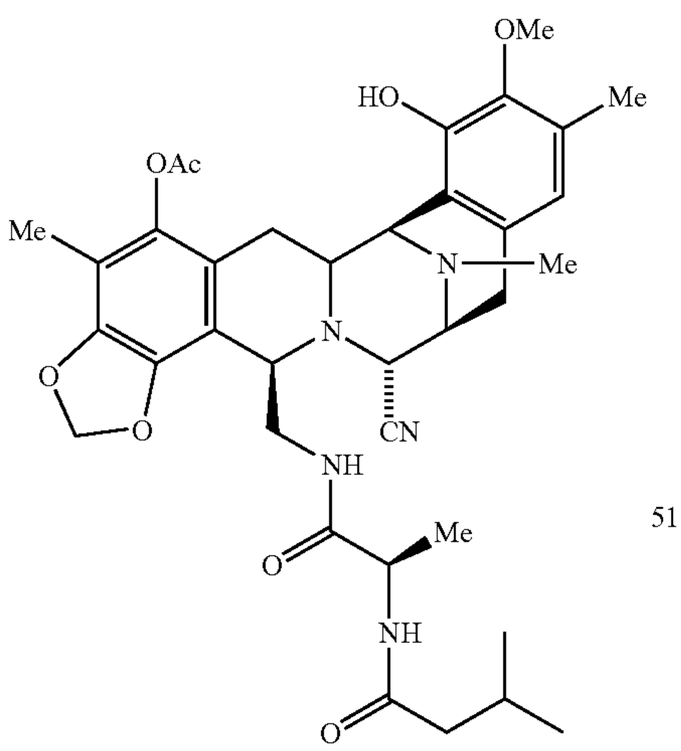
Compound	IC$_{50}$ (μM)					
	P-388	A-549	HT-29	MEL-28	CV-1	DU-145
 49	0.007	0.007	0.007	0.007		0.007
 50	0.0001	0.0001	0.0001	0.0001		0.0001
 51	0.0001	0.0001	0.0001	0.0001		0.0001

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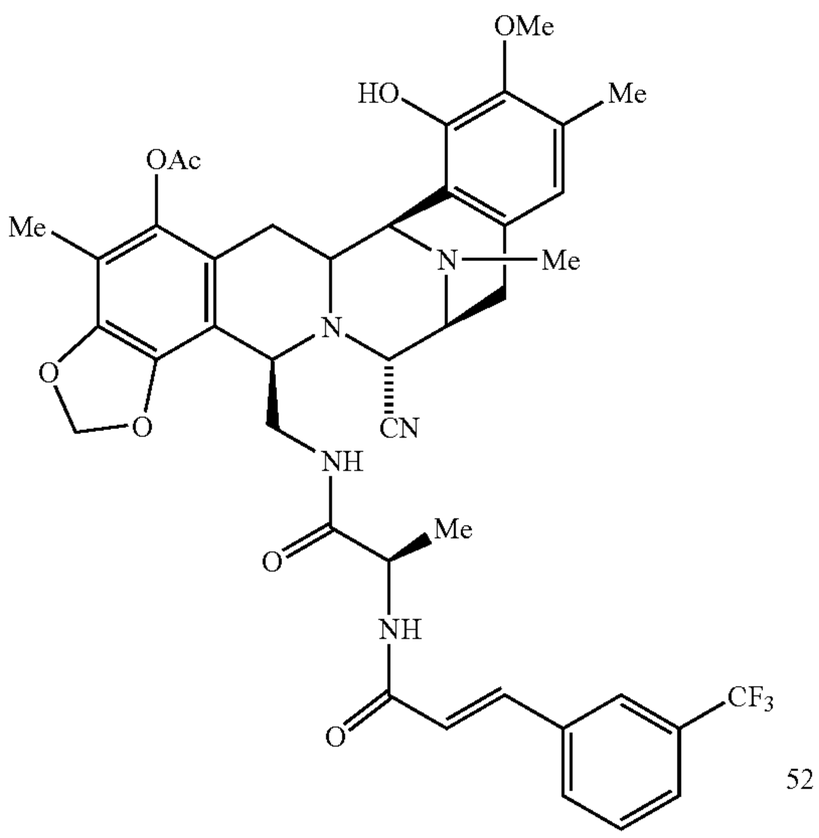
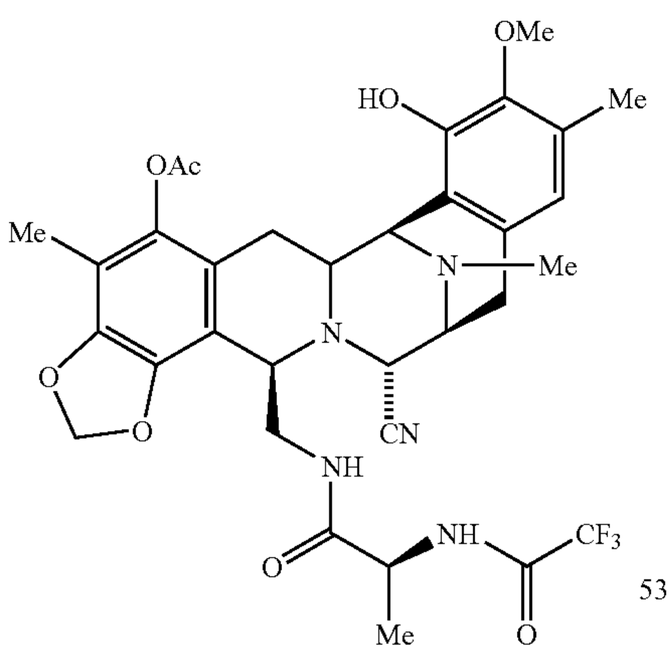
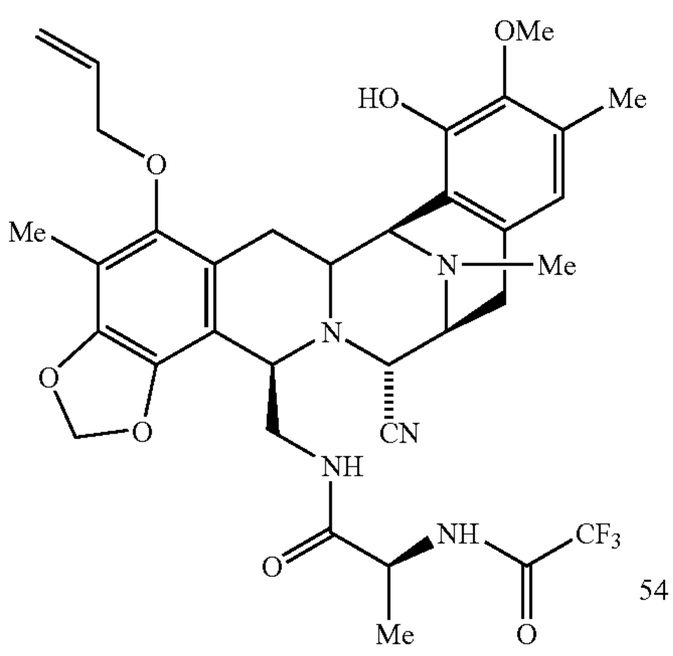
Compound	IC$_{50}$ (μM)					
	P-388	A-549	HT-29	MEL-28	CV-1	DU-145
 52	0.001	0.001	0.001	0.001		0.001
 53	0.0001	0.0001	0.0001	0.0001		0.0001
 54	0.001	0.001	0.001	0.001		0.001

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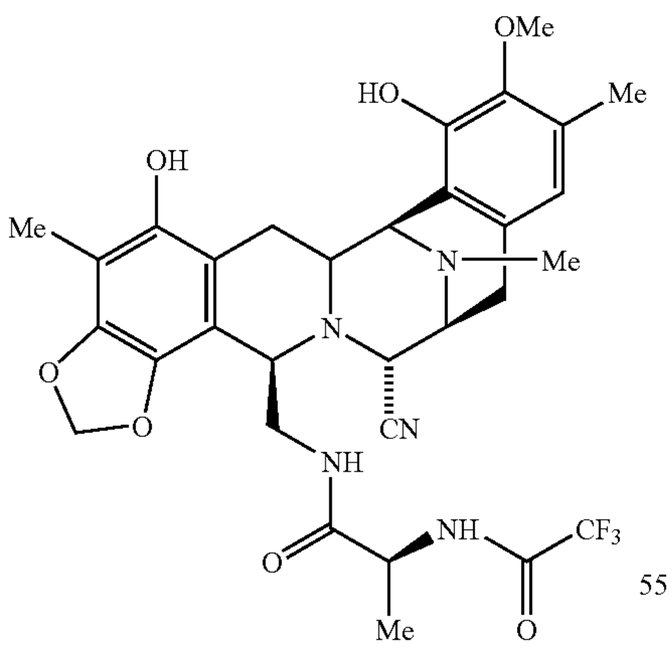
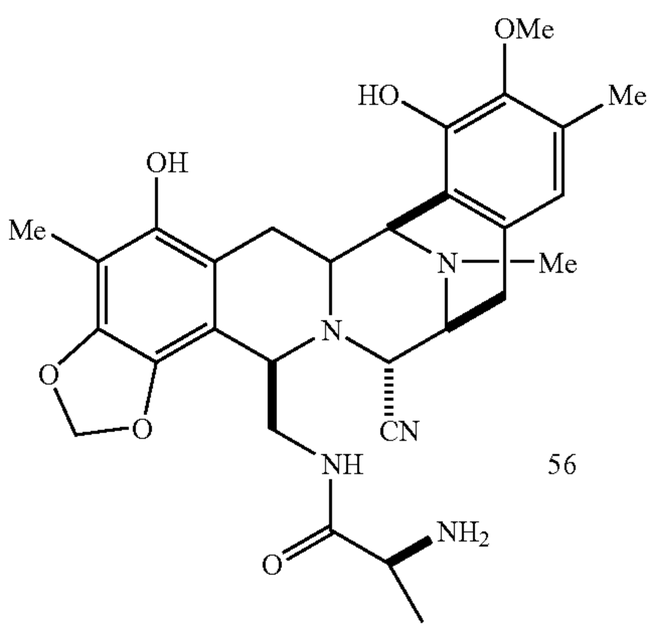
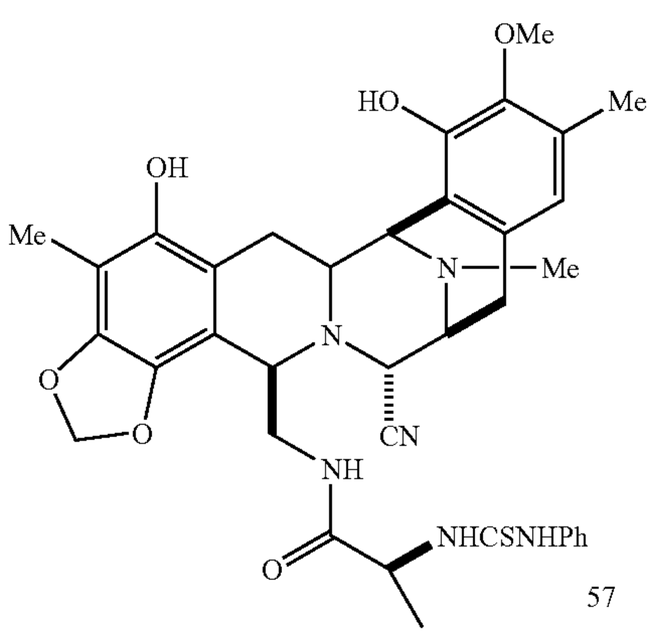
Compound	IC$_{50}$ (μM)					
	P-388	A-549	HT-29	MEL-28	CV-1	DU-145
 55	0.01	0.01	0.01	0.01		0.01
 56	0.18	0.9	0.18	0.8		0.9
 57	0.14	0.14	0.14	0.14		0.14

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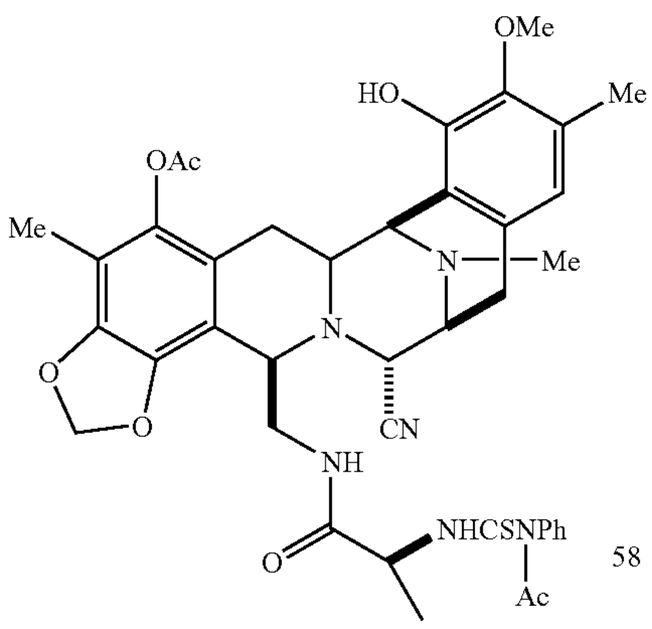
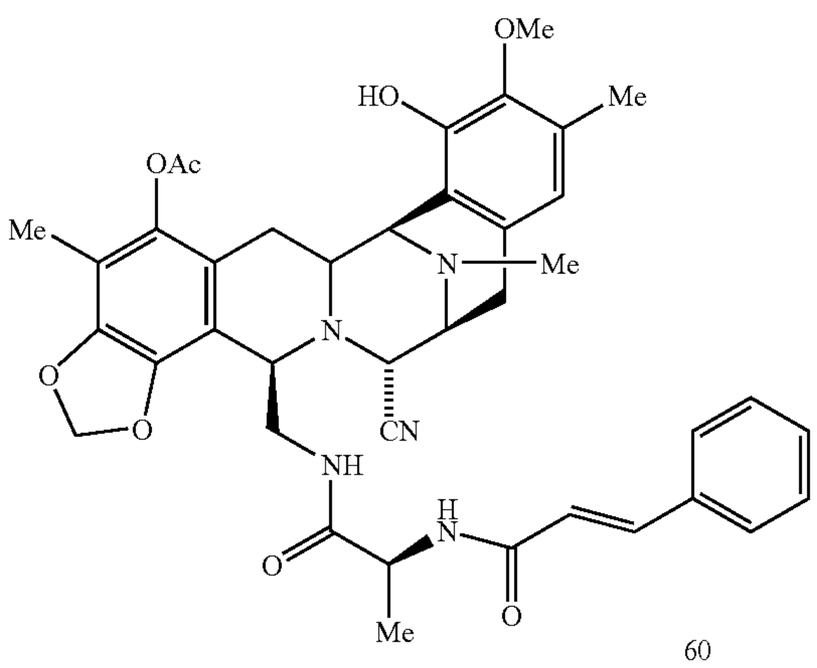
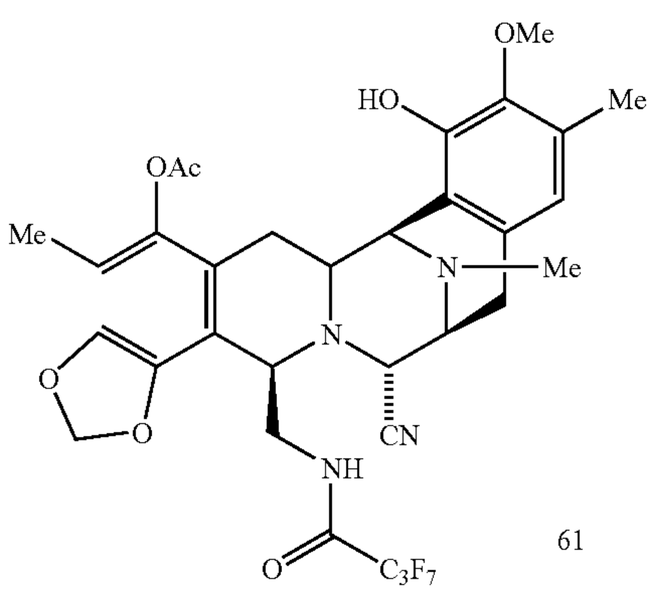
Compound	IC$_{50}$ (μM)					
	P-388	A-549	HT-29	MEL-28	CV-1	DU-145
 58	0.001	0.001	0.001	0.001		0.001
 60	0.001	0.001	0.0005	0.001		0.0005
 61	0.001	0.001	0.001	0.001		0.001

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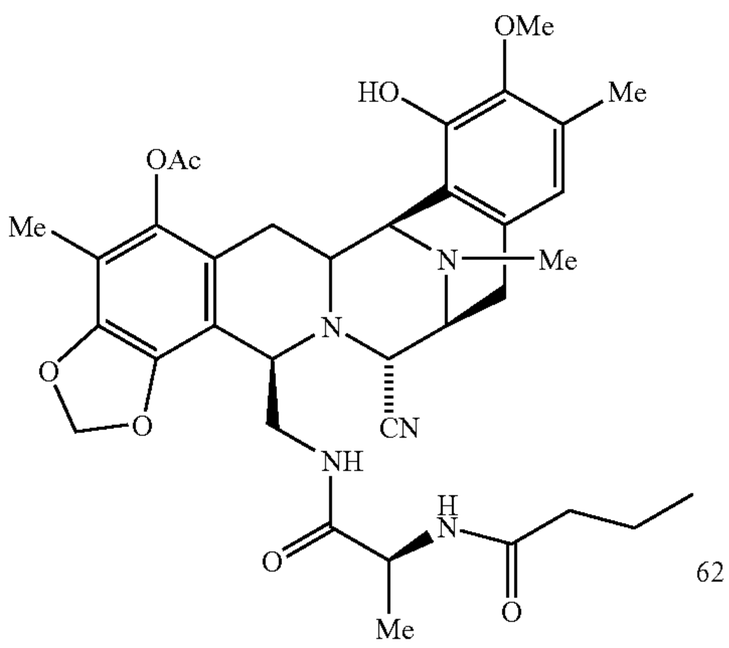
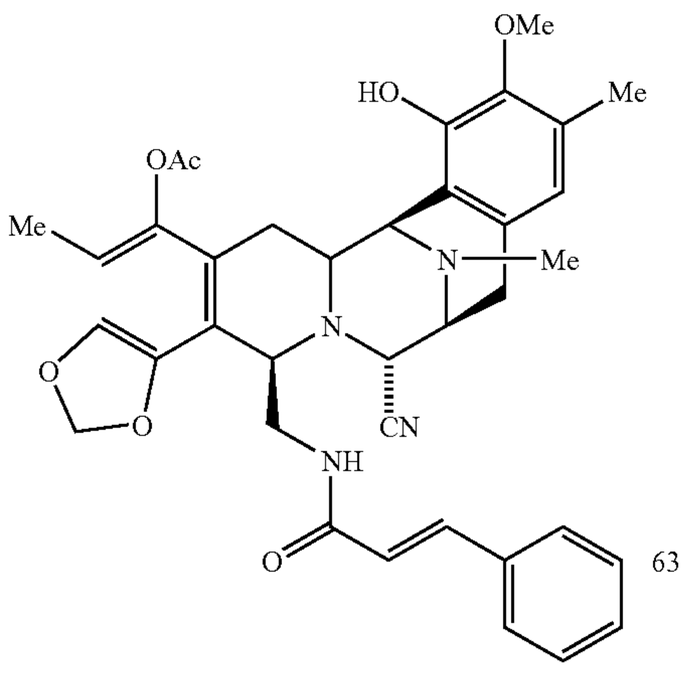
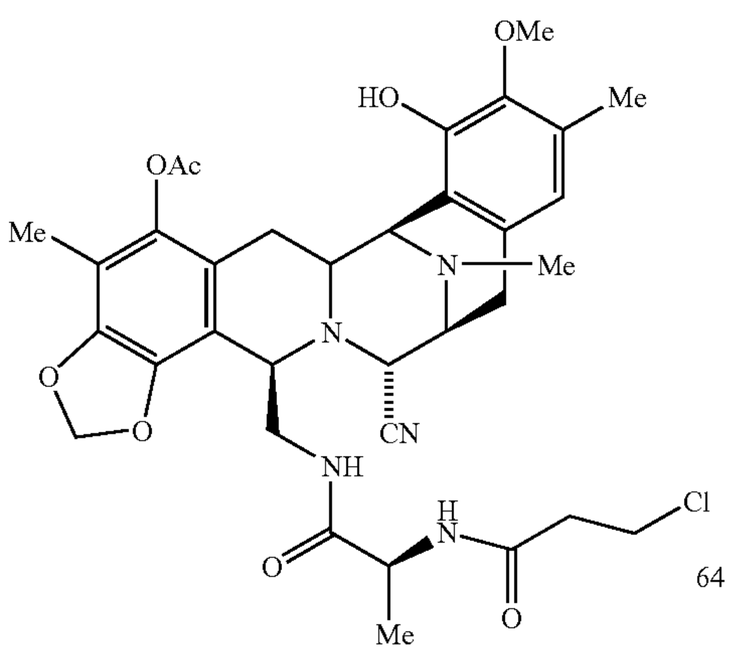
Compound	IC$_{50}$ (μM)					
	P-388	A-549	HT-29	MEL-28	CV-1	DU-145
 62	0.001	0.001	0.0005	0.0005		0.001
 63	0.0001	0.0001	0.0001	0.0001		0.0001
 64	0.001	0.001	0.001	0.001		0.001

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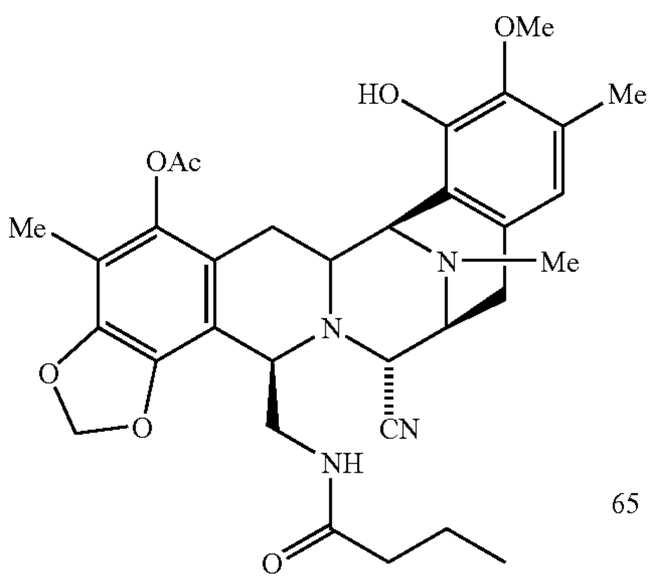
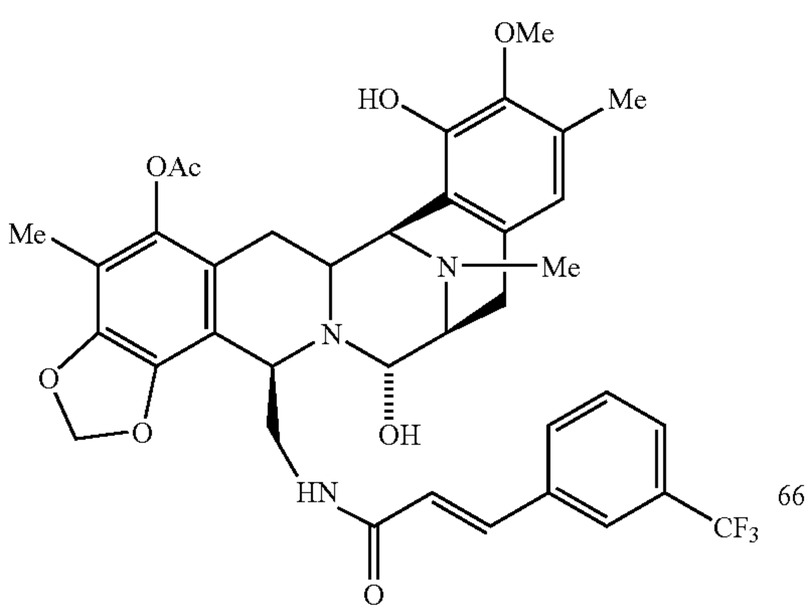
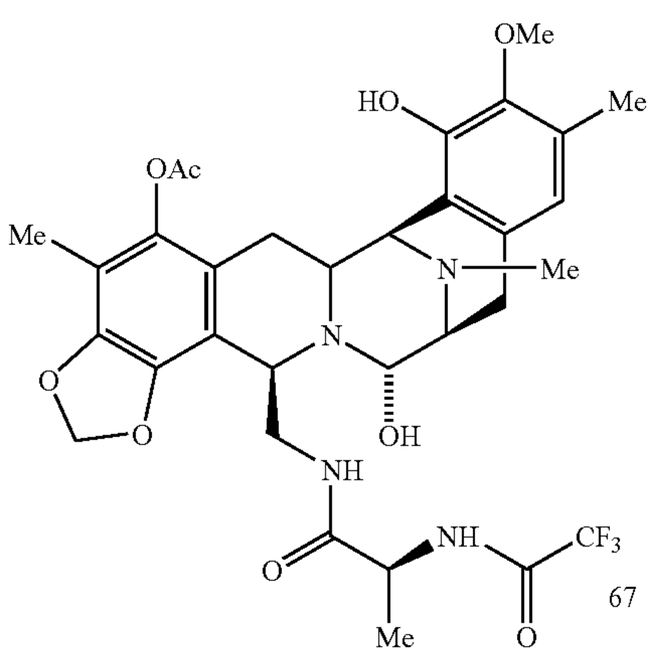
Compound	IC$_{50}$ (μM)					
	P-388	A-549	HT-29	MEL-28	CV-1	DU-145
 65	0.0001	0.0005	0.0001	0.0001		0.0005
 66	0.0001	0.0001	0.0001	0.0001		0.0001
 67	0.0001	0.0001	0.0001	0.0001		0.0001

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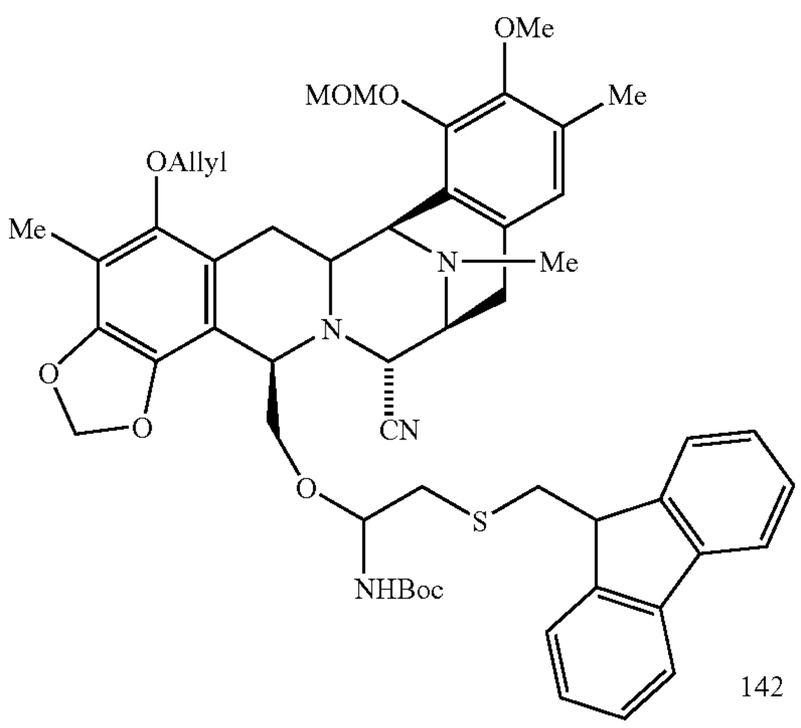
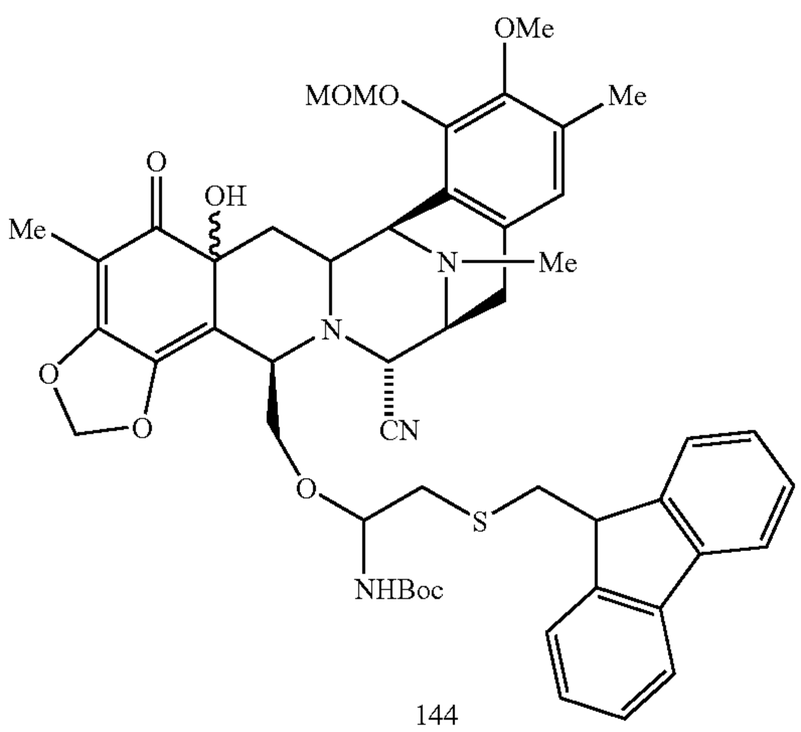
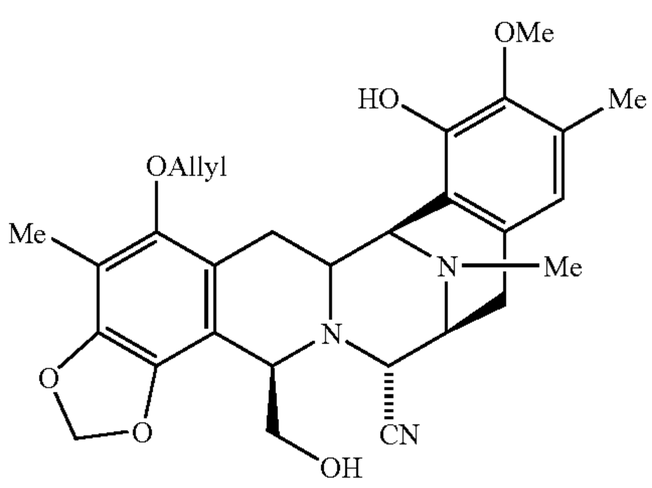
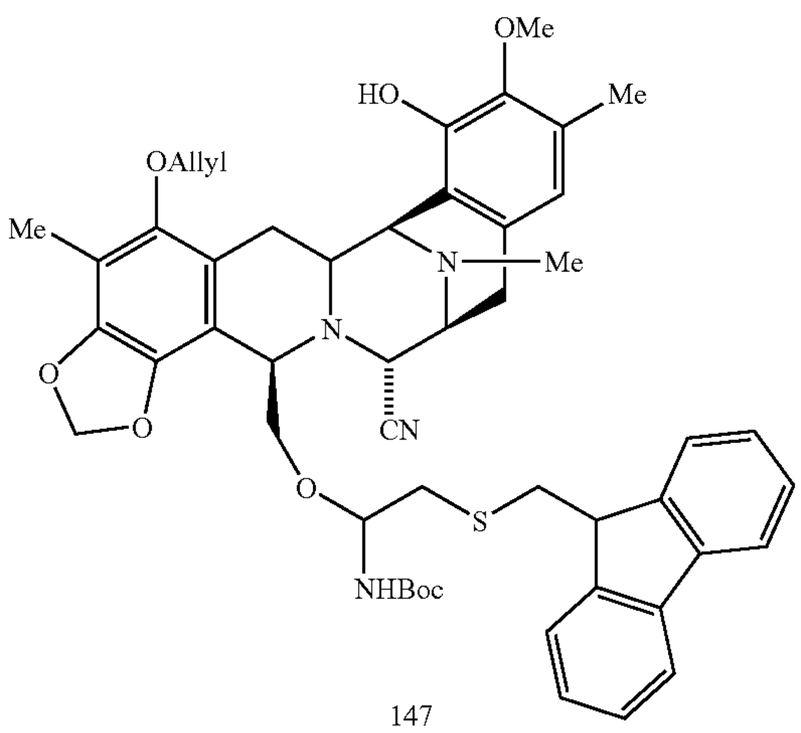
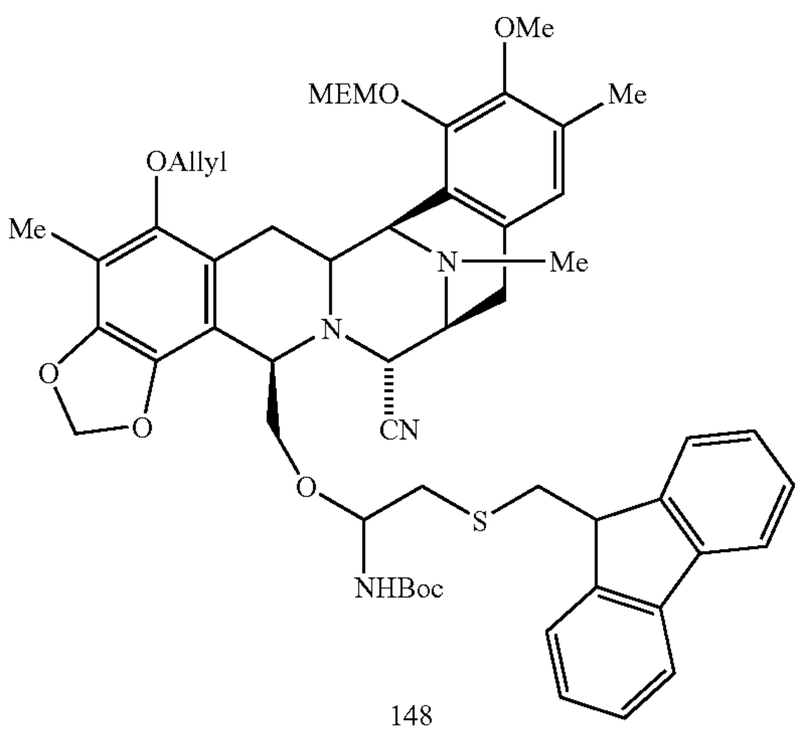
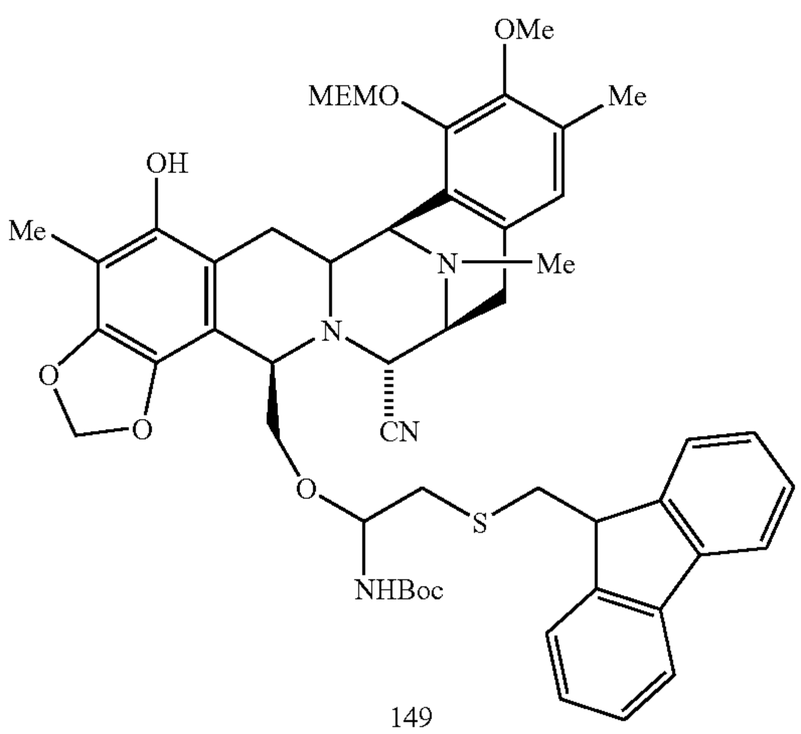
Compound	IC$_{50}$ (μM)					
	P-388	A-549	HT-29	MEL-28	CV-1	DU-145
 142		>1	>1			
 144		>1	>1			
 146	0.19	0.19	0.19	0.19		

-continued

Compound	IC$_{50}$ (μM)					
	P-388	A-549	HT-29	MEL-28	CV-1	DU-145
 147		0.0055	0.0055			
 148		>1	>1			
 149		0.01	0.01			

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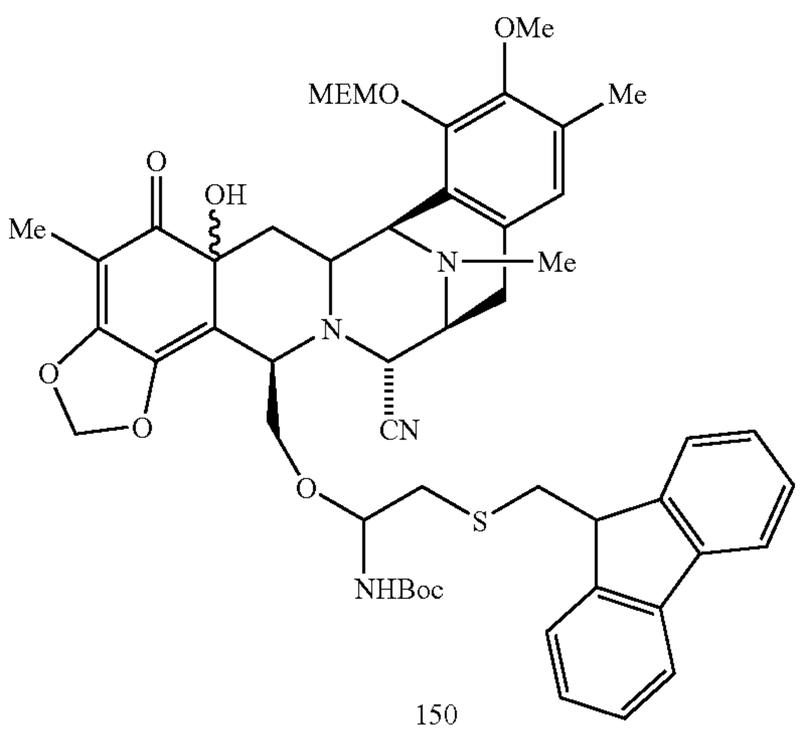
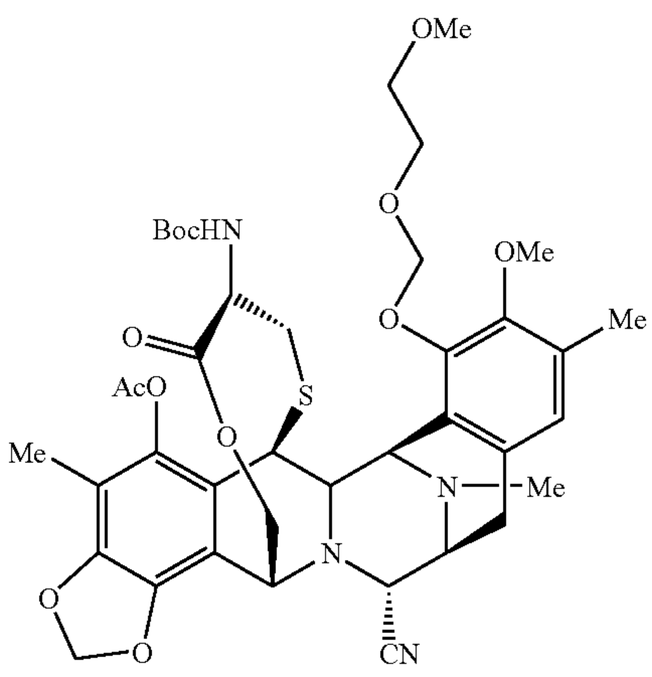
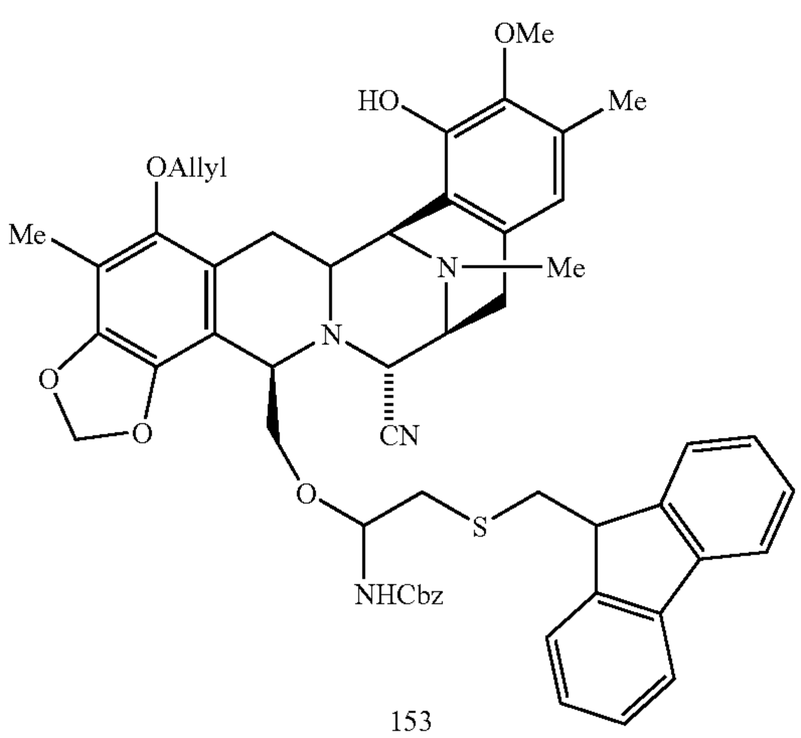
Compound	IC$_{50}$ (μM)					
	P-388	A-549	HT-29	MEL-28	CV-1	DU-145
 150		0.051	0.051			
 151		0.012	0.012			
 153		0.11	0.11			

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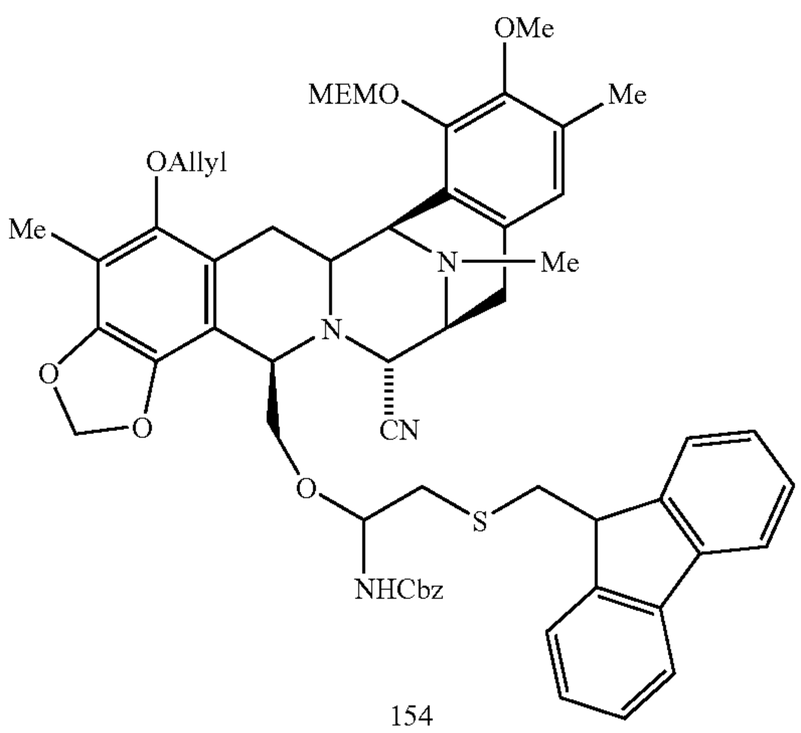
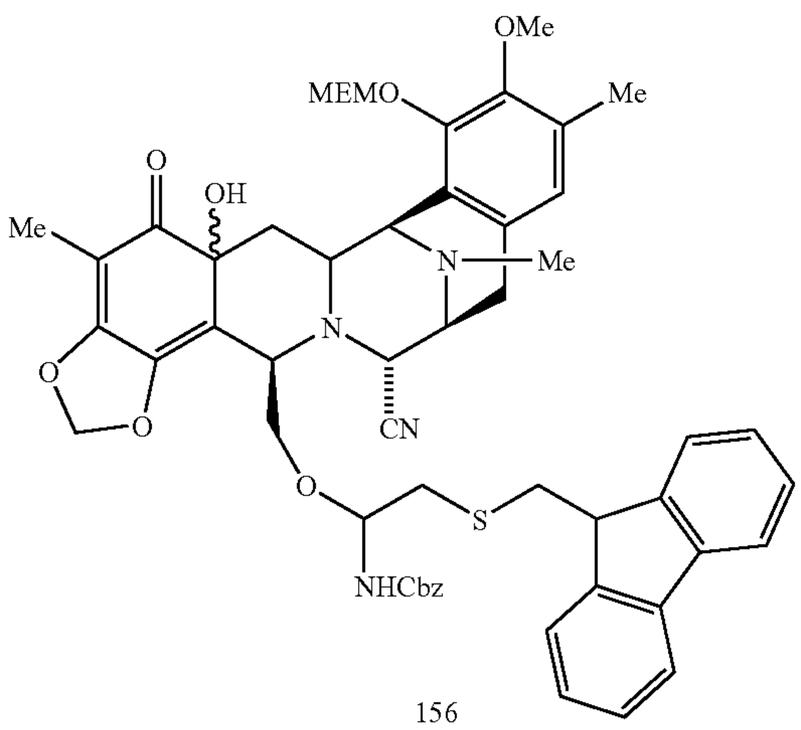
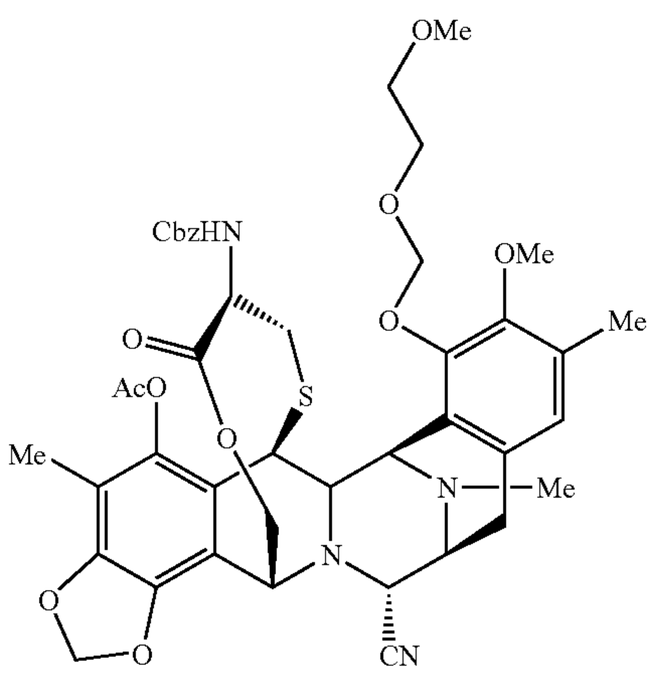
Compound	IC$_{50}$ (μM)					
	P-388	A-549	HT-29	MEL-28	CV-1	DU-145
 154		>1	>1			
 156		>1	>1			
 157		0.59	0.59			

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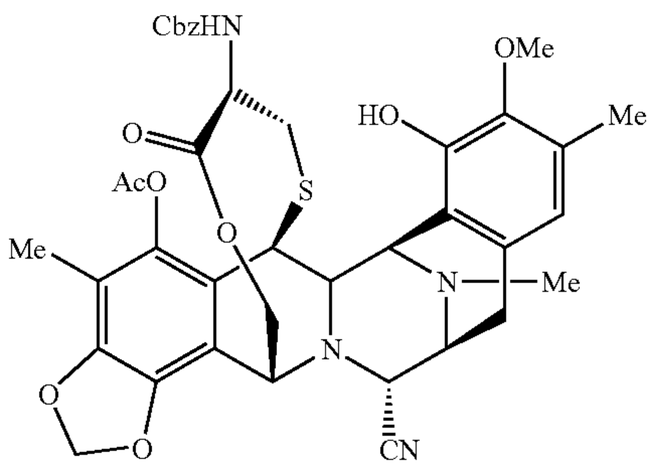
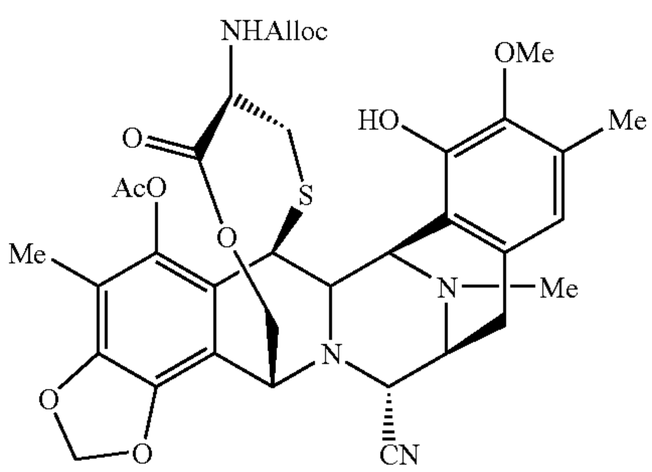
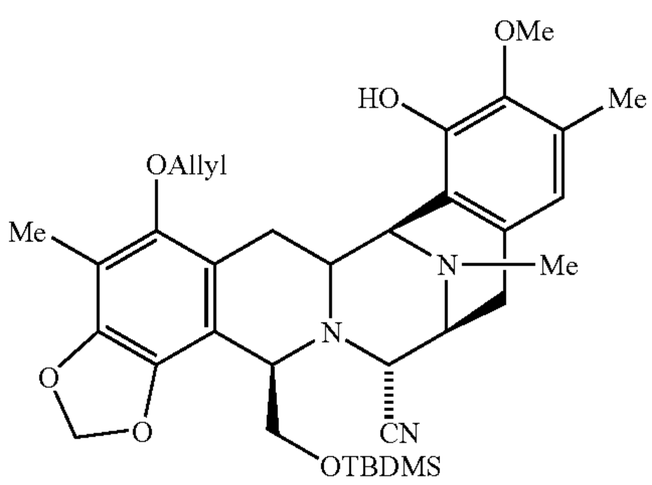
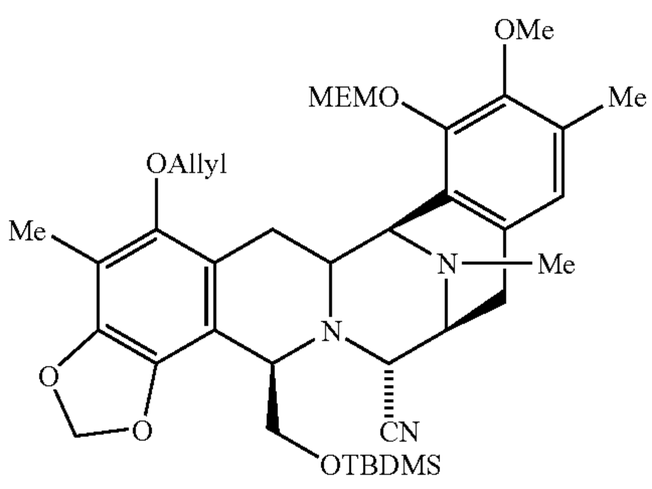
Compound	IC$_{50}$ (μM)					
	P-388	A-549	HT-29	MEL-28	CV-1	DU-145
 158		0.0013	0.0013			
 164		0.00015	0.00015			
 165		>1	>1			
 166		>1	>1			

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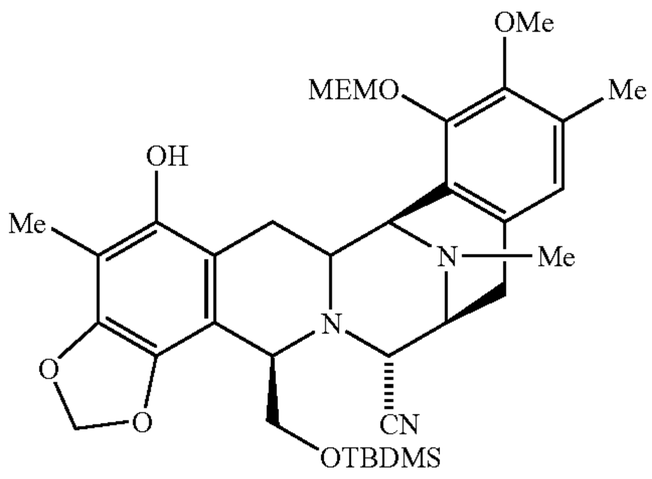
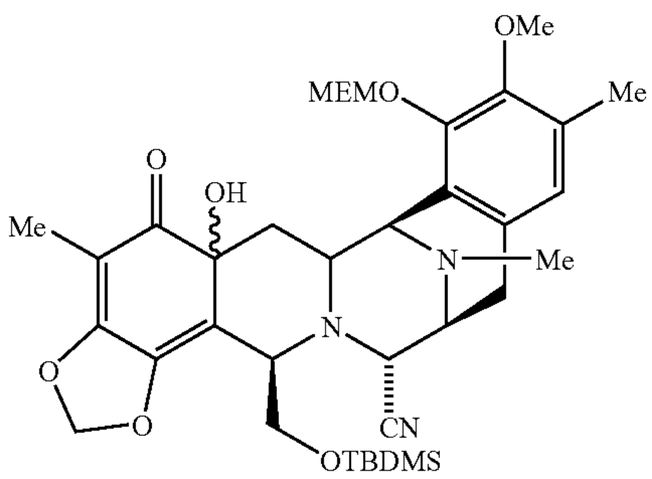
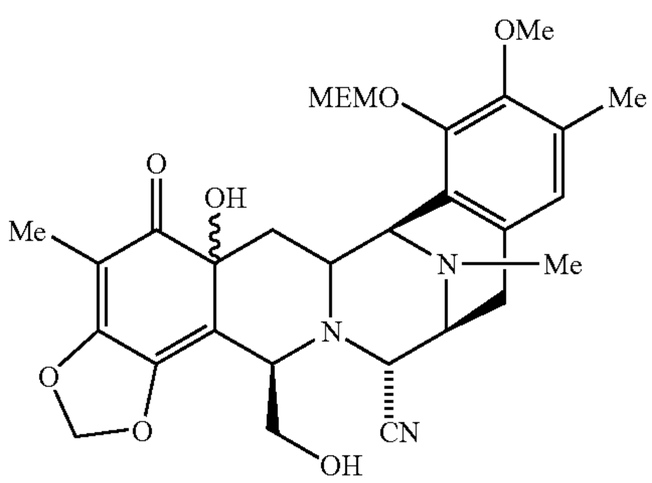
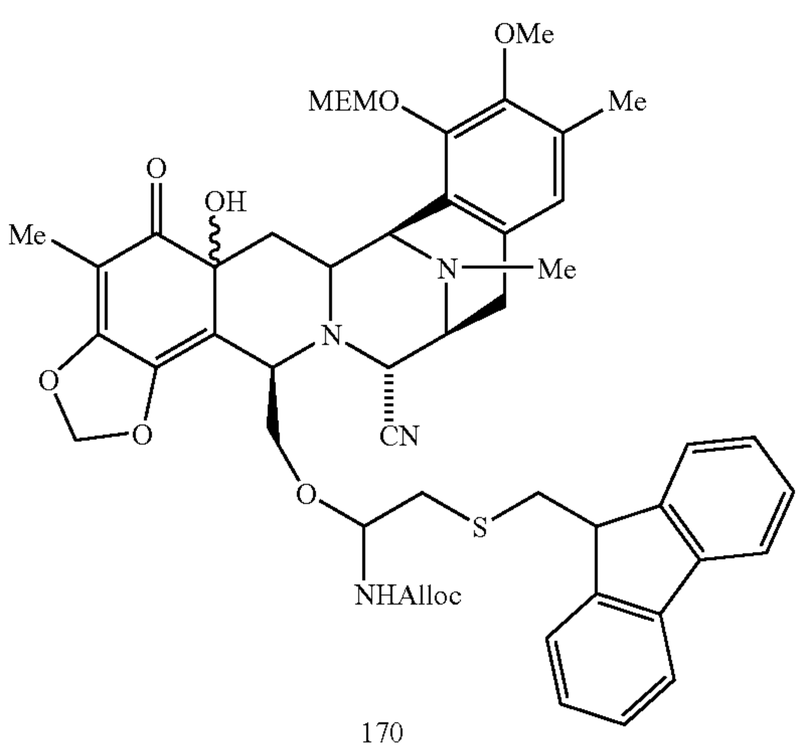
Compound	IC$_{50}$ (μM)					
	P-388	A-549	HT-29	MEL-28	CV-1	DU-145
 167		>1	>1			
 168		>1	>1			
 169		>1	>1			
 170		>1	>1			

-continued

Compound	IC$_{50}$ (μM)					
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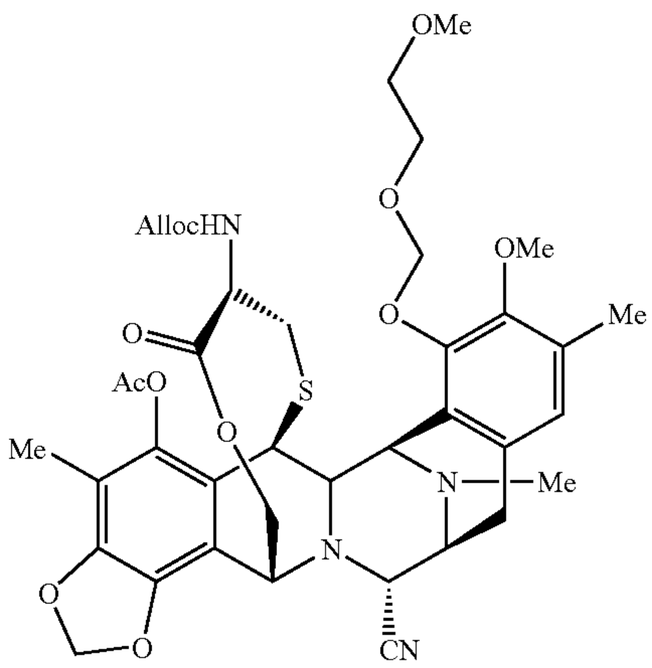
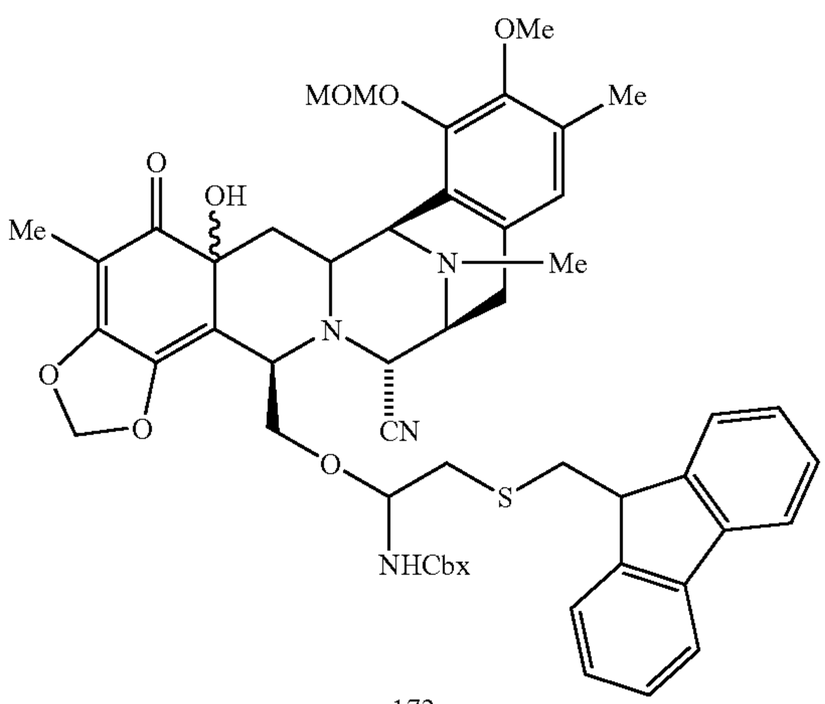
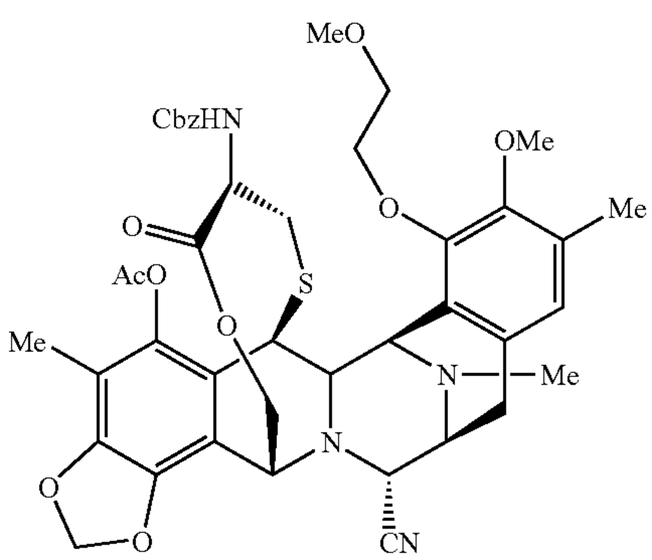
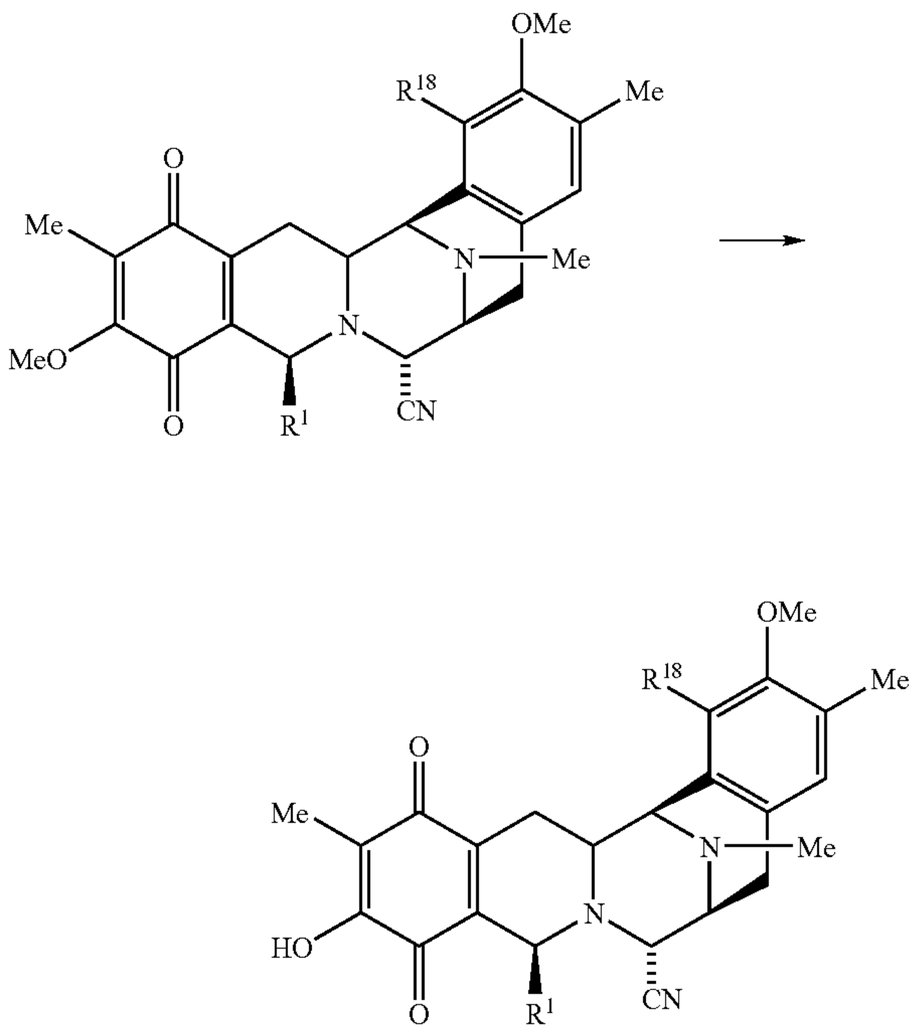
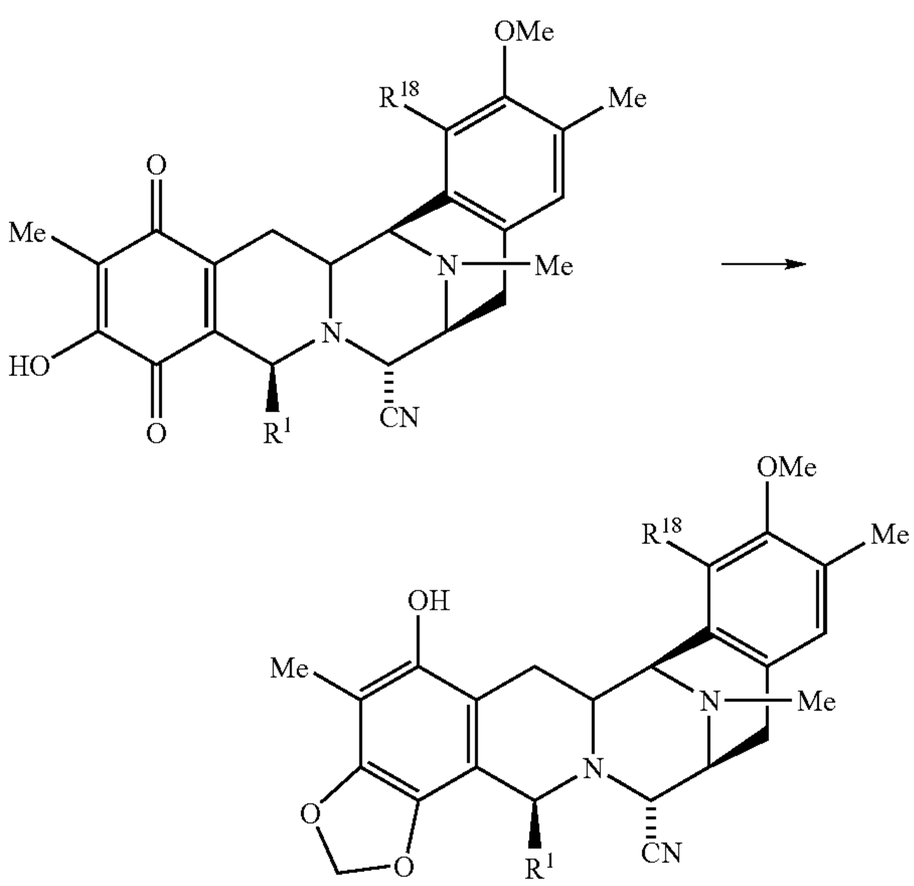
 171		0.012	0.012			
 172		>1	>1			
 173		0.062	0.062			

The active compounds of this invention thus include compounds with the 10-hydroxy group and the 1-labile group.

An important method of this invention includes the reaction:

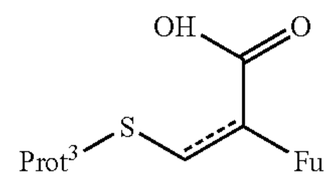


Another important method of this invention includes the reaction:



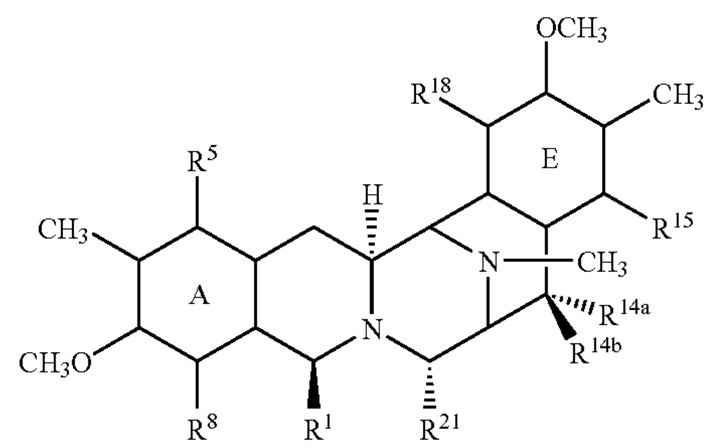
Another important method of this invention includes the reaction includes the reaction where a group R^1 is aminomethylene is converted to a hydroxymethylene group.

Another important method of this invention includes the reaction wherein a compound with a group R^1 which is hydroxymethylene is reacted with a reagent of the formula (XIX)



where Fu indicates a protected functional group, $Prot^3$ is a protecting group, and the dotted line shows an optional double bond.

Another important method of this invention includes the reaction for preparing a 21-cyano compound of formula (XVI) which comprises reacting a compound of formula (XV):



where R^1, R^5, R^8, R^{14a}, R^{14b}, R^{15} and R^{18} are as defined and R^{21} is a hydroxy group, with a source of cyanide ion, to give the desired 21-cyano compound.

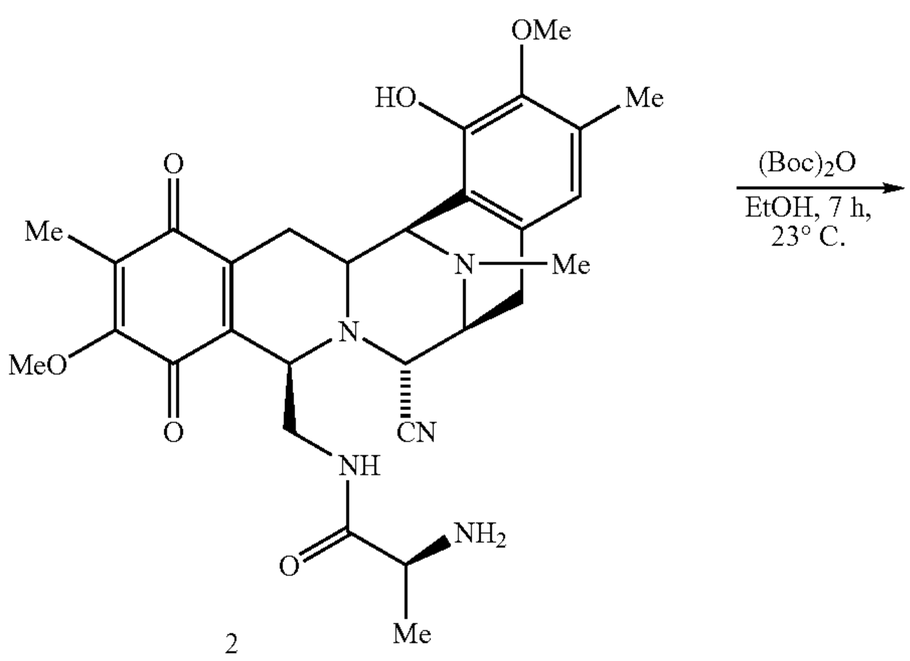
In addition, processes using other nucleophile-containing compounds, to produce similar compounds of formula (XVI) wherein the 21-position is protected by another nucleophilic group, a 21-Nuc group, are also envisaged. For example, a 21-Nuc compound of formula (XVI) with an alkylamino substituent at the 21-position can be produced by reacting the compound of formula (XV) wherein R^{21} is a hydroxy group with a suitable alkylamine. A 21-Nuc compound of formula (XVI) with an alkylthio substituent at the 21-position can also be produced by reacting the compound of formula (XV) wherein R^{21} is a hydroxy group with a suitable alkanethiol. Alternatively, a 21-Nuc compound of formula (XVI) with an □-carbonylalkyl substituent at the 21-position can be produced by reacting the compound of formula (XV) wherein R^{21} is a hydroxy group with a suitable carbonyl compound, typically in the presence of a base. Other synthetic routes are available for other 21-Nuc compounds.

Another important reaction of this invention involves treatment of a 21-cyano product of this invention to form a 21-hydroxy compound. Such compounds have interesting in vivo properties.

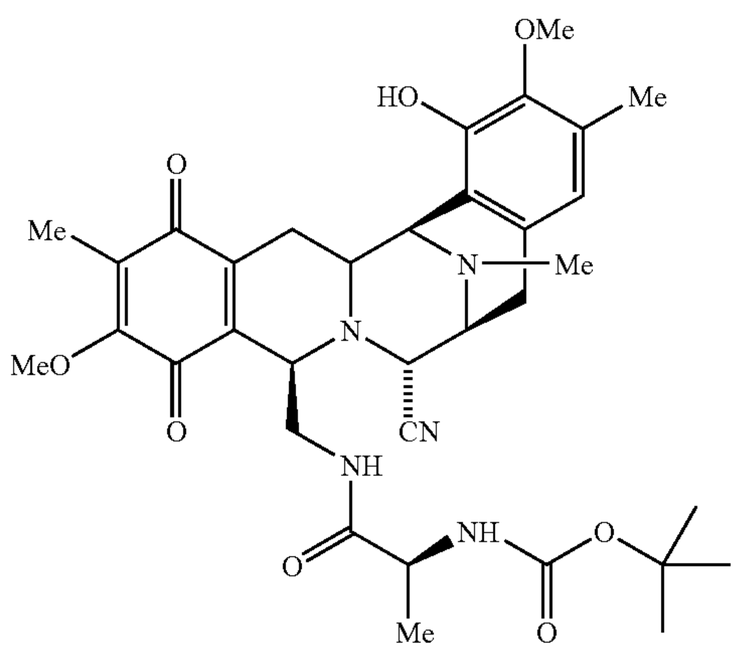
EXAMPLES

The present invention is illustrated by the following examples.

Example 1



2



14

To a solution of 2 (21.53 g, 39.17 mmol) in ethanol (200 ml), tert-butoxycarbonyl anhydride (7.7 g, 35.25 mmol) was added and the mixture was stirred for 7 h at 23° C. Then, the reaction was concentrated in vacuo and the residue was purified by flash column chromatography (SiO_2, hexane:ethyl acetate 6:4) to give 14 (20.6 g, 81%) as a yellow solid.

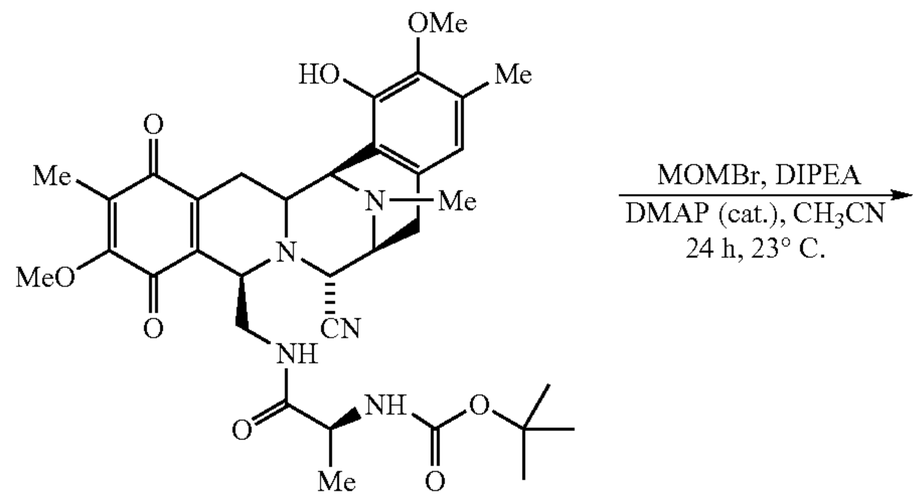
Rf: 0.52 (ethyl acetate:$CHCl_3$ 5:2).

^{1}H NMR (300 MHz, $CDCl_3$): δ 6.49 (s, 1H), 6.32 (bs, 1H), 5.26 (bs, 1H), 4.60 (bs, 1H), 4.14 (d, J=2.4 Hz, 1H), 4.05 (d, J=2.4 Hz, 1H), 3.94 (s, 3H), 3.81 (d, J=4.8 Hz, 1H), 3.7 (s, 3H), 3.34 (br d, J=7.2 Hz, 1H), 3.18-3.00 (m, 5H), 2.44 (d, J=18.3 Hz, 1H), 2.29 (s, 3H), 2.24 (s, 3H), 1.82 (s, 3H), 1.80-1.65 (m, 1H), 1.48 (s, 9H), 0.86 (d, J=5.7 Hz, 3H)

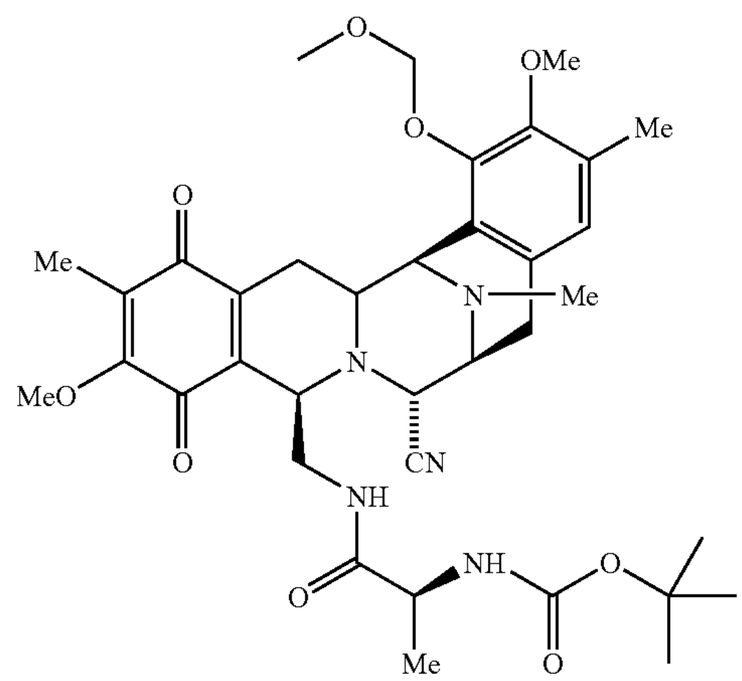
^{13}C NMR (75 MHz, $CDCl_3$): δ 185.5, 180.8, 172.7, 155.9, 154.5, 147.3, 143.3, 141.5, 135.3, 130.4, 129.2, 127.5, 120.2, 117.4, 116.9, 80.2, 60.7, 60.3, 58.5, 55.9, 55.8, 54.9, 54.4, 50.0, 41.6, 40.3, 28.0, 25.3, 24.0, 18.1, 15.6, 8.5.

ESI-MS m/z: Calcd. for $C_{34}H_{43}N_5O_8$: 649.7. Found $(M+H)^+$: 650.3.

Example 2



14



15

To a stirred solution of 14 (20.6 g, 31.75 mmol) in CH_3CN (159 ml), diisopropylethylamine (82.96 ml, 476.2 mmol), methoxymethylene bromide (25.9 ml, 317.5 mmol) and dimethylaminopyridine (155 mg, 1.27 mmol) were added at 0° C. The mixture was stirred at 23° C. for 24 h. The reaction was quenched at 0° C. with aqueous 0.1N HCl (750 ml) (pH=5), and extracted with CH_2Cl_2 (2×400 ml). The organic phase was dried (sodium sulphate) and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, gradient hexane:ethyl acetate 4:1 to hexane:ethyl acetate 3:2) to give 15 (17.6 g, 83%) as a yellow solid.

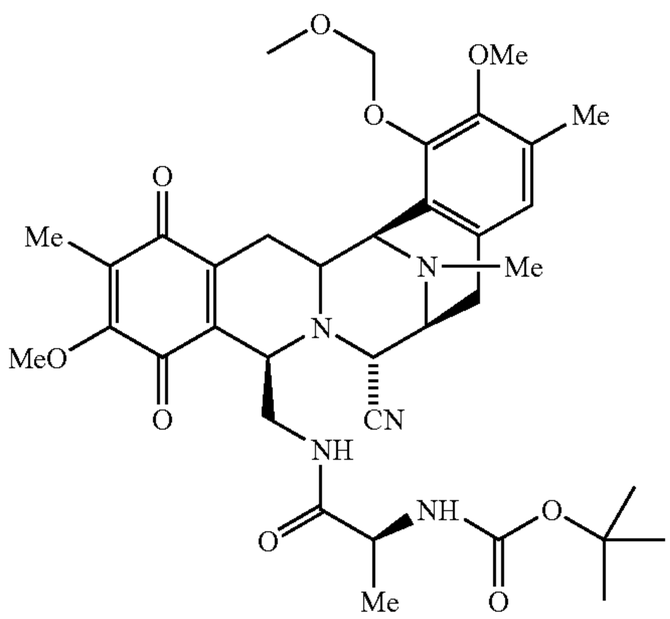
Rf: 0.38 (hexane:ethyl acetate 3:7).

^{1}H NMR (300 MHz, $CDCl_3$): δ 6.73 (s, 1H), 5.35 (bs, 1H), 5.13 (s, 2H), 4.50 (bs, 1H), 4.25 (d, J=2.7 Hz, 1H), 4.03 (d, J=2.7 Hz, 1H), 3.97 (s, 3H), 3.84 (bs, 1H), 3.82-3.65 (m, 1H), 3.69 (s, 3H), 3.56 (s, 3H), 3.39-3.37 (m, 1H), 3.20-3.00 (m, 5H), 2.46 (d, J=18 Hz, 1H), 2.33 (s, 3H), 2.23 (s, 3H), 1.85 (s, 3H), 1.73-1.63 (m, 1H), 1.29 (s, 9H), 0.93 (d, J=5.1 Hz, 3H)

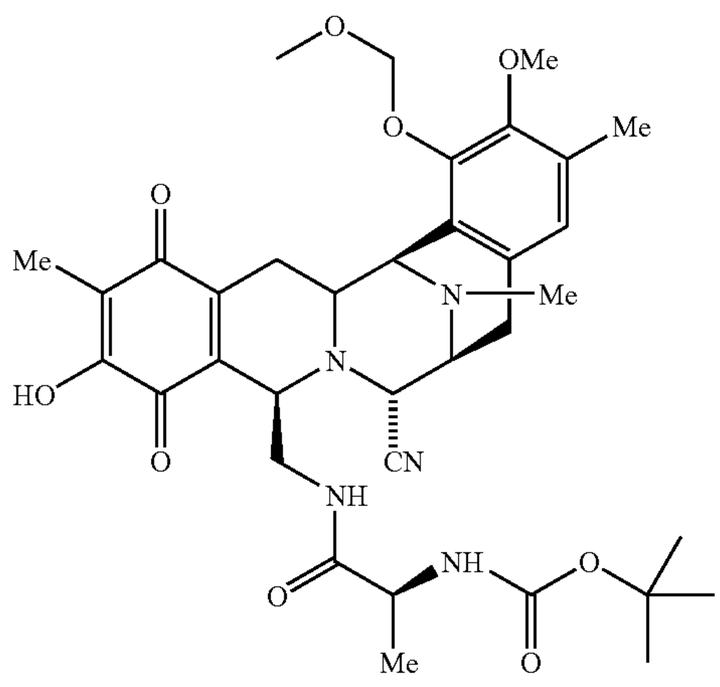
^{13}C NMR (75 MHz, $CDCl_3$): δ 185.4, 180.9, 172.4, 155.9, 154.5, 149.0, 148.4, 141.6, 135.1, 131.0, 129.9, 127.6, 124.4, 123.7, 117.3, 99.1, 79.3, 60.7, 59.7, 58.4, 57.5, 56.2, 55.9, 55.0, 54.2, 50.0, 41.5, 39.9, 28.0, 25.2, 24.0, 18.1, 15.6, 8.5.

ESI-MS m/z: Calcd. for $C_{36}H_{47}N_5O_9$: 693.8. Found $(M+H)^+$: 694.3.

Example 3



15



16

To a flask containing 15 (8 g, 1.5 ml) in methanol (1.6 l) an aqueous solution of 1M sodium hydroxide (3.2 l) was added at 0° C. The reaction was stirred for 2 h at this temperature and then, quenched with 6M HCl to pH=5. The mixture was extracted with ethyl acetate (3×11) and the combined organic layers were dried over sodium sulphate and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, gradient $CHCl_3$ to $CHCl_3$:ethyl acetate 2:1) to afford 16 (5.3 mg, 68%).

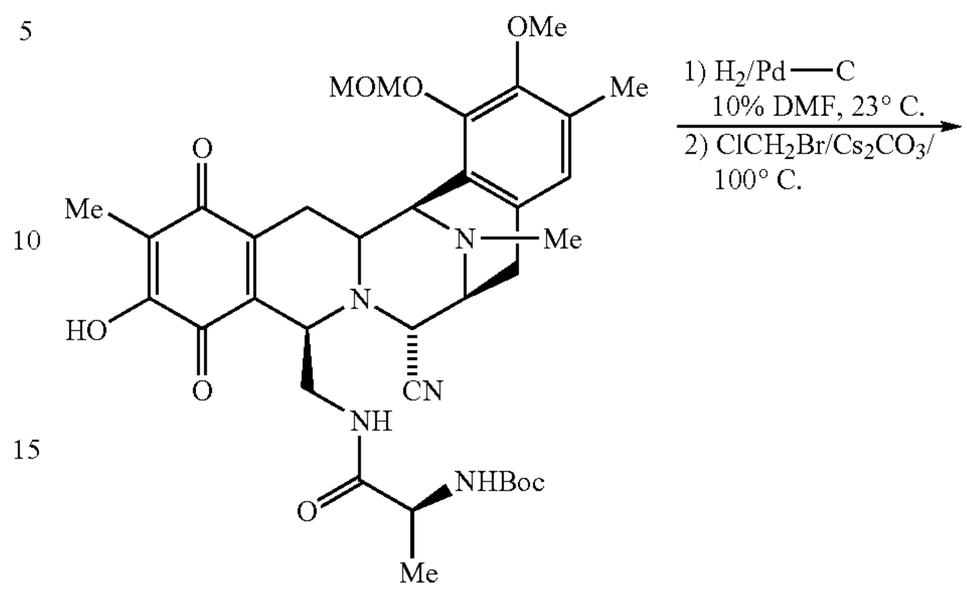
Rf: 0.48 (CH_3CN:H_2O 7:3, RP-C18)

^{1}H NMR (300 MHz, $CDCl_3$): δ 6.73 (s, 1H), 5.43 (bs, 1H), 5.16 (s, 2H), 4.54 (bs, 1H), 4.26 (d, J=1.8 Hz, 1H), 4.04 (d, J=2.7 Hz 1H), 3.84 (bs, 1H), 3.80-3.64 (m, 1H), 3.58 (s, 3H), 3.41-3.39 (m, 1H), 3.22-3.06 (m, 5H), 2.49 (d, J=18.6 Hz 1H), 2.35 (s, 3H), 2.30-2.25 (m, 1H), 2.24 (s, 3H), 1.87 (s, 3H), 1.45-1.33 (m, 1H), 1.19 (s, 9H), 1.00 (br d, J=6.6 Hz 3H)

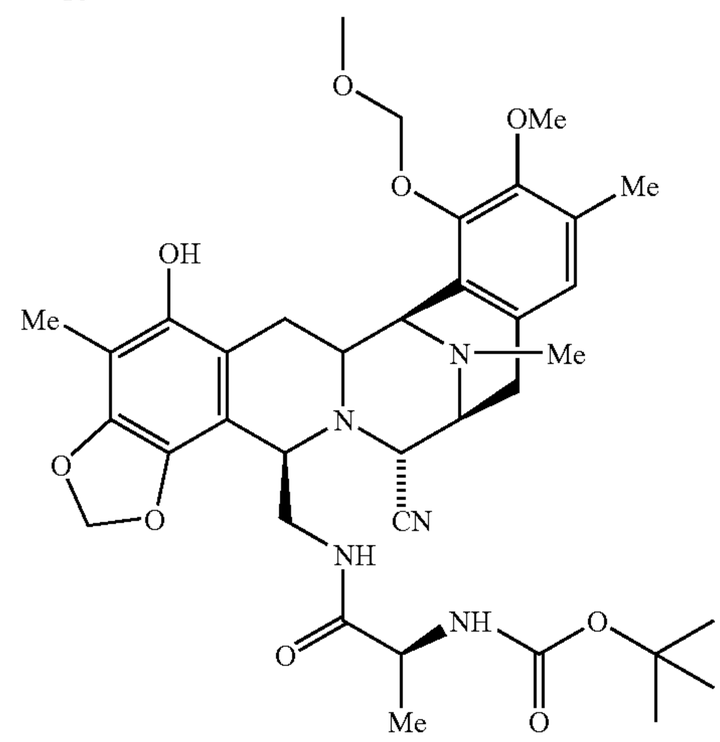
^{13}C NMR (75 MHz, □$CDCl_3$): δ 184.9, 180.9, 172.6, 154.7, 151.3, 149.1, 148.6, 144.7, 132.9, 131.3, 129.8, 124.5, 123.7, 117.3, 116.8, 99.1, 79.4, 59.8, 58.6, 57.7, 56.2, 55.6, 54.9, 54.5, 50.1, 41.6, 40.1, 28.0, 25.3, 24.4, 18.1, 15.7, 8.0.

ESI-MS m/z: Calcd. for $C_{35}H_{45}N_5O_9$: 679.7. Found $(M+H)^+$: 680.3.

Example 4



16



17

To a degassed solution of compound 16 (1.8 g, 2.64 mmol) in DMF (221 ml) 10% Pd/C (360 mg) was added and stirred under H_2 (atmospheric pressure) for 45 min. The reaction was filtered through celite under argon, to a flask containing anhydrous Cs_2CO_3 (2.58 g, 7.92 mmol). Then, bromochloromethane (3.40 ml 52.8 mmol), was added and the tube was sealed and stirred at 100° C. for 2 h. The reaction was cooled, filtered through a pad of celite and washed with CH_2Cl_2. The organic layer was concentrated and dried (sodium sulphate) to afford 17 as a brown oil that was used in the next step with no further purification.

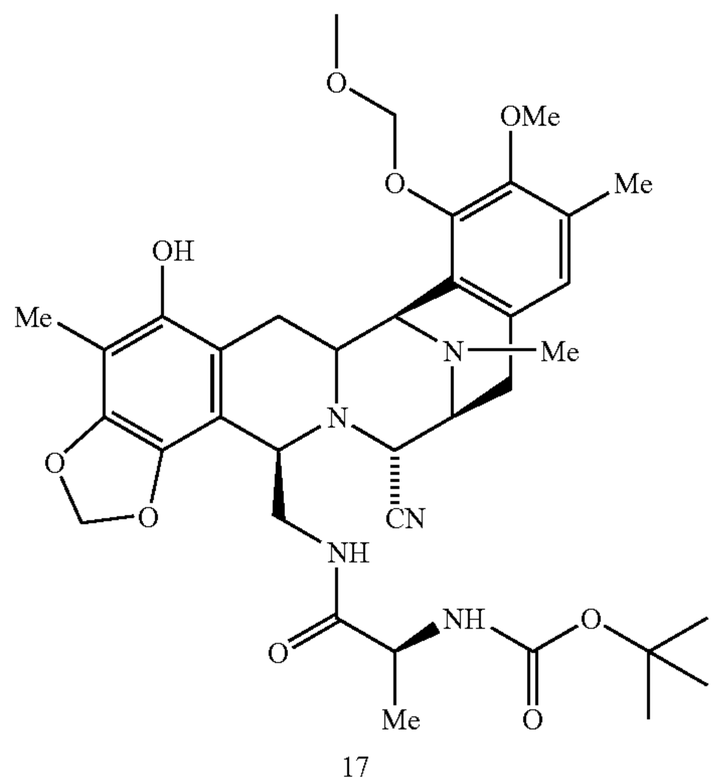
Rf: 0.36 (hexane:ethyl acetate 1:5, SiO_2).

^{1}H NMR (300 MHz, $CDCl_3$): δ 6.68 (s, 1H), 6.05 (bs, 1H), 5.90 (s, 1H), 5.79 (s, 1H), 5.40 (bs, 1H), 5.31-5.24 (m, 2H), 4.67 (d, J=8.1 Hz, 1H), 4.19 (d, J=2.7 Hz, 1H), 4.07 (bs, 1H), 4.01 (bs, 1H), 3.70 (s, 3H), 3.67 (s, 3H), 3.64-2.96 (m, 5H), 2.65 (d, J=18.3 Hz, 1H), 2.33 (s, 3H), 2.21 (s, 3H), 2.04 (s, 3H), 2.01-1.95 (m, 1H), 1.28 (s, 9H), 0.87 (d, J=6.3 Hz, 3H)

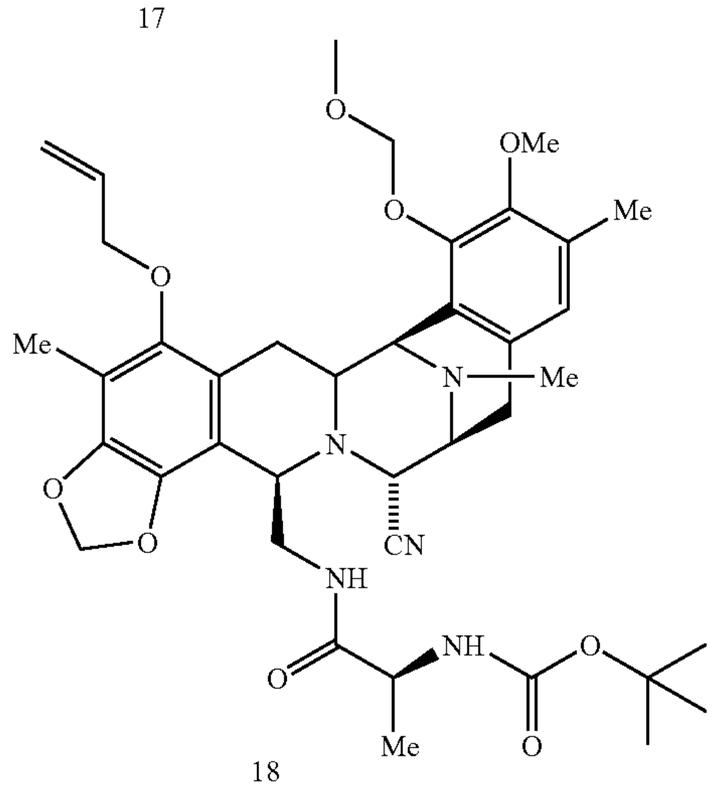
^{13}C NMR (75 MHz, $CDCl_3$): δ 172.1, 162.6, 154.9, 149.1, 145.7, 135.9, 130.8, 130.7, 125.1, 123.1, 117.8, 100.8, 99.8, 76.6, 59.8, 59.2, 57.7, 57.0, 56.7, 55.8, 55.2, 49.5, 41.6, 40.1, 36.5, 31.9, 31.6, 29.7, 28.2, 26.3, 25.0, 22.6, 18.2, 15.8, 14.1, 8.8.

ESI-MS m/z: Calcd. for $C_{36}H_{47}N_5O_9$: 693.34. Found $(M+H)^+$: 694.3.

Example 5



17



18

To a flask containing a solution of 17 (1.83 g, 2.65 mmol) in DMF (13 ml), Cs_2CO_3 (2.6 g, 7.97 mmol), and allyl bromide (1.15 ml, 13.28 mmol) were added at 0° C. The resulting mixture was stirred at 23° C. for 1 h. The reaction was filtered through a pad of celite and washed with CH_2Cl_2. The organic layer was dried and concentrated (sodium sulphate). The residue was purified by flash column chromatography (SiO_2, $CHCl_3$:ethyl acetate 1:4) to afford 18 (1.08 mg, 56%) as a white solid.

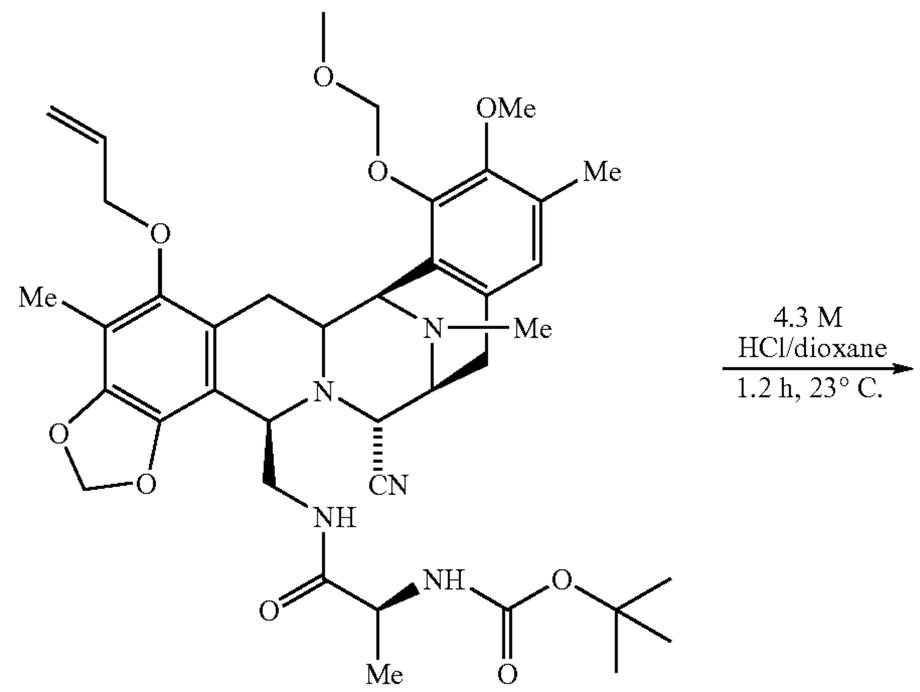
Rf: 0.36 ($CHCl_3$:ethyl acetate 1:3).

^{1}H NMR (300 MHz, $CDCl_3$): δ 6.70 (s, 1H), 6.27-6.02 (m, 1H), 5.94 (s, 1H), 5.83 (s, 1H), 5.37 (dd, J_1=1.01 Hz, J_2=16.8 Hz, 1H), 5.40 (bs, 1H), 5.25 (dd, J_1=1.0 Hz, J_2=10.5 Hz, 1H), 5.10 (s, 2H), 4.91 (bs, 1H), 4.25-4.22 (m, 1H), 4.21 (d, J=2.4 Hz, 1H), 4.14-4.10 (m, 1H), 4.08 (d, J=2.4 Hz, 1H), 4.00 (bs, 1H), 3.70 (s, 3H), 3.59 (s, 3H), 3.56-3.35 (m, 2H), 3.26-3.20 (m, 2H), 3.05-2.96 (dd, J_1=8.1 Hz, J_2=18 Hz, 1H), 2.63 (d, J=18 Hz, 1H), 2.30 (s, 3H), 2.21 (s, 3H), 2.09 (s, 3H), 1.91-1.80 (m, 1H), 1.24 (s, 9H), 0.94 (d, J=6.6 Hz, 3H)

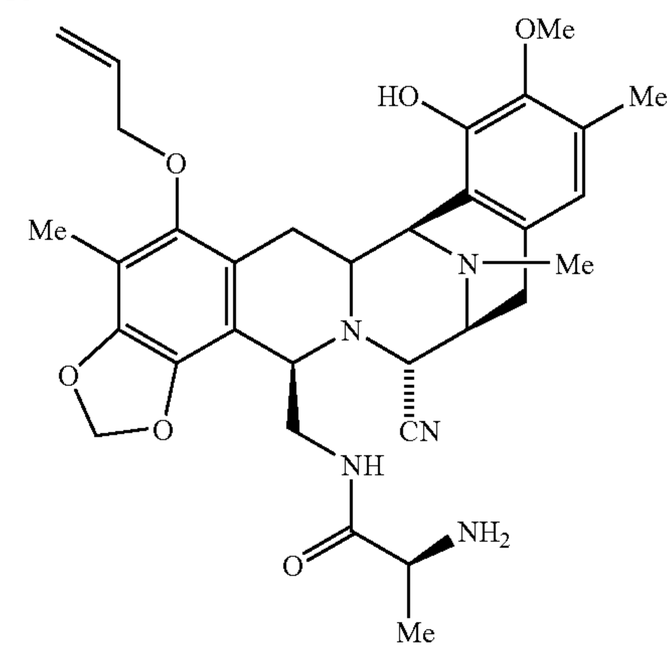
^{13}C NMR (75 MHz, $CDCl_3$): δ 172.0, 154.8, 148.8, 148.6, 148.4, 144.4, 138.8, 133.7, 130.9, 130.3, 125.1, 124.0, 120.9, 117.8, 117.4, 112.8, 112.6, 101.1, 99.2, 73.9, 59.7, 59.3, 57.7, 56.9, 56.8, 56.2, 55.2, 40.1, 34.6, 31.5, 28.1, 26.4, 25.1, 22.6, 18.5, 15.7, 14.0, 9.2.

ESI-MS m/z: Calcd. for $C_{39}H_{51}N_5O_9$: 733.4. Found $(M+H)^+$: 734.4.

Example 6



18



19

To a solution of 18 (0.1 g, 0.137 mmol) in dioxane (2 ml), 4.2M HCl/dioxane (1.46 ml) was added and the mixture was stirred for 1.2 h at 23° C. The reaction was quenched at 0° C. with sat. Aqueous sodium bicarbonate (60 ml) and extracted with ethyl acetate (2×70 ml). The organic layers were dried (sodium sulphate) and concentrated in vacuo to afford 19 (267 mg, 95%) as a white solid that was used in subsequent reactions with no further purification.

Rf: 0.17 (ethyl acetate:methanol 10:1, SiO_2)

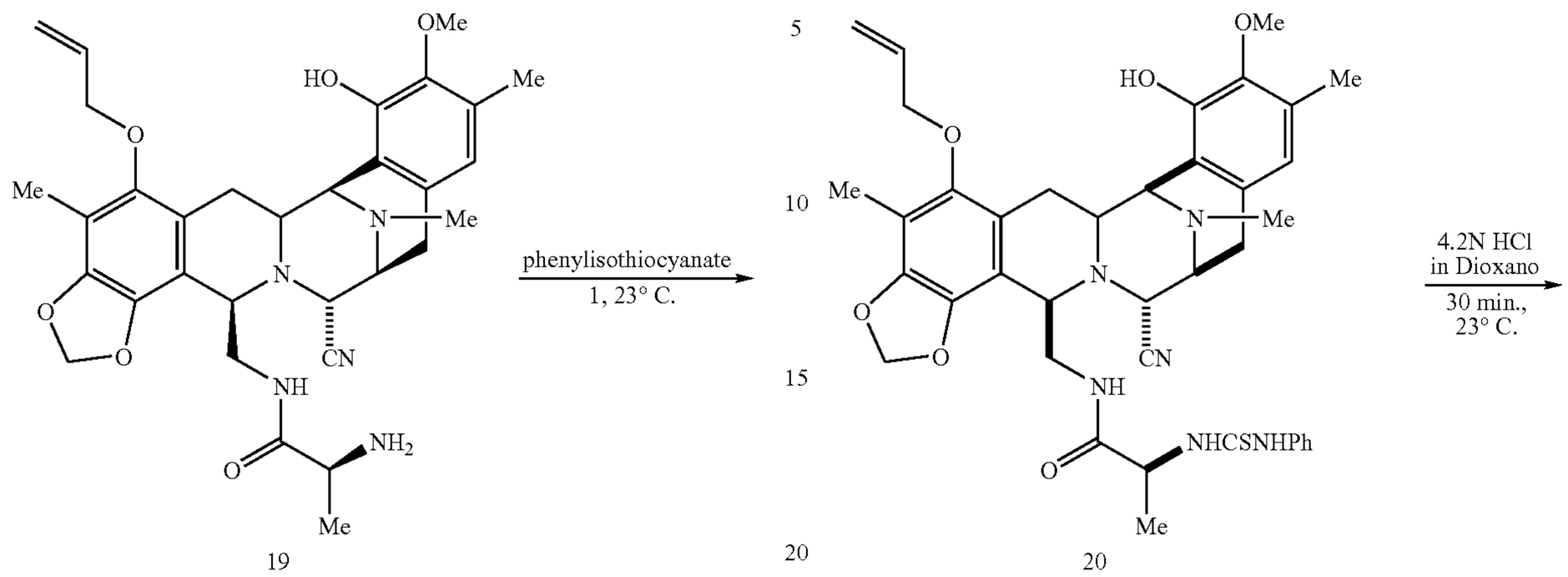
^{1}H NMR (300 MHz, $CDCl_3$): δ 6.49 (s, 1H), 6.12-6.00 (m, 1H), 5.94 (s, 1H), 5.86 (s, 1H), 5.34 (dd, J=1.0 Hz, J=17.4 Hz, 1H), 5.25 (dd, J=1.0 Hz, J-10.2 Hz, 1H), 4.18-3.76 (m, 5H), 3.74 (s, 3H), 3.71-3.59 (m, 1H), 3.36-3.20 (m, 4H), 3.01-2.90 (m, 1H), 2.60 (d, J=18.0 Hz, 1H), 2.29 (s, 3H), 2.24 (s, 3H), 2.11 (s, 3H), 1.97-1.86 (m, 1H), 0.93 (d, J=8.7 Hz, 3H)

^{13}C NMR (75 MHz, $CDCl_3$): δ 175.5, 148.4, 146.7, 144.4, 142.4, 138.9, 133.7, 131.3, 128.3, 120.8, 117.9, 117.4, 113.8, 112.4, 101.1, 74.2, 60.5, 59.1, 56.5, 56.1, 56.3, 56.0, 55.0, 50.5, 41.6, 39.5, 29.5, 26.4, 24.9, 21.1, 15.5, 9.33.

ESI-MS m/z: Calcd. for $C_{32}H_{39}N_5O_6$: 589. Found $(M+H)^+$: 590.

Example 7

Example 8



To a solution of 19 (250 mg, 0.42 mmol) in CH_2Cl_2 (1.5 mmol), phenyl isothiocyanate (0.3 ml, 2.51 mmol) was added and the mixture was stirred at 23° C. for 1 h. The reaction was concentrated in vacuo and the residue was purified by flash column chromatography (SiO_2, gradient Hexane to 5:1 hexane:ethyl acetate) to afford 20 (270 mg, 87%) as a white solid.

Rf: 0.56 ($CHCl_3$:ethyl acetate 1:4).

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.00 (bs, 1H), 7.45-6.97 (m, 4H), 6.10 (s, 1H), 6.08-6.00 (m, 1H), 5.92 (s, 1H), 5.89 (s, 1H), 5.82 (s, 1H), 5.40 (dd, J=1.5 Hz, J=17.1 Hz, 1H), 3.38 (bs, 1H), 5.23 (dd, J=1.5 Hz, J=10.5 Hz, 1H), 4.42-4.36 (m, 1H), 4.19-4.03 (m, 5H), 3.71 (s, 3H), 3.68-3.17 (m, 4H), 2.90 (dd, J=7.8 Hz, J=18.3 Hz, 1H), 2.57 (d, J=18.3 Hz, 1H), 2.25 (s, 3H), 2.12 (s, 3H), 2.10 (s, 3H), 1.90 (dd, J=12.3 Hz, J=16.5 Hz, 1H), 0.81 (d, J=6.9 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$): δ 178.4, 171.6, 148.6, 146.8, 144.3, 142.7, 138.7, 136.2, 133.6, 130.7, 129.8, 126.6, 124.2, 124.1, 120.9, 120.5, 117.7, 117.4, 116.7, 112.6, 112.5, 101.0, 74.0, 60.6, 59.0, 57.0, 56.2, 56.1, 55.0, 53.3, 41.4, 39.7, 26.3, 24.8, 18.3, 15.5, 9.2.

ESI-MS m/z: Calcd. for $C_{39}H_{44}N_6O_6S$: 724.8 Found $(M+H)^+$: 725.3.

To a solution of 20 (270 mg, 0.37 mmol) in dioxane (1 ml), 4.2N HCl/dioxane (3.5 ml) was added and the reaction was stirred at 23° C. for 30 min. Then, ethyl acetate (20 ml) and H_2O (20 ml) were added and the organic layer was decanted. The aqueous phase was basified with saturated aqueous sodium bicarbonate (60 ml) (pH=8) at 0° C. and then, extracted with CH_2Cl_2 (2×50 ml). The combined organic extracts were dried (sodium sulphate), and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, ethyl acetate:methanol 5:1) to afford compound 21 (158 mg, 82%) as a white solid.

Rf: 0.3 (ethyl acetate:methanol 1:1).

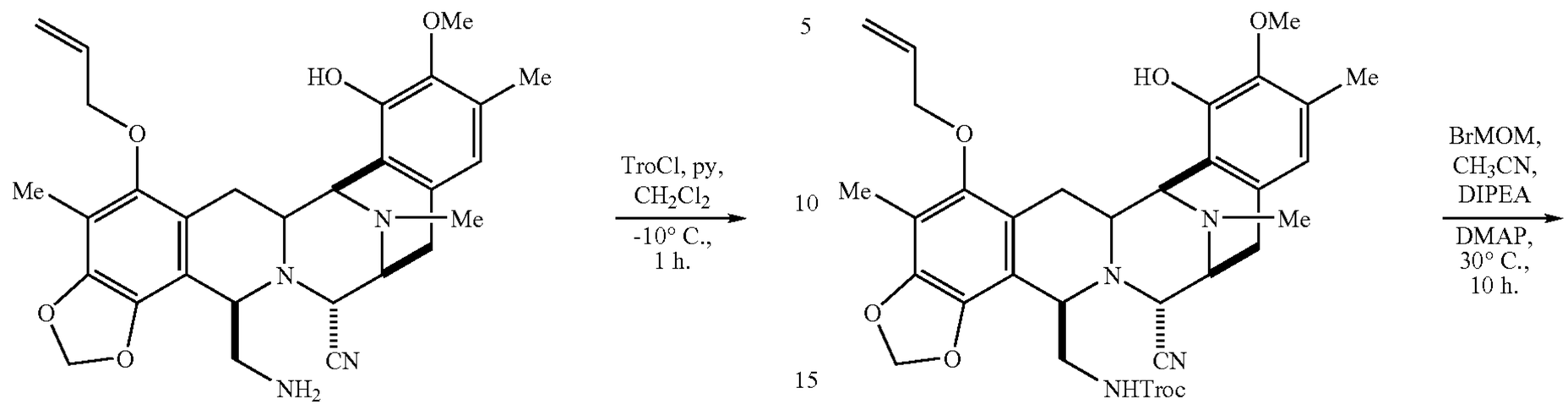
^{1}H NMR (300 MHz, $CDCl_3$): δ 6.45 (s, 1H), 6.12-6.03 (m, 1H), 5.91 (s, 1H), 5.85 (s, 1H), 5.38 (dd, J_1=1.2 Hz, J_2=17.1 Hz, 1H), 5.24 (dd, J_1=1.2 Hz, J_2=10.5 Hz, 1H), 4.23-4.09 (m, 4H), 3.98 (d, J=2.1 Hz, 1H), 3.90 (bs, 1H), 3.72 (s, 3H), 3.36-3.02 (m, 5H), 2.72-2.71 (m, 2H), 2.48 (d, J=18.0 Hz, 1H), 2.33 (s, 3H), 2.22 (s, 3H), 2.11 (s, 3H), 1.85 (dd, J_1=11.7 Hz, J_2=15.6 Hz, 1H)).

^{13}C NMR (75 MHz, $CDCl_3$): δ 148.4, 146.7, 144.4, 142.8, 138.8, 133.8, 130.5, 128.8, 121.5, 120.8, 118.0, 117.5, 116.9, 113.6, 112.2, 101.1, 74.3, 60.7, 59.9, 58.8, 56.6, 56.5, 55.3, 44.2, 41.8, 29.7, 26.5, 25.7, 15.7, 9.4.

ESI-MS m/z: Calcd. for $C_{29}H_{34}N_4O_5$: 518.3. Found $(M+H)^+$: 519.2.

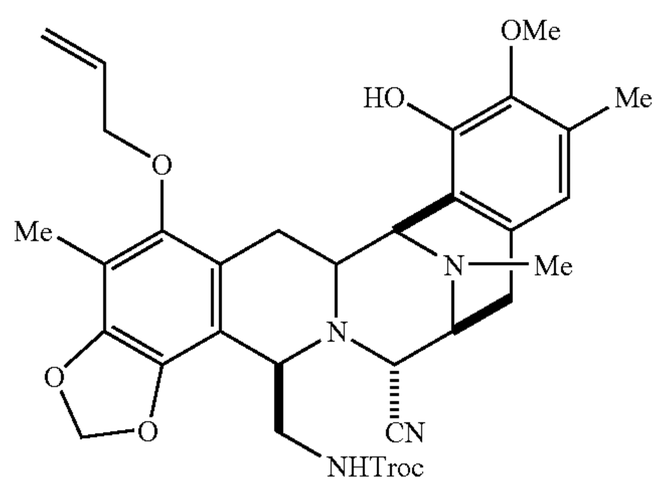
Example 9

Example 10



21

22



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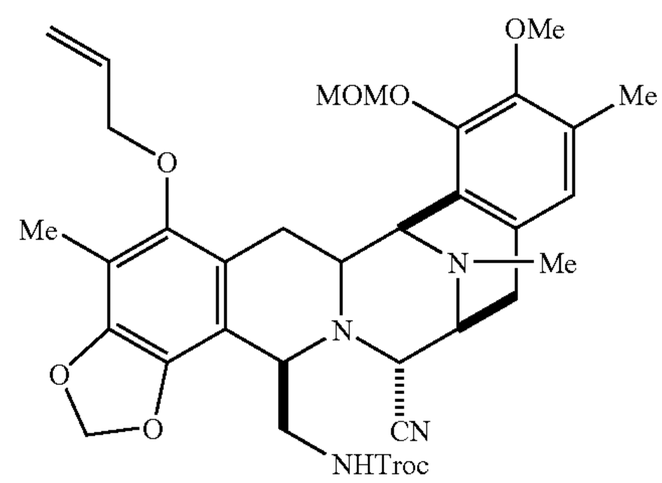


23

To a solution of 21 (0.64 g, 1.22 mmol) in CH_2Cl_2 (6.13 ml), pyridine (0.104 ml, 1.28 mmol) and 2,2,2-trichloroethyl chloroformate (0.177 ml, 1.28 mmol) were added at −10° C. The mixture was stirred at this temperature for 1 h and then, the reaction was quenched by addition of 0.1N HCl (10 ml) and extracted with CH_2Cl_2 (2×10 ml). The organic layer was dried over sodium sulphate and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2; (hexane:ethyl acetate 1:2) to afford 22 (0.84 g, 98%) as a white foam solid.

Rf: 0.57 (ethyl acetate:methanol 5:1).

^{1}H NMR (300 MHz, $CDCl_3$): δ 6.50 (s, 1H), 6.10-6.00 (m, 1H), 6.94 (d, J=1.5 Hz, 1H), 5.87 (d, J=1.5 Hz, 1H), 5.73 (bs, 1H), 5.37 (dq, J_1=1.5 Hz, J_2=17.1 Hz, 1H), 5.26 (dq, J_1=1.8 Hz, J_2=10.2 Hz, 1H), 4.60 (d, J=12 Hz, 1H), 4.22-4.10 (m, 4H), 4.19 (d, J=12 Hz, 1H), 4.02 (m, 2H), 3.75 (s, 3H), 3.37-3.18 (m, 5H), 3.04 (dd, J_1=8.1 Hz, J_2=18 Hz, 1H), 2.63 (d, J=18 Hz, 1H), 2.31 (s, 3H), 2.26 (s, 3H), 2.11 (s, 3H), 1.85 (dd, J_1=12.3 Hz, J_2=15.9 Hz, 1H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 154.3, 148.5, 146.7, 144.5, 142.8, 139.0, 133.8, 130.7, 128.7, 121.3, 120.8, 117.8, 117.7, 116.8, 112.7, 101.2, 77.2, 74.3, 60.7, 59.9, 57.0, 56.4, 55.3, 43.3, 41.7, 31.6, 26.4, 25.3, 22.6, 15.9, 14.1, 9.4.

ESI-MS m/z: Calcd. for $C_{32}H_{35}Cl_3N_4O_7$: 694.17. Found $(M+H)^+$: 695.2.

To a solution of 22 (0.32 g, 0.46 mmol) in CH_3CN (2.33 ml), diisopropylethylamine (1.62 ml, 9.34 mmol), bromomethyl methyl ether (0.57 ml, 7.0 mmol) and dimethylaminopyridine (6 mg, 0.046 mmol) were added at 0° C. The mixture was heated at 30° C. for 10 h. Then, the reaction was diluted with dichloromethane (30 ml) and poured in an aqueous solution of HCl at pH=5 (10 ml). The organic layer was dried over sodium sulphate and the solvent was eliminated under reduced pressure to give a residue which was purified by flash column chromatography (SiO_2, hexane:ethyl acetate 2:1) to afford 23 (0.304 g, 88%) as a white foam solid.

Rf: 0.62 (hexane:ethyl acetate 1:3).

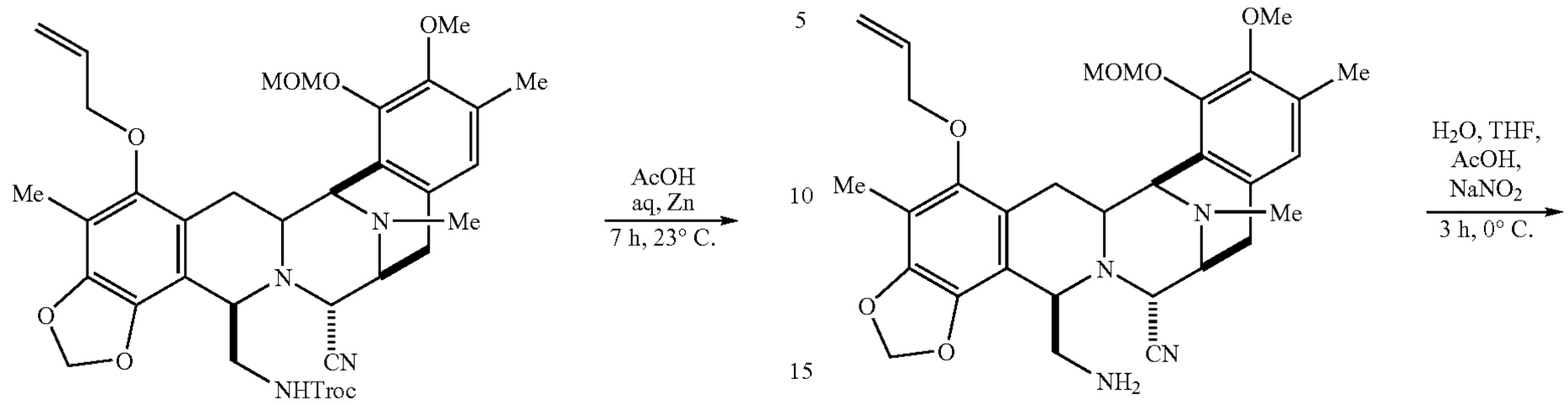
^{1}H NMR (300 MHz, $CDCl_3$): δ 6.73 (s, 1H), 6.10 (m, 1H), 5.94 (d, J=1.5 Hz, 1H), 5.88 (d, J=1.5 Hz, 1H), 5.39 (dq, J_1=1.5 Hz, J_2=17.1 Hz, 1H), 5.26 (dq, J_1=1.8 Hz, J_2=10.2 Hz, 1H), 5.12 (s, 2H), 4.61 (d, J=12 Hz, 1H), 4.55 (t, J=6.6 Hz, 1H), 4.25 (d, J=12 Hz, 1H), 4.22-4.11 (m, 4H), 4.03 (m, 2H), 3.72 (s, 3H), 3.58 (s, 3H), 3.38-3.21 (m, 5H), 3.05 (dd, J_1=8.1 Hz, J_2=18 Hz, 1H), 2.65 (d, J=18 Hz, 1H), 2.32 (s, 3H), 2.23 (s, 3H), 2.12 (s, 3H), 1.79 (dd, J_1=12.3 Hz, J_2=15.9 Hz, 1H);

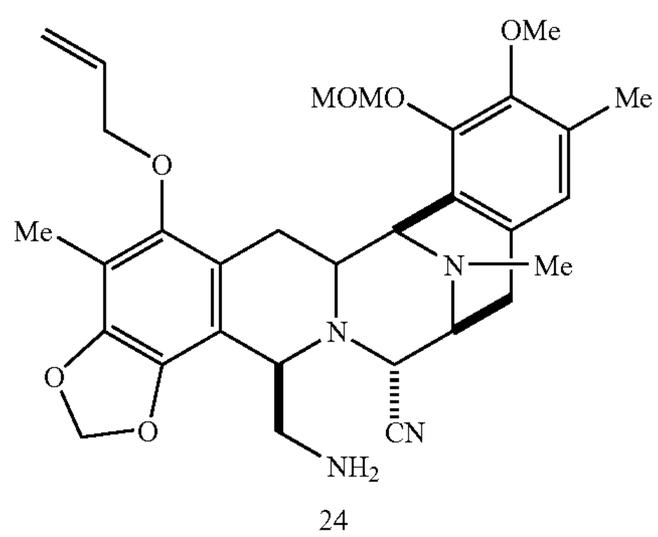
^{13}C NMR (75 MHz, $CDCl_3$) δ 154.3, 148.6, 148.4, 144.5, 139.0, 133.6, 130.6, 130.1, 125.07, 124.7, 124.0, 121.1, 117.7, 112.6, 101.2, 99.2, 77.2, 74.4, 74.1, 59.8, 59.8, 57.7, 57.0, 56.8, 56.68, 55.3, 43.2, 41.5, 26.4, 25.2, 15.9, 9.3.

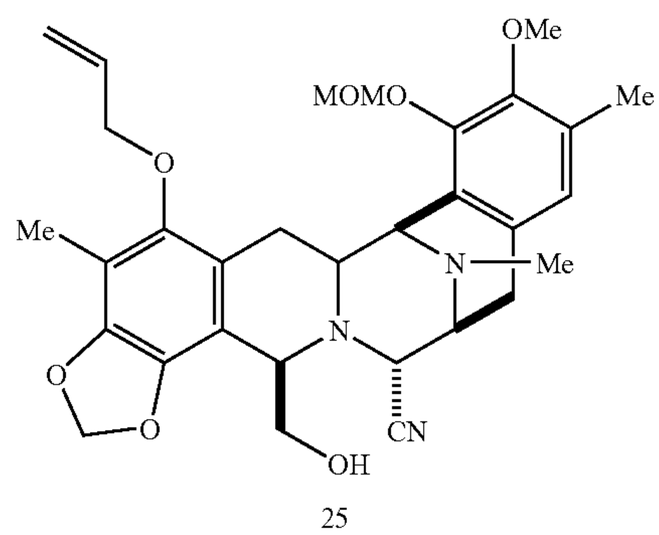
ESI-MS m/z: Calcd. for $C_{34}H_{39}Cl_3N_4O_8$: 738.20. Found $(M+H)^+$: 739.0.

Example 11

Example 12







To a suspension of 23 (0.304 g, 0.41 mmol) in 90% aqueous acetic acid (4 ml), powder zinc (0.2 g, 6.17 mmol) was added and the reaction was stirred for 7 hour at 23° C. The mixture was filtered through a pad of celite which was washed with CH_2Cl_2. The organic layer was washed with an aqueous sat. solution of sodium bicarbonate (pH=9) (15 ml) and dried over sodium sulphate. The solvent was eliminated under reduced pressure to give 24 (0.191 g, 83%) as a white solid.

Rf: 0.3 (ethyl acetate:methanol 5:1).

^{1}H NMR (300 MHz, $CDCl_3$): δ 6.68 (s, 1H), 6.09 (m, 1H), 5.90 (d, J=1.5 Hz, 1H), 5.83 (d, J=1.5 Hz, 1H), 5.39, (dq, J_1=1.5 Hz, J_2=17.1 Hz, 1H), 5.25 (dq, J_1=1.5 Hz, J_2=10.2 Hz, 1H), 5.10 (s, 2H), 4.22-4.09 (m, 3H), 3.98 (d, J=2.4 Hz, 1H), 3.89 (m, 1H), 3.69 (s, 3H), 3.57 (s, 3H), 3.37-3.17 (m, 3H), 3.07 (dd, J_1=8.1 Hz, J_2=18 Hz, 1H), 2.71 (m, 2H), 2.48 (d, J=18 Hz, 1H), 2.33 (s, 3H), 2.19 (s, 3H), 2.17 (s, 3H), 1.80 (dd, J_1=12.3 Hz, J_2=15.9 Hz, 1H)

^{13}C NMR (75 MHz, $CDCl_3$): δ 148.5, 148.2, 144.3, 138.7, 133.7, 130.7, 129.9, 125.0, 123.9, 121.3, 117.9, 117.5, 113.6, 112.0, 101.0, 99.2, 74.0, 59.8, 59.7, 58.8, 57.6, 57.0, 56.2, 55.2, 44.2, 41.5, 31.5, 26.4, 25.6, 22.5, 16.7, 14.0, 9.2.

ESI-MS m/z: Calcd. for $C_{31}H_{38}N_4O_6$: 562.66. Found $(M+H)^+$: 563.1.

To a solution of 24 (20 mg, 0.035 mmol), in H_2O (0.7 mmol) and THF (0.7 mmol), $NaNO_2$ (12 mg, 0.17 mmol) and 90% aqueous AcOH (0.06 ml) were added at 0° C. and the mixture was stirred at 0° C. for 3 h. After dilution with CH_2Cl_2 (5 ml), the organic layer was washed with water (1 ml), dried over sodium sulphate and concentrated in vacuo.

The residue was purified by flash column chromatography (SiO_2, hexane:ethyl acetate 2:1) to afford 25 (9.8 mg, 50%) as a white solid.

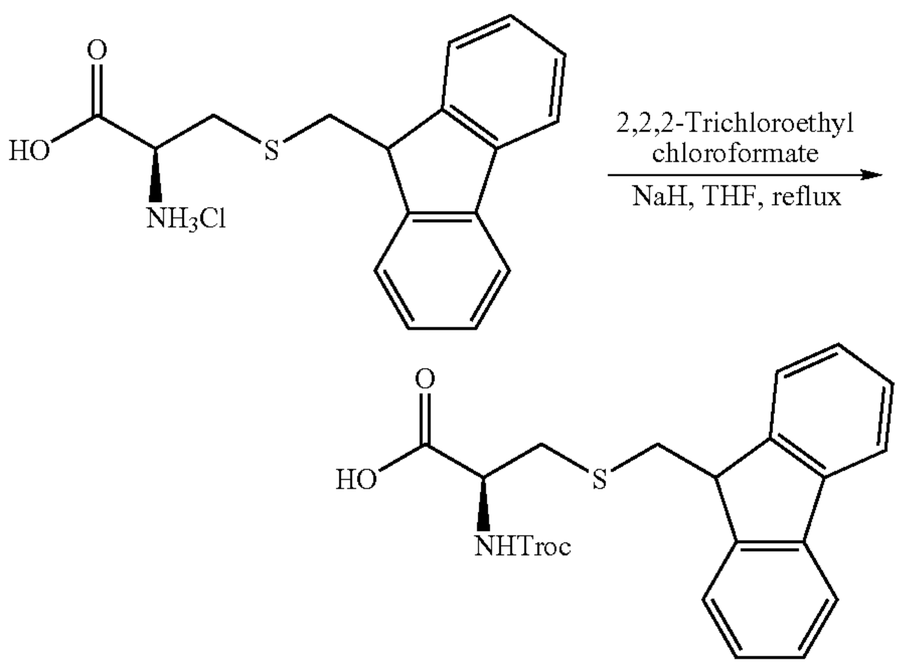
Rf: 0.34 (hexane:ethyl acetate 1:1).

^{1}H NMR (300 MHz, $CDCl_3$): δ 6.71 (s, 1H), 6.11 (m, 1H), 5.92□(d, J=1.5 Hz, 1H), 5.87 (d, J=1.5 Hz, 1H), 5.42 (dq, J_1=1.5 Hz, J_2=17.1 Hz, 1H), 5.28 (dq, J_1=1.5 Hz, J_2=10.2 Hz, 1H), 5.12 (s, 2H), 4.26-4.09 (m, 3H), 4.05 (d, J=2.4 Hz, 1H), 3.97 (t, J=3.0 Hz, 1H), 3.70 (s, 3H), 3.67-3.32 (m, 4H), 3.58 (s, 3H), 3.24 (dd, J_1=2.7 Hz, J_2=15.9 Hz, 1H), 3.12 (dd, J_1=8.1 Hz, J_2=18.0 Hz, 1H), 2.51 (d, J=18 Hz, 1H), 2.36 (s, 3H), 2.21 (s, 3H), 2.12 (s, 3H), 1.83 (dd, J_1=12.3 Hz, J_2=15.9 Hz, 1H)

^{13}C NMR (75 MHz, $CDCl_3$) δ 148.7, 148.4, 138.9, 133.7, 131.1, 129.4, 125.1, 123.9, 120.7, 117.6, 117.5, 113.2, 112.3, 101.1, 99.2, 74.0, 63.2, 59.8, 59.7, 57.9, 57.7, 57.0, 56.5, 55.2, 41.6, 29.6, 26.1, 25.6, 22.6, 15.7, 9.2.

ESI-MS m/z: Calcd. for $C_{31}H_{37}N_3O_7$: 563.64. Found $(M+H)^+$: 564.1.

Example 13



29

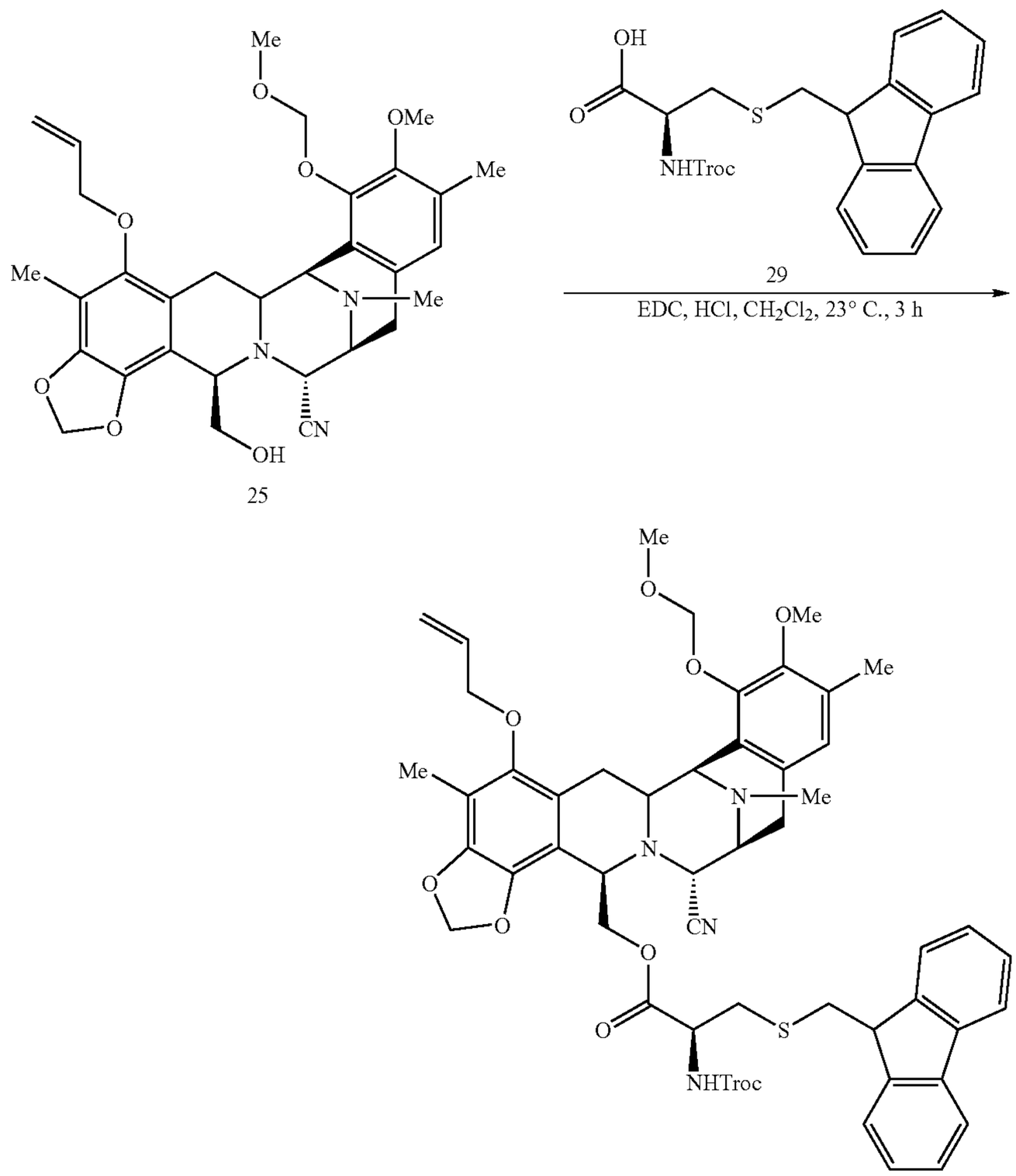
The starting material (2.0 g, 5.90 mmol) was added to a suspension of sodium hydride (354 mg, 8.86 mmol) in THF (40 ml) at 23° C., following the suspension was treated with allyl chloroformate (1.135 ml, 8.25 mmol) at 23° C. and then refluxed for 3 hours. The suspension was cooled, filtered off, the solid washed with ethyl acetate (100 ml), and the filtrate was concentrated. The oil crude was ground with hexane (100 ml) and kept at 4° C. overnight. After, the solvent was decanted and the light yellow slurry was treated with CH_2Cl_2 (20 ml), and precipitated with hexane (100 ml). After 10 minutes, the solvent was decanted again. The operation was repeated until appearing a white solid. The white solid was filtered off and dried to afford compound 29 (1.80 g, 65%) as a white solid.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.74 (d, J=7.5 Hz, 2H), 7.62 (d, J=6.9 Hz, 2H), 7.33 (t, J=7.5 Hz, 2H), 7.30 (t, J=6.3 Hz, 2H), 5.71 (d, J=7.8 Hz, 1H), 4.73 (d, J=7.8 Hz, 2H), 4.59 (m, 1H), 4.11 (t, J=6.0 Hz, 1H), 3.17 (dd, J=6.0 Hz, J=2.7 Hz, 2H), 3.20 (dd, J=5.4 Hz, J=2.1 Hz, 2H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 173.6, 152.7, 144.0, 139.7, 137.8, 126.0, 125.6, 123.4, 118.3, 73.4, 52.4, 45.5, 35.8, 33.7.

ESI-MS m/z: Calcd. for $C_{20}H_{18}Cl_3NO_4S$: 474.8. Found $(M+Na)^+$: 497.8

Example 14



25

30

A mixture of compound 25 (585 mg, 1.03 mmol) and compound 29 (1.47 mg, 3.11 mmol) were azeotroped with anhydrous toluene (3×0.10 ml). To a solution of 25 and 29 in anhydrous CH_2Cl_2 (40 ml) was added DMAP (633 mg, 5.18 mmol) and EDC-HCl (994 mg, 5.18 mmol) at 23° C. The reaction mixture was stirred at 23° C. for 3 hours. The mixture was partitioned with saturated aqueous solution of sodium bicarbonate (50 ml) and the layers were separated. The aqueous layer was washed with CH_2Cl_2 (50 ml). The combined organic layers were dried over sodium sulphate, filtered and concentrated. The crude was purified by flash column chromatography (ethyl acetate/hexane 1:3) to obtain 30 (1.00 g, 95%) as a pale cream yellow solid.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.72 (m, 2H), 7.52 (m, 2H), 7.38 (m, 2H), 7.28 (m, 2H), 6.65 (s, 1H), 6.03 (m, 1H), 5.92 (d, J=1.5 Hz, 1H), 5.79 (d, J=1.5 Hz, 1H), 5.39 (m, 1H), 5.29 (dq, J=10.3 Hz, J=1.5 Hz, 1H), 5.10 (s, 2H), 4.73 (d, J=11.9 Hz, 1H), 4.66 (d, J=11.9 Hz, 1H), 4.53 (m, 1H), 4.36-3.96 (m, 9H), 3.89 (t, J=6.4 Hz, 1H), 3.71 (s, 3H), 3.55 (s, 3H), 3.33 (m, 1H), 3.20 (m, 2H), 2.94 (m, 3H), 2.59 (m, 1H), 2.29 (s, 3H), 2.23 (s, 3H), 2.02 (s, 3H), 1.83 (dd, J=16.0 Hz, J=11.9 Hz, 1H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 169.7, 154.0, 148.8, 148.4, 145.7, 144.5, 140.9, 139.0, 133.7, 130.9, 130.6, 127.6, 127.0, 124.8, 124.6, 124.1, 120.8, 119.9, 118.2, 117.7, 117.3, 112.7, 112.1, 101.3, 99.2, 74.7, 73.9, 64.4, 59.8, 57.7, 57.0, 56.8, 55.4, 53.3, 46.7, 41.4, 36.5, 34.7, 31.5, 26.4, 24.9, 22.6, 15.7, 14.0, 9.1

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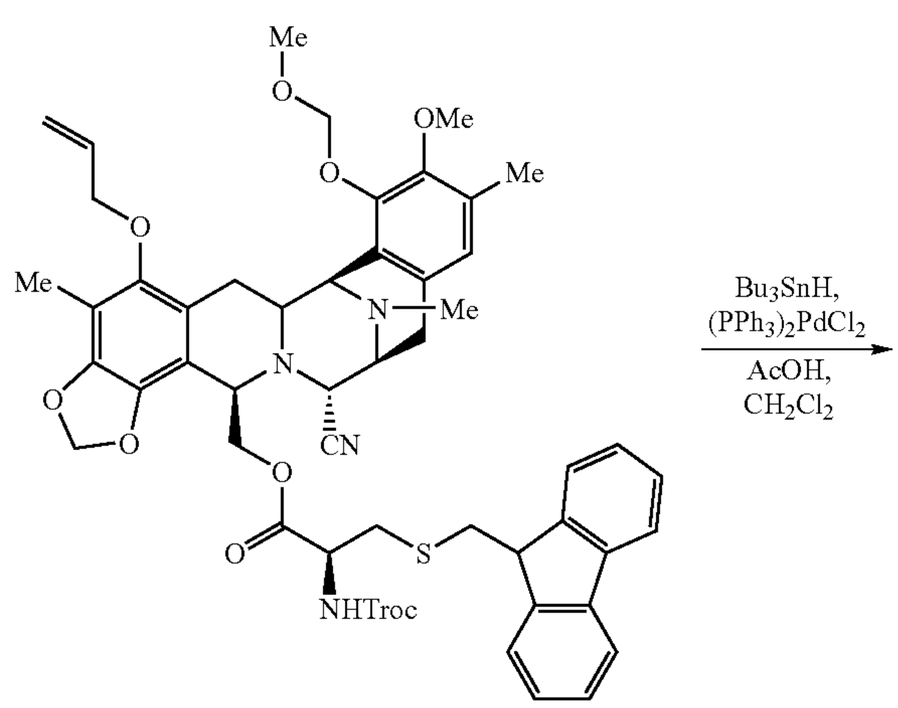
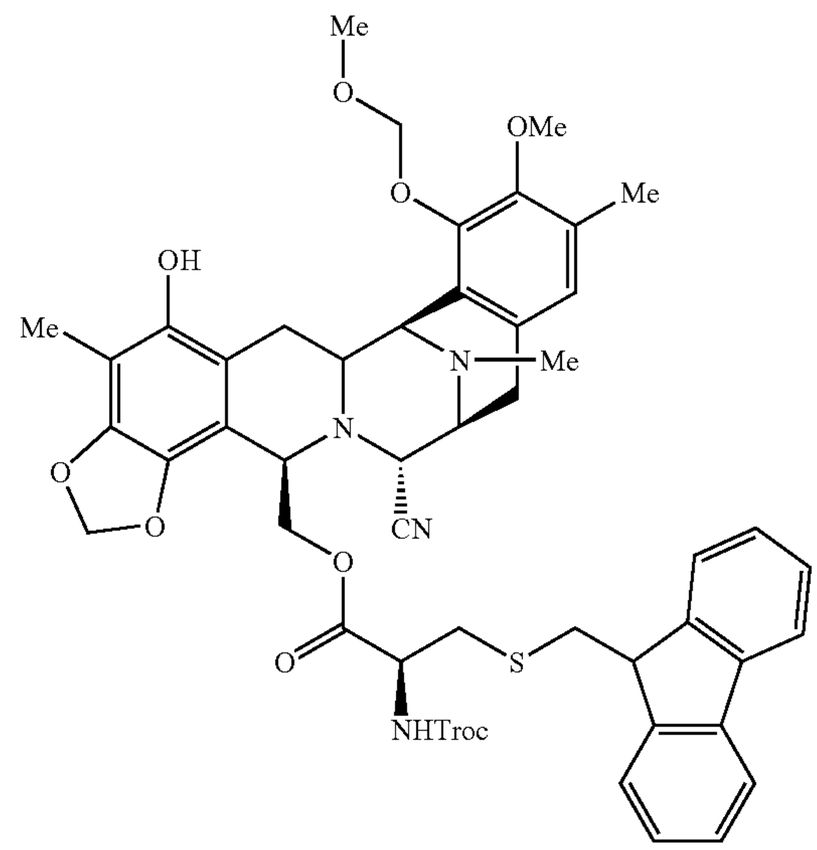
ESI-MS m/z: Calcd. for $C_{51}H_{53}Cl_3N_4O_{10}S$: 1020.4. Found $(M+H)^+$: 1021.2

Example 15



30

-continued



31

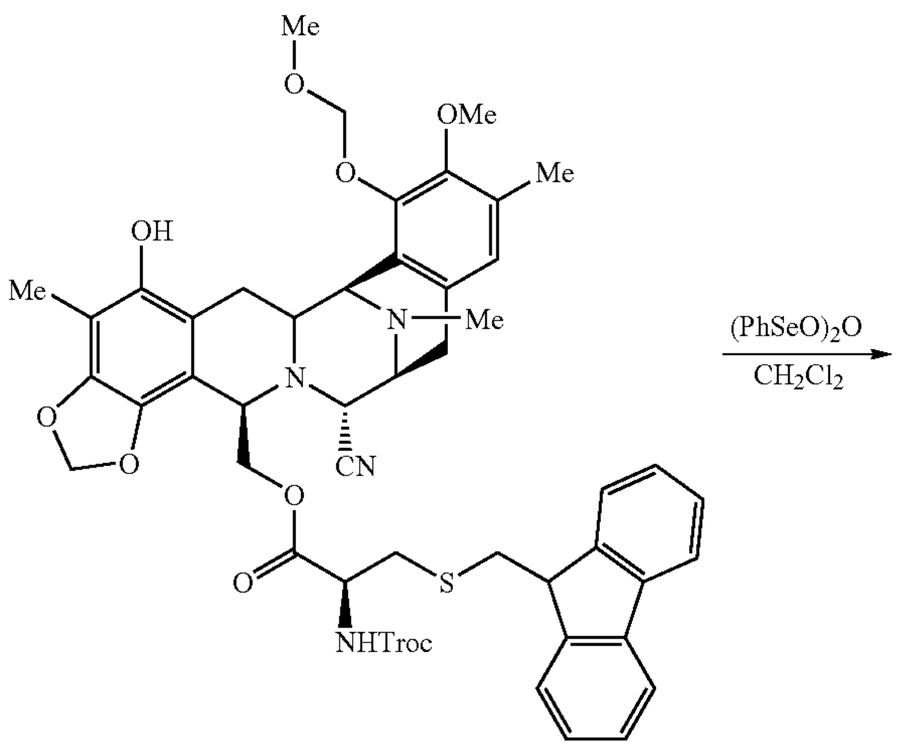
To a solution of 30 (845 mg, 0.82 mmol), acetic acid (500 mg, 8.28 mmol) and $(PPh_3)_2PdCl_2$ (29 mg, 0.04 mmol) in anhydrous CH_2Cl_2 20 ml at 23° C. was added, dropwise, Bu_3SnH (650 mg, 2.23 mmol). The reaction mixture was stirred at this temperature for 15 min., bubbling was. The crude was quenched with water (50 ml) and extracted with CH_2Cl_2 (3×50 ml). The organic layers were dried over sodium sulphate, filtered and concentrated. The crude was purified by flash column chromatography (ethyl acetate/hexane in gradient from 1:5 to 1:3) to obtain compound 31 (730 mg, 90%) as a pale cream yellow solid.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.72 (m, 2H), 7.56 (m, 2H), 7.37 (m, 2H), 7.30 (m, 2H), 6.65 (s, 1H), 5.89 (s, 1H), 5.77 (s, 1H), 5.74 (s, 1H), 5.36 (d, J=5.9 Hz, 1H), 5.32 (d, J=5.9 Hz, 1H), 5.20 (d, J=9.0, 1H), 4.75 (d, J=12.0 Hz, 1H), 4.73 (m, 1H), 4.48 (d, J=11.9 Hz, 1H), 4.08 (m, 4H), 3.89 (m, 1H), 3.86, (t, J=6.2 Hz, 1H), 3.70 (s, 3H), 3.69 (s, 3H), 3.38 (m, 1H), 3.25 (m, 1H), 3.02-2.89 (m, 4H), 2.67 (s, 1H), 2.61 (s, 1H), 2.51 (dd, J=14.3 Hz, J=4.5 Hz, 1H), 2.29 (s, 3H), 2.23 (s, 3H), 1.95 (s, 3H), 1.83 (m, 1H).

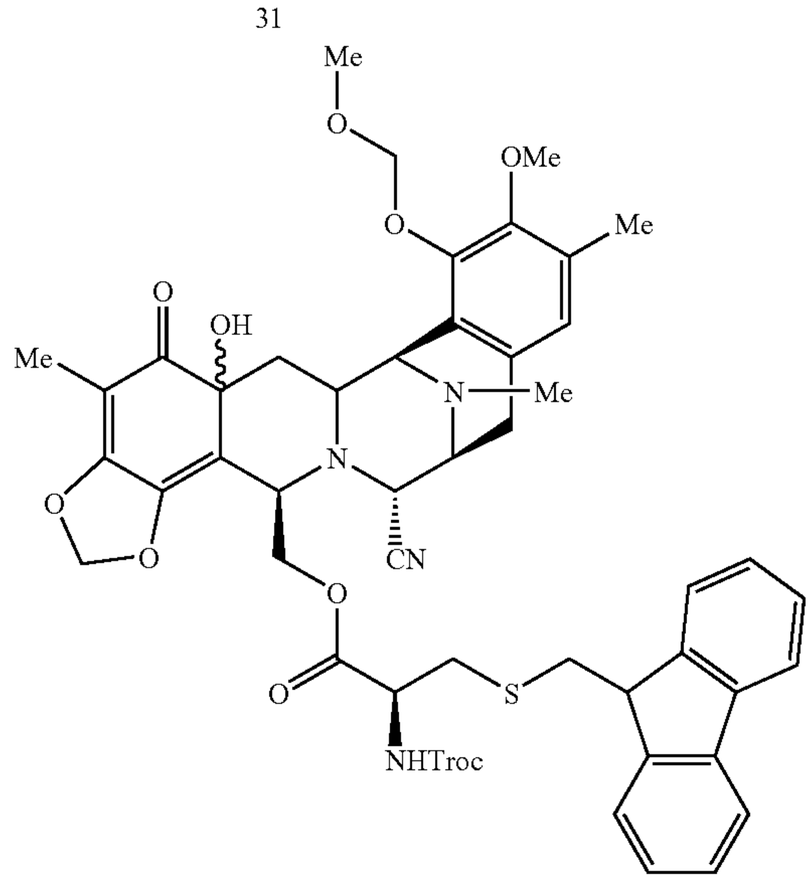
^{13}C-NMR (75 MHz, $CDCl_3$): δ 168.2, 152.5, 148.1, 146.2, 144.4, 144.3, 143.3, 139.6, 134.6, 129.7, 129.6, 126.2, 125.6, 123.4, 123.3, 121.6, 118.5, 116.3, 110.7, 110.2, 105.1, 99.4, 98.5, 75.2, 73.3, 61.7, 58.4, 57.9, 56.3, 56.1, 55.1, 54.7, 53.9, 51.9, 45.2, 40.1, 35.6, 33.3, 24.8, 23.3, 14.5, 7.3.

ESI-MS m/z: Calcd. for $C_{48}H_{49}Cl_3N_4O_{10}S$: 980.3. Found $(M+H)^+$: 981.2

Example 16



31



32

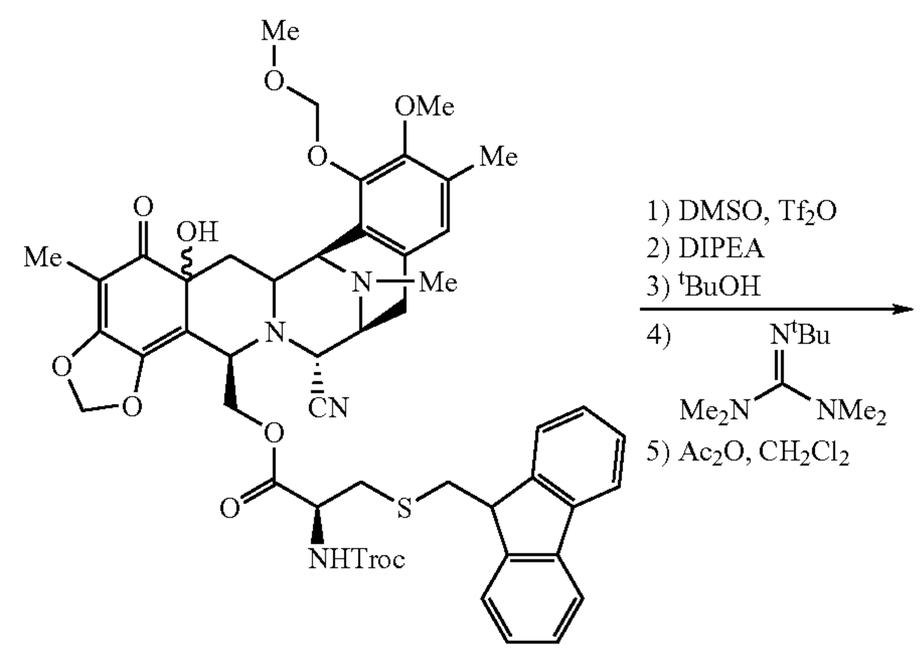
To a solution of 31 (310 mg, 0.32 mmol), in anhydrous CH_2Cl_2 (15 ml) at −10° C. was added a solution of benzeneseleninic anhydride 70% (165 mg, 0.32 mmol), in anhydrous CH_2Cl_2 (7 ml), via cannula, keeping the temperature at −10° C. The reaction mixture was stirred at −10° C. for 5 min. A saturated solution of sodium bicarbonate (30 ml) was added at this temperature. The aqueous layer was washed with more CH_2Cl_2 (40 ml). The organic layers were dried over sodium sulphate, filtered and concentrated. The crude was purified by flash column chromatography (ethyl acetate/hexane in gradient from 1:5 to 1:1) to obtain 32 (287 mg, 91%, HPLC: 91.3%) as a pale cream yellow solid and as a mixture of two isomers (65:35) which were used in the next step.

^{1}H-NMR (300 MHz, $CDCl_3$): 8 (Mixture of isomers) 7.76 (m, 4H), 7.65 (m, 4H), 7.39 (m, 4H), 7.29 (m, 4H), 6.62 (s, 1H), 6.55 (s, 1H), 5.79-5.63 (m, 6H), 5.09 (s, 1H), 5.02 (d, J=6.0 Hz, 1H), 4.99 (d, J=6.0 Hz, 1H), 4.80-4.63 (m, 6H), 4.60 (m, 1H), 4.50 (m, 1H), 4.38 (d, J=12.8 Hz, J=7.5 Hz, 1H), 4.27 (dd, J=12.8 Hz, J=7.5 Hz, 1H), 4.16-3.90 (m, 10H), 3.84 (s, 3H), 3.62 (s, 3H), 3.50 (s, 3H), 3.49 (s, 3H), 3.33-2.83 (m, 14H), 2.45-2.18 (m, 2H), 2.21 (s, 6H), 2.17 (s, 6H), 1.77 (s, 6H), 1.67 (m, 2H).

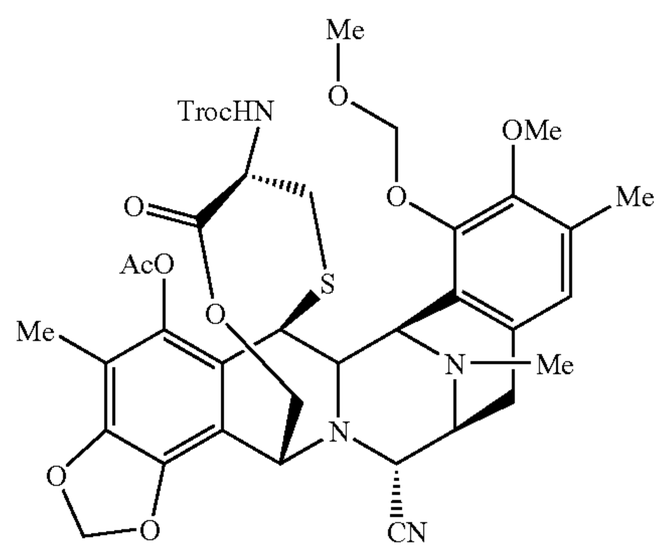
^{13}C-NMR (75 MHz, $CDCl_3$): δ (Mixture of isomers) 168.6, 168.4, 158.6, 154.8, 152.8, 152.5, 147.3, 147.2, 146.8, 144.1, 144.0, 140.8, 139.7, 137.1, 129.8, 129.3, 128.4, 128.7, 126.5, 125.5, 123.7, 123.6, 123.5, 123.4, 122.2, 121.3, 118.3, 115.8, 115.5, 110.2, 106.9, 103.5, 103.2, 100.1, 99.6, 97.9, 97.7, 93.8, 73.4, 70.9, 69.2, 64.9, 62.5, 59.3, 58.9, 58.4, 56.7, 56.3, 56.2, 55.4, 55.2, 55.1, 54.9, 54.7, 54.3, 54.1, 53.8, 52.8, 45.5, 40.5, 40.0, 39.8, 35.8, 35.5, 33.9, 33.7, 30.1, 28.8, 24.2, 24.1, 21.2, 14.5, 14.4, 12.7, 6.0, 5.7.

ESI-MS m/z: Calcd. for $C_{48}H_{49}Cl_3N_4O_{11}S$: 996.3. Found $(M+H)^+$: 997.2

Example 17



32



33

The reaction flask was flamed twice, purged vacuum/Argon several times and kept under Argon atmosphere for the reaction. To a solution of DMSO (39.1 ml, 0.55 mmol, 5 equivalents.) in anhydrous CH_2Cl_2 (4.5 ml) was dropwise added triflic anhydride (37.3 ml, 0.22 mmol, 2 equivalents.) at −78° C. The reaction mixture was stirred at −78° C. for 20 minutes, then a solution of 32 (110 mg, 0.11 mmol, HPLC: 91.3%) in anhydrous CH_2Cl_2 (1 ml, for the main addition and 0.5 ml for wash) at −78° C. was added, via cannula. During the addition the temperature was kept at −78° C. in both flasks and the colour changed from yellow to brown. The reaction mixture was stirred at −40° C. for 35 minutes. During this period of time the solution was turned from yellow to dark green. After this time, $^iPr_2NEt$ (153 ml, 0.88 mmol, 8 equivalents.) was dropwise added and the reaction mixture was kept at 0° C. for 45 minutes, the colour of the solution turned to

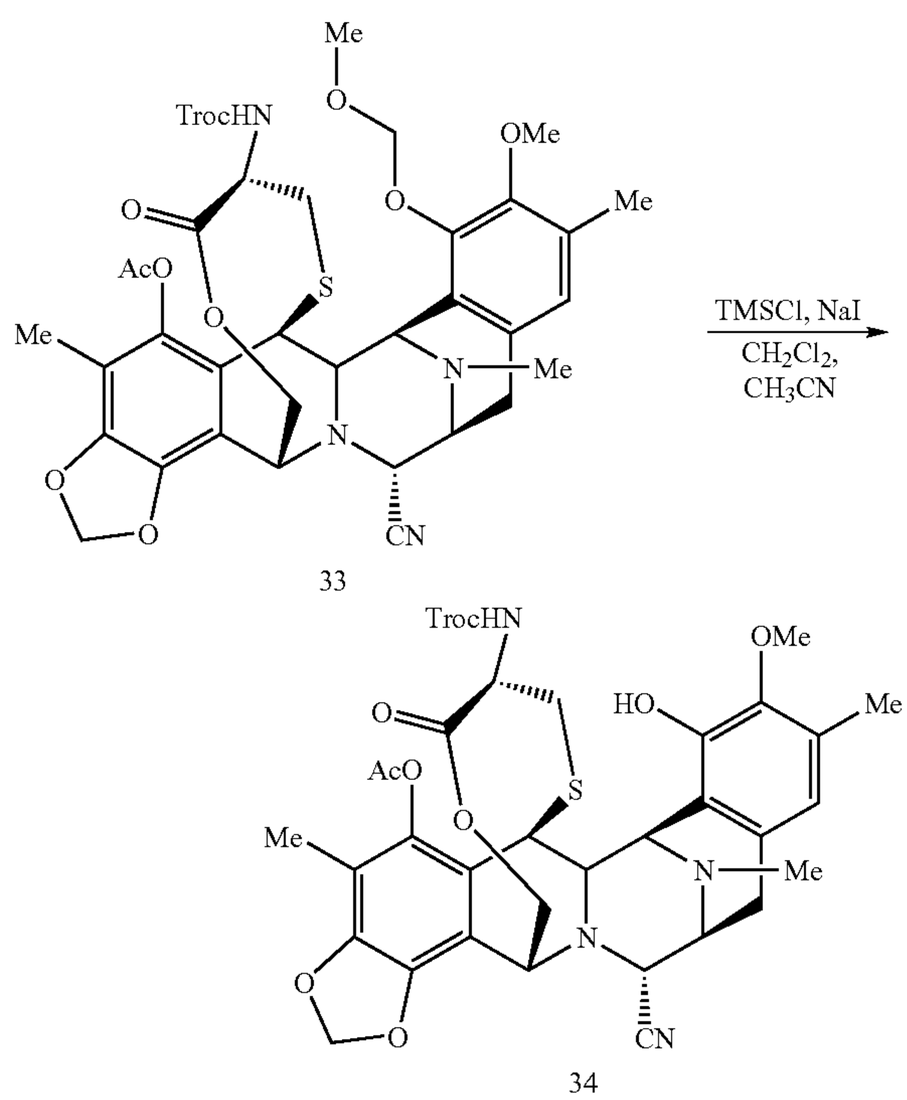
brown during this time. Then t-butanol (41.6 ml, 0.44 mmol, 4 equivalents.) and 2-^{t}Butyl-1,1,3,3-tetramethylguanidine (132.8 ml, 0.77 mmol, 7 equivalents.) were dropwise added and the reaction mixture was stirred at 23° C. for 40 minutes. After this time, acetic anhydride (104.3 ml, 1.10 mmol, 10 equivalents.) was dropwise added and the reaction mixture was kept at 23° C. for 1 hour more. Then the reaction mixture was diluted with CH_2Cl_2 (20 ml) and washed with aqueous saturated solution of NH_4Cl (50 ml), sodium bicarbonate (50 ml), and sodium chloride (50 ml). The combined organic layers were dried over sodium sulphate, filtered and concentrated. The residue was purified by flash column chromatography (eluent:ethyl acetate/hexane gradient from 1:3 to 1:2) to afford compound 33 (54 mg, 58%) as a pale yellow solid.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 6.85 (s, 1H), 6.09 (s, 1H), 5.99 (s, 1H), 5.20 (d, J=5.8 Hz, 1H), 5.14 (d, J=5.3 Hz, 1H), 5.03 (m, 1H), 4.82 (d, J=12.2, 1H), 4.63 (d, J=12.0 Hz, 1H), 4.52 (m, 1H), 4.35-4.17 (m, 4H), 3.76 (s, 3H), 3.56 (s, 3H), 3.45 (m, 2H), 2.91 (m, 2H), 2.32 (s, 3H), 2.28 (s, 3H), 2.21 (s, 3H), 2.12 (m, 2H), 2.03 (s, 3H).

^{13}C-NMR (75 MHz, $CDCl_3$): δ 168.5, 167.2, 152.7, 148.1, 147.1, 144.5, 139.6, 139.1, 130.5, 129.0, 123.7, 123.5, 123.3, 118.8, 116.5, 112.1, 100.6, 97.8, 73.3, 60.5, 59.4, 59.2, 58.3, 57.6, 57.4, 56.1, 53.3, 53.1, 40.6, 40.0, 31.0, 22.2, 18.9, 14.4, 8.1.

ESI-MS m/z: Calcd. for $C_{36}H_{39}Cl_3N_4O_{11}S$: 842.1. Found $(M+H)^+$: 843.1

Example 18

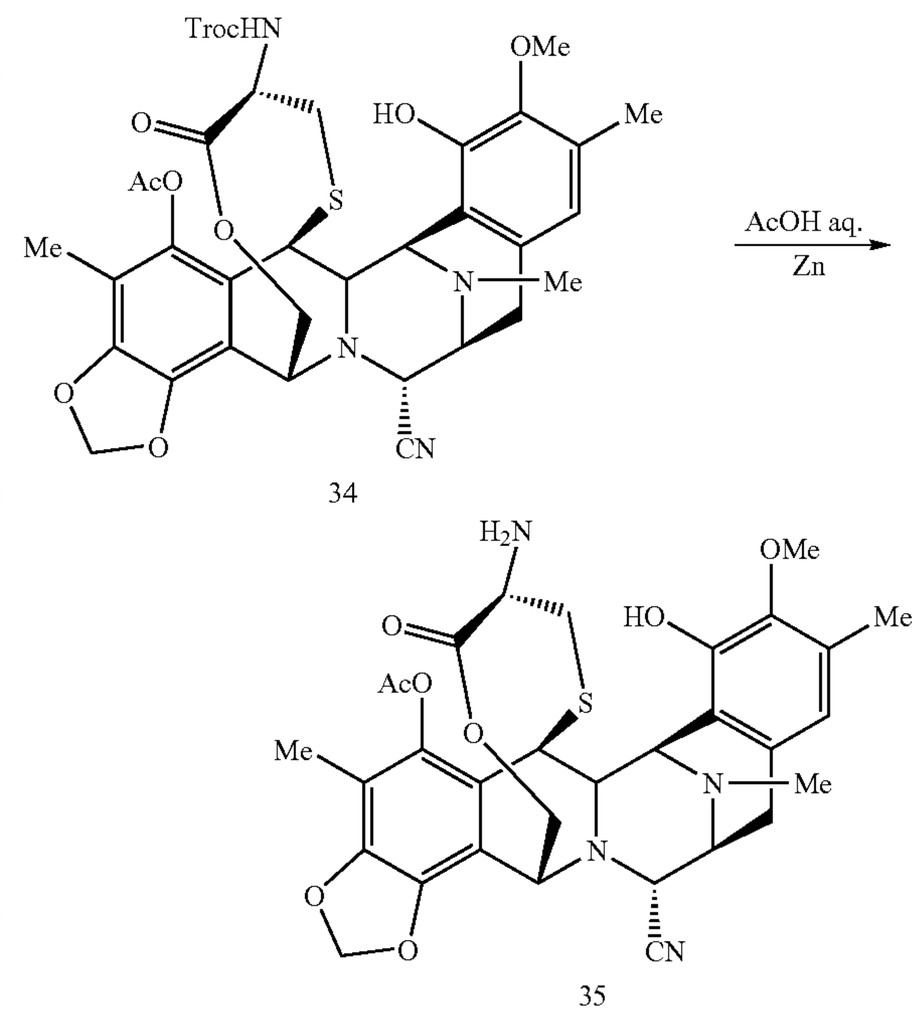


To a solution of 33 (12 mg, 0.014 mmol) in dry dichloromethane (1.2 ml) and HPLC grade acetonitrile (1.2 ml) was added at 23° C. sodium iodide (21 mg, 0.14 mmol) and freshly distilled (over calcium hydride at atmospheric pressure) trimethylsilyl chloride (15.4 mg, 0.14 mmol). The reaction mixture turned to orange colour. After 15 min the solution was diluted with dichloromethane (10 ml) and was washed with a freshly aqueous saturated solution of $Na_2S_2O_4$ (3×10 ml). The organic layer was dried over sodium sulphate, filtered and concentrated. It was obtained compound 34 (13 mg, quantitative) as pale yellow solid which was used without further purification.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 6.85 (s, 1H), 6.09 (s, 1H), 5.99 (s, 1H), 5.27 (d, J=5.8 Hz, 1H), 5.14 (d, J=5.3 Hz, 1H), 5.03 (d, J=11.9 Hz, 1H), 4.82 (d, J=12.2, 1H), 4.63 (d, J=13.0 Hz, 1H), 4.52 (m, 1H), 4.34 (m, 1H), 4.27 (bs, 1H), 4.18 (m, 2H), 3.76 (s, 3H), 3.56 (s, 3H), 3.44 (m, 1H), 3.42 (m, 1H), 2.91 (m, 2H), 2.32 (s, 3H), 2.28 (s, 3H), 2.21 (s, 3H), 2.03 (s, 3H).

ESI-MS m/z: Calcd. for $C_{34}H_{35}N_4O_{10}S$: 798.1. Found $(M+H)^+$: 799.1

Example 19



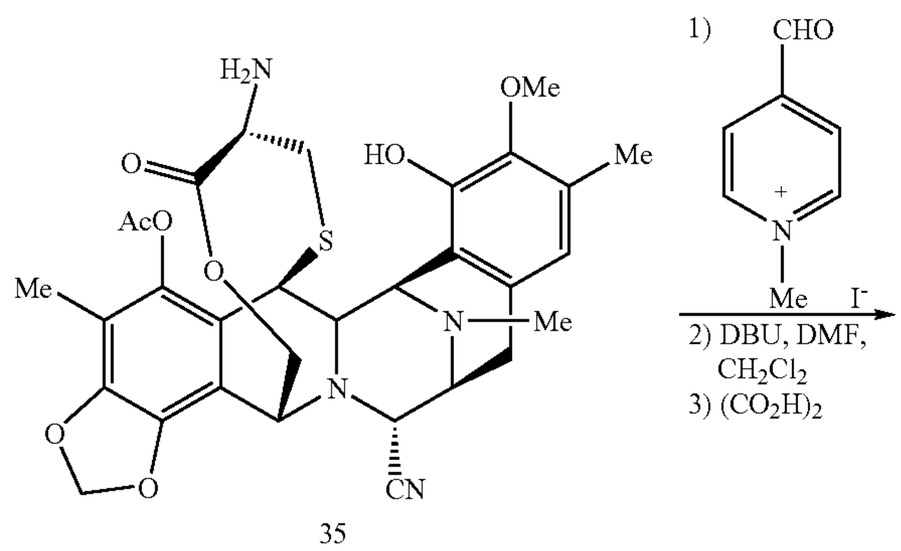
To a solution of 34 (13 mg, 0.016 mmol) in a mixture of acetic acid/H_2O (90:10, 1 ml) was added powder Zinc (5.3 mg, 0.081 mmol) at 23° C. The reaction mixture was heated at 70° C. for 6 h. After this time, was cooled to 23° C., diluted with CH_2Cl_2 (20 ml) and washed with aqueous saturated solution of sodium bicarbonate (15 ml) and aqueous solution of Et_3N (15 ml). The organic layer was dried over sodium sulphate, filtered and concentrated. The residue was purified by flash column chromatography with Silica-NH_2 (eluent: ethyl acetate/hexane gradient from 0:100 to 50:50) to afford compound 35 (6.8 mg, 77% for two steps) as a pale yellow solid.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 6.51 (s, 1H), 6.03 (dd, J=1.3 Hz, J=26.5 Hz, 2H), 5.75 (bs, 1H), 5.02 (d, J=11.6 Hz, 1H), 4.52 (m, 1H), 4.25 (m, 2H), 4.18 (d, J=2.5 Hz, 1H), 4.12 (dd, J=1.9 Hz, J=11.5 Hz, 1H), 3.77 (s, 3H), 3.40 (m, 2H), 3.26 (t, J=6.4 Hz, 1H), 2.88 (m, 2H), 2.30-2.10 (m, 2H), 2.30 (s, 3H), 2.28 (s, 3H), 2.18 (s, 3H), 2.02 (s, 3H).

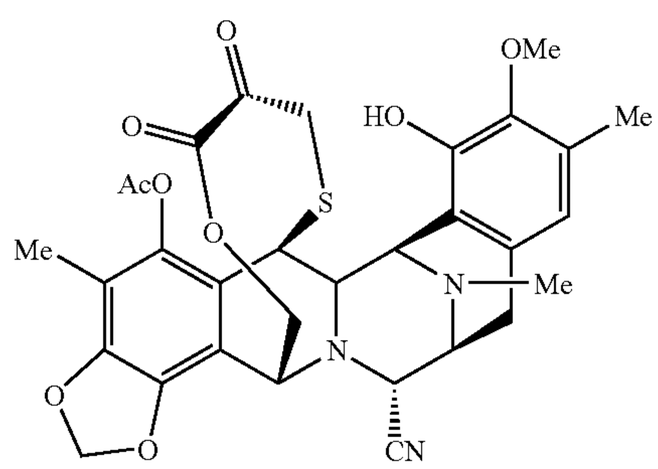
^{13}C-NMR (75 MHz, $CDCl_3$): δ 174.1, 168.4, 147.8, 145.4, 142.9, 140.8, 140.1, 131.7, 130.2, 129.1, 128.3, 120.4, 118.3, 117.9, 113.8, 111.7, 101.7, 61.2, 59.8, 59.2, 58.9, 54.4, 53.8, 54.4, 41.3, 41.5, 34.1, 23.6, 20.3, 15.5, 9.4.

ESI-MS m/z: Calcd. for $C_{31}H_{34}N_4O_8S$: 622.7. Found $(M+H)^+$: 623.2.

Example 20



35



36

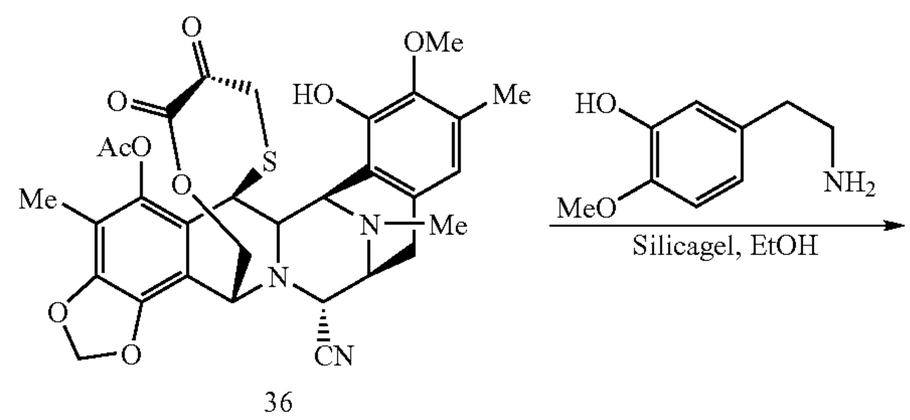
A solution of N-methylpyridine-4-carboxaldehyde iodide (378 mg, 1.5 mmol) in anhydrous DMF (5.8 mL) was treated with anhydrous toluene (2×10 mL) to eliminate the amount of water by azeotropic removal of the toluene. A solution of 35 (134 mg, 0.21 mmol), previously treated with anhydrous toluene (2×10 mL), in anhydrous CH_2Cl_2 (distilled over CaH_2, 7.2 mL) was added, via cannula, at 23° C. to this orange solution. The reaction mixture was stirred at 23° C. for 4 hours. After this time DBU (32.2 □L, 0.21 mmol) was dropwise added at 23° C. and it was stirred for 15 minutes at 23° C. A freshly aqueous saturated solution of oxalic acid (5.8 mL) was added to the reaction mixture and was stirred for 30 minutes at 23° C. Then the reaction mixture was cooled to 0° C. and $NaHCO_3$ was portionwise added followed by addittion of aqueous saturated solution of $NaHCO_3$. The mixture was extracted with Et_2O. K_2CO_3 was added to the aqueous layer and it was extrated with Et_2O. The combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. The crude was purified by flash column chromatography (AcOEt/hexane from 1/3 to 1/1) to afford compound 36 (77 mg, 57%) as pale yellow solid.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 6.48 (s, 1H), 6.11 (d, J=1.3 Hz, 1H), 6.02 (d, J=1.3 Hz, 1H), 5.70 (bs, 1H), 5.09 (d, J=11.3 Hz, 1H), 4.66 (bs, 1H), 4.39 (m, 1H), 4.27 (d, J=5.6 Hz, 1H), 4.21 (d, J=10.5 Hz, 1H), 4.16 (d, J=2.6 Hz, 1H), 3.76 (s, 3H), 3.54 (d, J=5.1 Hz, 1H), 3.42 (d, 3=8.5 Hz, 1H), 2.88-2.54 (m, 3H), 2.32 (s, 3H), 2.24 (s, 3H), 2.14 (s, 3H), 2.04 (s, 3H).

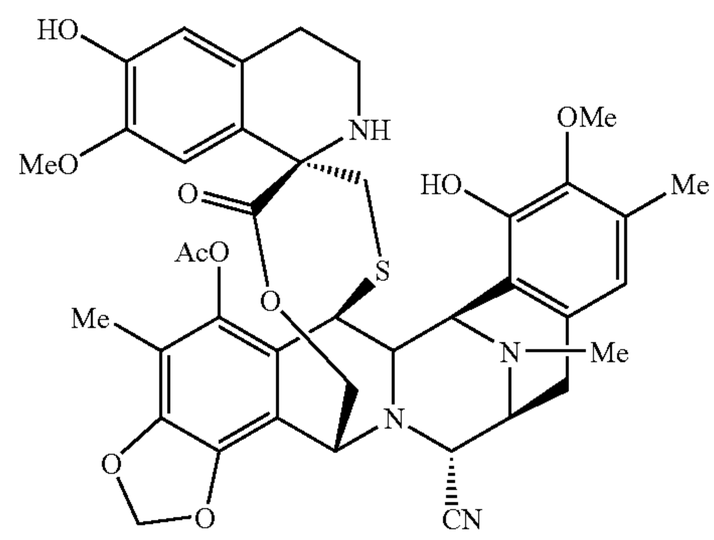
^{13}C-NMR (75 MHz, $CDCl_3$): δ 186.7, 168.5, 160.5, 147.1, 146.4, 142.9, 141.6, 140.7, 130.4, 129.8, 121.7 (2C), 120.0, 117.8, 117.1, 113.5, 102.2, 61.7, 61.4, 60.3, 59.8, 58.9, 54.6, 41.6, 36.9, 29.7, 24.1, 20.3, 15.8, 14.1, 9.6.

ESI-MS m/z: Calcd. for $C_{31}H_{31}N_3O_9S$: 621.7. Found $(M+H)^+$: 622.2

Example 21



36



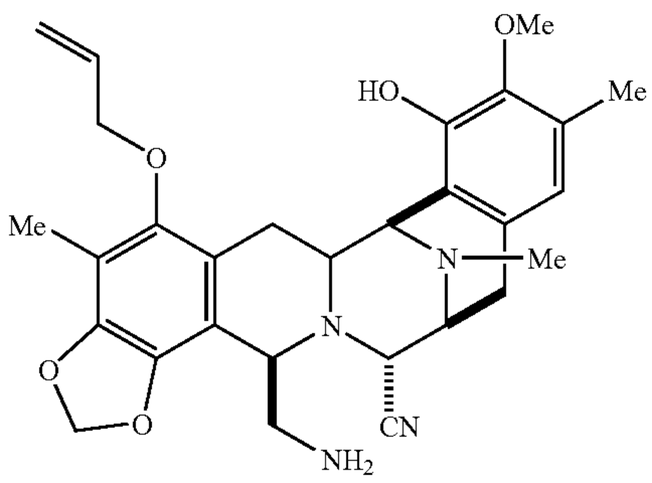
Et-770

To a solution of 36 (49 mg, 0.08 mmol) and 2-[3-hydroxy-4-methoxyphenyl]ethylamine (46.2 mg, 0.27 mmol) in ethanol (2.5 ml) was added silica gel (105 mg) at 23° C. The reaction mixture was stirred at 23° C. for 14 h. It was diluted with hexane and poured into a column of chromatography (ethyl acetate/hexane from 1/3 to 1/1) to afford Et-770 (55 mg, 90%) as a pale yellow solid.

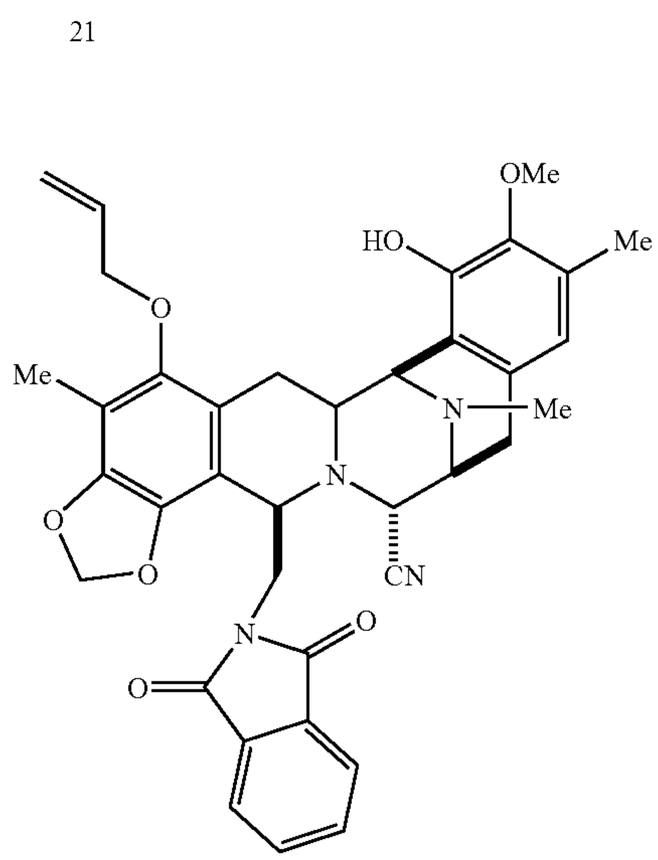
^{1}H-NMR (300 MHz, $CDCl_3$): δ 6.60 (s, 1H), 6.47 (s, 1H), 6.45 (s, 1H), 6.05 (s, 1H), 5.98 (s, 1H), 5.02 (d, J=111.4 Hz, 1H), 4.57 (bs, 1H), 4.32 (bs, 1H), 4.28 (d, J=5.3 Hz, 1H), 4.18 (d, J=2.5 Hz, 1H), 4.12 (dd, J=2.1 Hz, J=11.5 Hz, 1H), 3.78 (s, 3H), 3.62 (s, 3H), 3.50 (d, J=5.0 Hz, 1H), 3.42 (m, 1H), 3.10 (ddd, J_1=4.0 Hz, J_2=10.0 Hz, J_3=11.0 Hz, 1H), 2.94 (m, 2H), 2.79 (m, 1H), 2.61 (m, 1H), 2.47 (m, 1H), 2.35 (m, 1H), 2.32 (s, 3H), 2.27 (s, 3H), 2.20 (s, 3H), 2.09 (m, 1H), 2.04 (s, 3H).

ESI-MS m/z: Calcd. for $C_{40}H_{42}N_4O_{10}S$: 770.7. Found $(M+H)^+$: 771.2

Example 22



21



27

To a solution of 21 (22 mg, 0.042 mmol) in CH_2Cl_2 (0.8 ml) was added phthalic anhydride (6.44 mg, 0.042 mmol) and the reaction mixture was stirred for 2 h at 23° C. Then, carbonyldiimidazole (1 mg, 0.006 mmol) was added and the mixture was stirred at 23° C. for 7 h. Then, carbonyldiimidazole (5.86 mg, 0.035 ml) was added and the reaction was stirred at 23° C. for an additional 17 h. The solution was diluted with CH_2Cl_2 (15 ml) and washed with 0.1 N HCl (15 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, hexane:ethyl acetate 2:1) to afford 27 (26.4 mg, 96%) as a white solid.

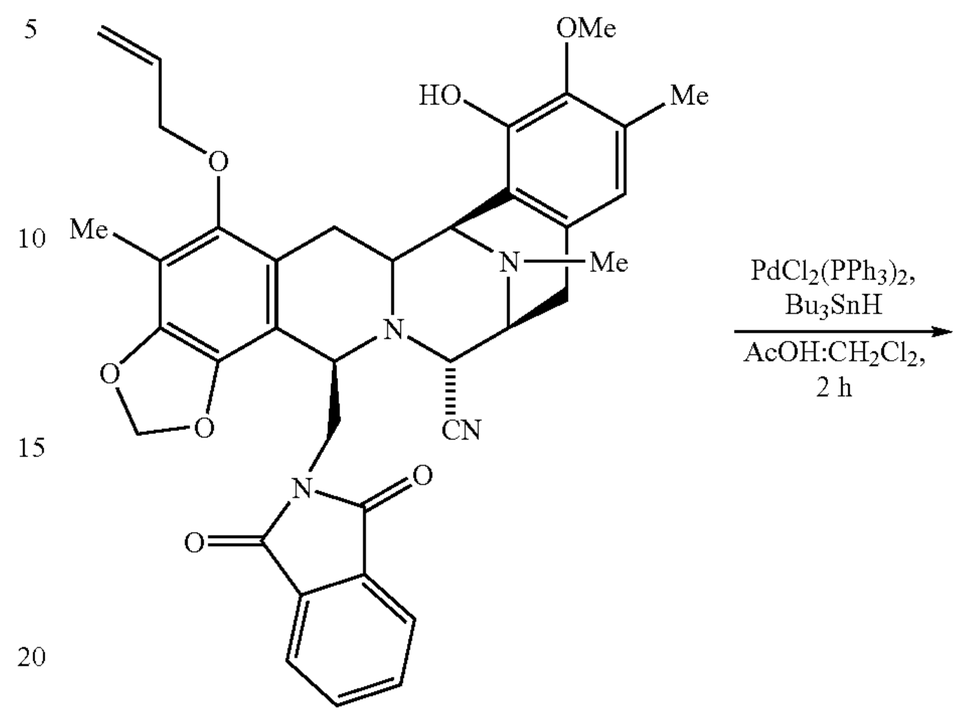
Rf: 0.58 (ethyl acetate).

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.73-7.64 (m, 4H), 6.40 (s, 1H), 6.12-6.01 (m, 1H), 5.63 (s, 1H), 5.58 (d, J=1.5 Hz, 1H), 5.37 (dd, J_1=1.8 Hz, J_2=17.4 Hz), 5.23 (dd, J_1=1.8 Hz, J_2=10.5 Hz, 1H), 5.12 (d, J=1.5 Hz, 1H), 4.22-4.15 (m, 3H), 4.08 (d, J=1.8 Hz, 1H), 3.68 (s, 3H), 3.59-3.55 (m 2H), 3.35 (d, J=8.1 Hz, 1H), 3.27-3.16 (m, 2H), 3.05 (dd, J_1=8.1 Hz, J_2=18.3 Hz, 1H), 2.64 (d, J=18.0 Hz, 1H), 2.30 (s, 3H), 2.24 (s, 3H), 2.09 (s, 3H), 1.80 (dd, J_1=11.4 Hz, J_2=15 Hz, 1H);

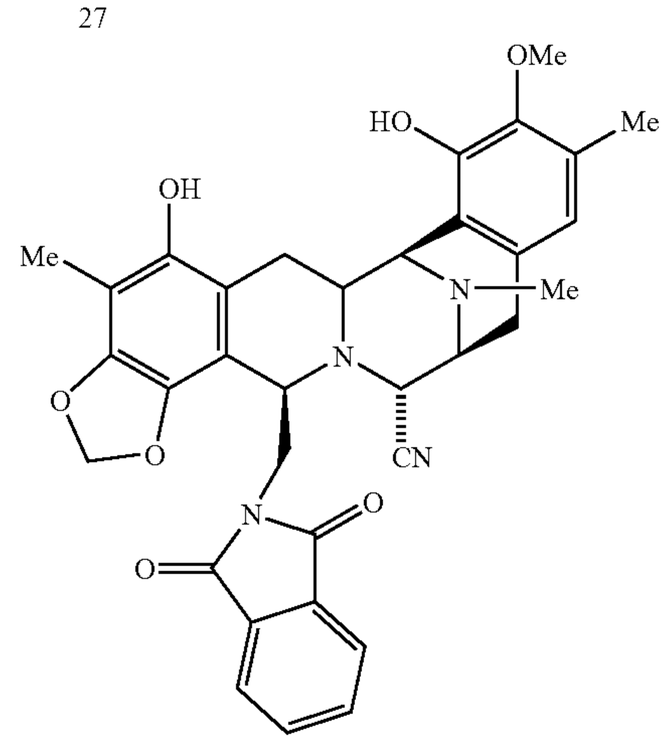
^{13}C NMR (75 MHz, $CDCl_3$): δ 167.7, 148.9, 146.4, 144.2, 142.6, 139.5, 134.0, 133.5, 132.0, 131.0, 128.3, 123.0, 121.3, 120.9, 118.1, 117.5, 116.8, 113.6, 112.4, 100.8, 74.5, 60.6, 60.5, 57.7, 56.6, 55.6, 55.5, 42.3, 41.7, 26.6, 25.5, 15.9, 9.46.

ESI-MS m/z: Calcd. for $C_{37}H_{35}N_4O_7$: 648.79. Found $(M+H)^+$: 649.3.

Example 23



27



28

To a solution of 27 (26 mg, 0.041 mmol) in CH_2Cl_2 (11 ml), acetic acid (11 ml), $(PPh_3)_2PdCl_2$ (2.36 mg) and Bu_3SnH (28 ml, 0.10 mmol) were added at 23° C. After stirring at that temperature for 2 h the reaction was poured into a pad of flash column (SiO_2, gradient Hex to hexane:ethyl acetate 2:1) to afford 28 (24.7 mg, 99%) as a white solid.

Rf: 0.33 (hexane:ethyl acetate 2:1).

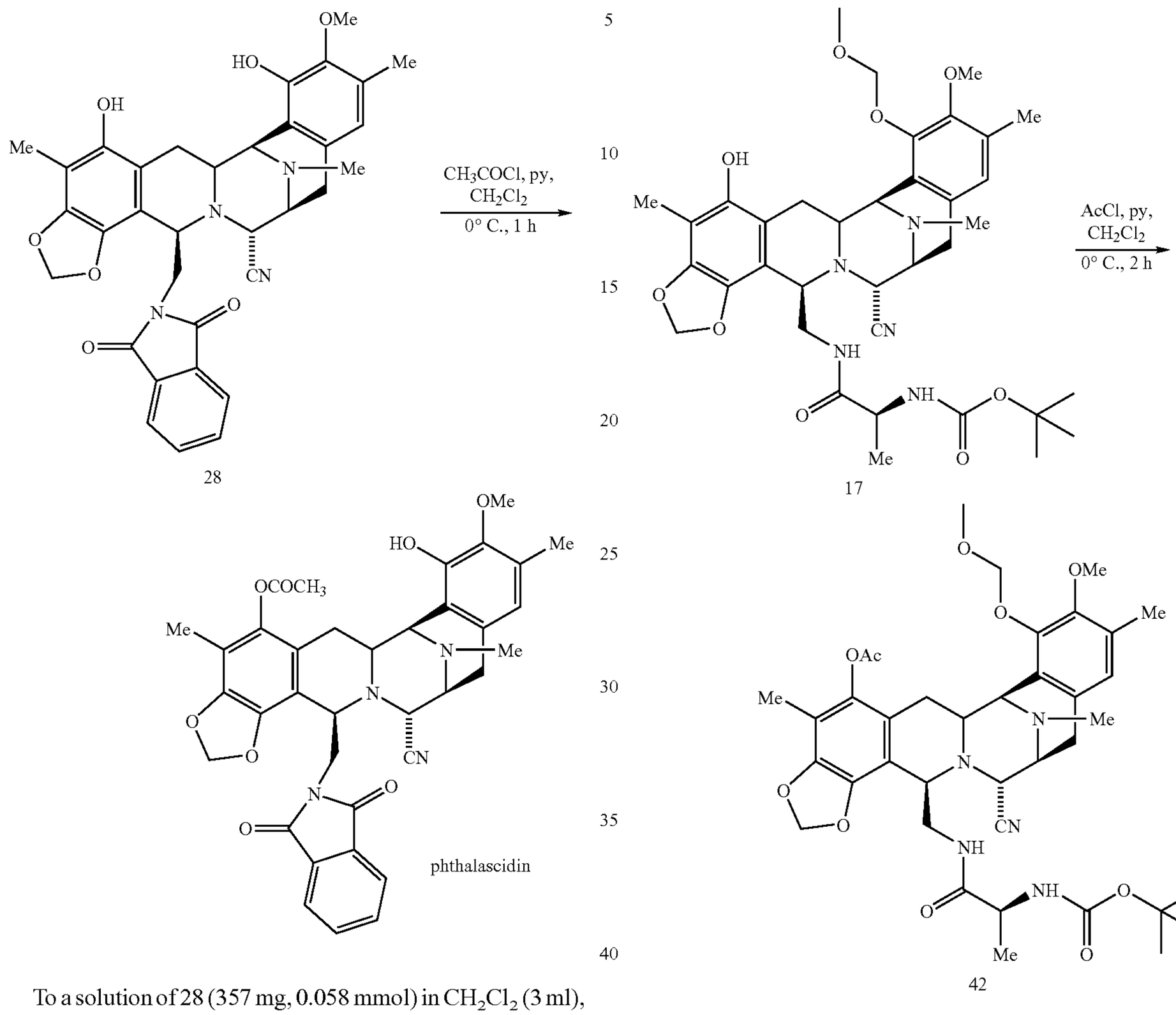
^{1}H NMR (300 MHz, $CDCl_3$): δ 7.75-7.70 (m, 2H), 7.69-7.65 (m, 2H), 6.39 (s, 1H), 5.82 (bs, 1H), 5.50 (d, J=1.5 Hz, 1H), 5.0 (d, J=1.5 Hz, 1H), 4.45 (bs, 1H), 4.23-4.19 (m, 2H), 4.10-4.09 (m, 1H), 3.73 (s, 3H), 3.60-3.48 (m, 2H), 3.36-3.33 (m, 1H), 3.26-3.20 (m, 1H), 3.14-3.08 (m, 1H), 3.98 (d, J=14.4 Hz, 1H), 2.61 (d, J=18.3 Hz, 1H), 2.30 (s, 3H), 2.23 (s, 3H), 2.06 (s, 3H), 1.85 (dd, J_1=12 Hz, J_2=15.3 Hz);

^{13}C NMR (75 MHz, $CDCl_3$): δ 167.8, 146.4, 145.1, 143.9, 142.7, 137.1, 133.5, 131.9, 130.8, 128.4, 122.9, 120.8, 118.0, 116.8, 114.0, 113.4, 106.4, 100.4, 60.6, 60.5, 57.8, 56.6, 55.5, 55.2, 42.6, 41.5, 25.6, 25.5, 15.8, 8.9.

ESI-MS m/z: Calcd. for $C_{34}H_{32}N_4O_7$: 608.6. Found $(M+H)^+$: 609.2.

Example 24

Example 25



To a solution of 28 (357 mg, 0.058 mmol) in CH_2Cl_2 (3 ml), acetyl chloride (41.58 ml, 0.58 mmol) and pyridine (47.3 ml, 0.58 ml) were added at 0° C. The reaction mixture was stirred for 1 h and then, the solution was diluted with CH_2Cl_2 (15 ml) and washed with 0.1 N HCl (15 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography (RP-18, $CH_3CN:H_2O$ 60:40) to afford phthalascidin (354 mg, 94%) as a white solid.

Rf: 0.37 ($CH_3CN:H_2O$ 7:3, RP-18).

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.72-7.68 (m, 2H), 7.67-7.63 (m, 2H), 6.38 (s, 1H), 5.69 (d, J=1.2 Hz, 1H), 5.64 (d, J=1.2 Hz, 1H), 5.30 (bs, 1H), 4.25-4.21 (m, 2H), 4.02 (d, J=2.1 Hz, 1H), 3.64-3.62 (m, 5H), 3.33 (d, J=8.4 Hz, 1H), 3.21-3.16 (m, 1H), 3.02 (dd, J_1=8.1 Hz, J_2=18 Hz, 1H), 2.76 (dd, J_1=1.8 Hz, J_2=15.6 Hz, 1H), 2.63 (d, J=17.7 Hz, 1H), 2.29 (s, 3H), 2.28 (s, 3H), 2.21 (s, 3H), 2.0 (s, 3H), 1.73 (dd, J_1=12.0 Hz, J_2=15.3 Hz, 1H))

^{13}C NMR (75 MHz, $CDCl_3$)): δ 168.5, 167.6, 146.2, 144.2, 142.5, 141.0, 140.5, 133.4, 131.8, 130.7, 128.2, 120.9, 120.8, 117.9, 116.4, 113.6, 101.1, 60.4, 60.0, 57.0, 56.3, 55.6, 55.4, 41.6, 41.5, 26.5, 25.2, 20.2, 15.7, 9.4.

ESI-MS m/z: Calcd. for $C_{36}H_{34}N_4O_8$: 650. Found $(M+H)^+$: 651.2.

To a solution of 17 (300 mg, 0.432 mmol) in CH_2Cl_2 (2 ml), acetyl chloride (30.7 ml, 0.432 mmol) and pyridine (34.9 ml, 0.432 mmol) were added at 0° C. The reaction mixture was stirred for 2 h at that temperature and then, the solution was diluted with CH_2Cl_2 (15 ml) and washed with 0.1 N HCl (15 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure to afford 42 (318 mg, 100%) as a white solid that was used in subsequent reactions with no further purification.

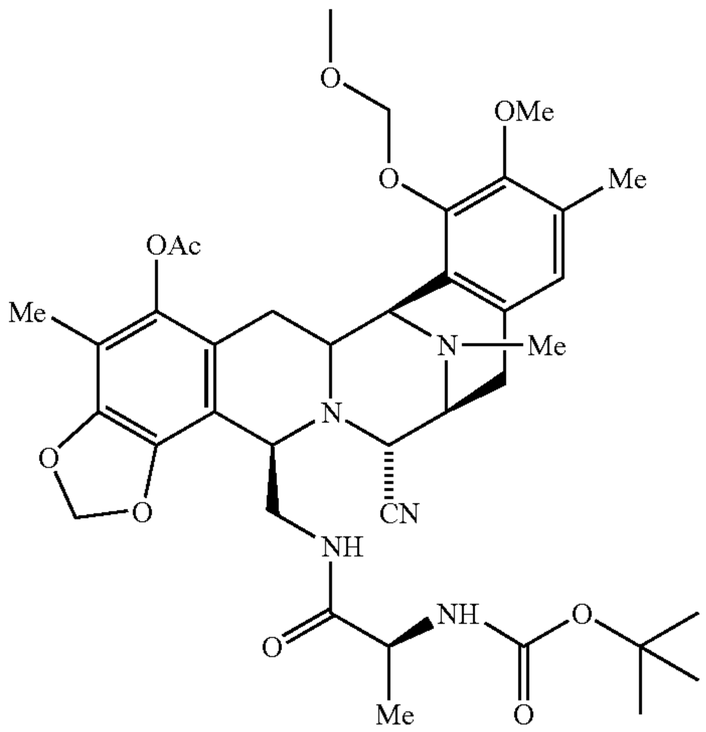
Rf: 0.5 (ethyl acetate:methanol 5:1).

^{1}H NMR (300 MHz, $CDCl_3$). δ 6.66 (s, 1H), 5.93 (d, J=1.2 Hz, 1H), 5.83 (d, J=1.2 Hz, 1H), 5.42 (t, J=6.6 Hz, 1H), 5.07 (d, J=5.7 Hz, 1H), 4.98 (d, J=5.7 Hz, 1H), 4.16 (d, J=1.8 Hz, 1H), 4.11 (d, J=2.7 Hz, 1H), 3.98 (bs, 1H), 3.73-3.61 (m, 2H), 3.64 (s, 3H), 3.52-3.48 (m, 1H), 3.50 (s, 3H), 3.33 (d, J=9.6 Hz, 1H), 3.17-3.14 (m, 1H), 2.97-2.87 (m, 1H), 2.75-2.70 (d, J=16.8 Hz, 1H), 2.26 (s, 6H), 2.16 (s, 3H), 1.96 (s, 3H), 1.70 (dd, J_1=11.7 Hz, J_2=15.6 Hz, 1H), 1.33 (s, 9H), 0.59 (d, J=6.0 Hz, 3H).

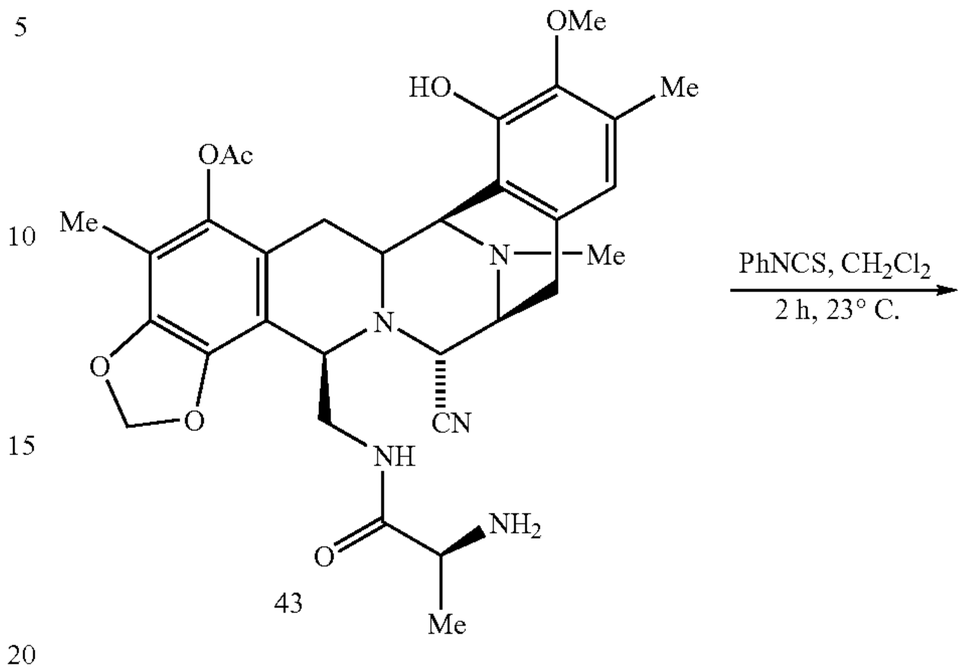
^{13}C NMR (75 MHz, $CDCl_3$)): δ 172.0, 168.3, 162.3, 148.2, 144.4, 140.4, 140.2, 130.9, 130.5, 125.3, 123.4, 120.8, 117.6, 112.7, 111.7, 101.4, 99.1, 79.2, 59.5, 58.8, 57.5, 57.4, 56.4, 55.5, 55.0, 41.3, 39.0, 28.2, 26.4, 24.6, 19.9, 18.4, 15.4, 9.1.

ESI-MS m/z: Calcd. for $C_{38}H_{49}N_5O_{10}$: 735.82. Found $(M+H)^+$: 736.3.

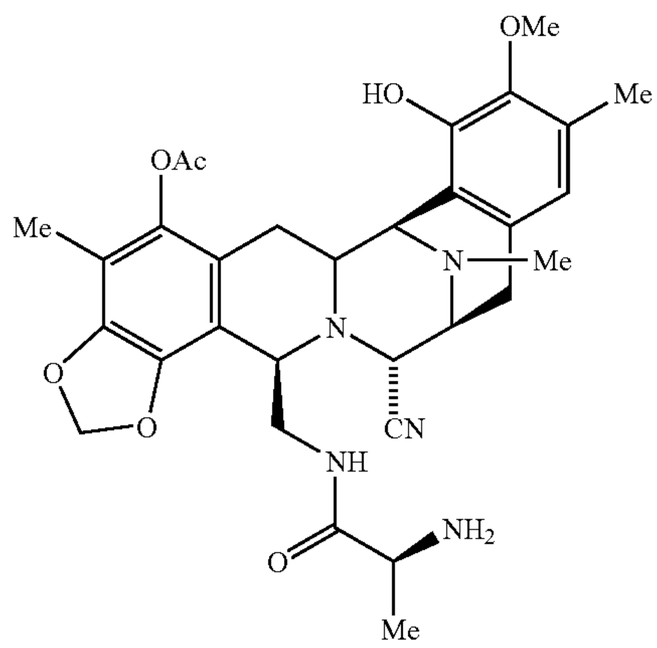
Example 26



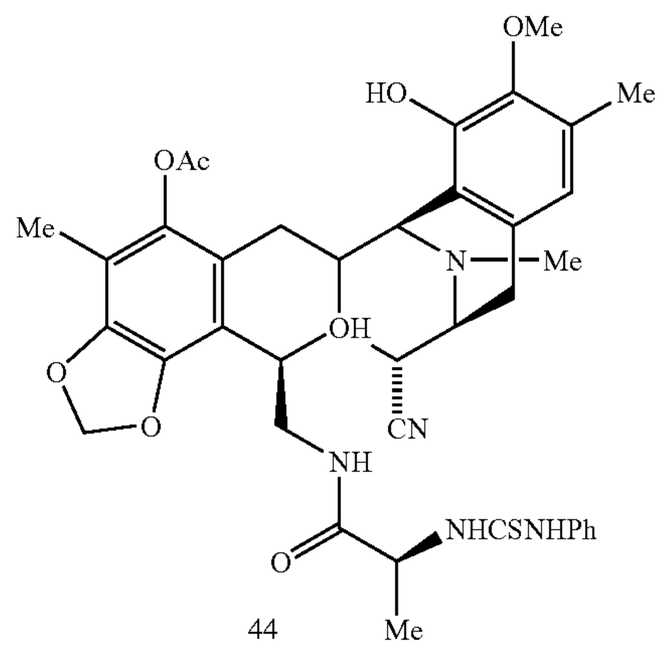
42



43



43



44

To a solution of 42 (318 mg, 0.432 mmol) in CH_2Cl_2 (2.16 mmol), trifluoroacetic acid (1.33 ml, 17.30 mmol) was added and the reaction mixture was stirred for 3.5 h at 23° C. The reaction was quenched at 0° C. with saturated aqueous sodium bicarbonate (60 ml) and extracted with CH_2Cl_2 (2×70 ml). The combined organic layers were dried (sodium sulphate) and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, ethyl acetate:methanol 20:1) to afford 43 (154 mg, 60%) as a white solid.

Rf: 0.22 (ethyl acetate:methanol 5:1).

^{1}H NMR (300 MHz, $CDCl_3$). δ 6.47 (s, 1H), 6.22 (bs, 1H), 5.95 (d, J=1.2 Hz, 1H), 5.88 (d, J=1.2 Hz, 1H), 4.08-4.06 (m, 2H), 4.01 (bs, 1H), 3.69 (s, 3H), 3.49 (d, J=3.6 Hz, 1H), 3.33 (d, J=8.1 Hz, 1H), 3.26-3.22 (m, 1H), 2.95 (dd, J_1=8.1 Hz, J_2=18 Hz, 1H), 2.80-2.76 (m, 2H), 2.58 (d, J=18 Hz, 1H), 2.29 (s, 3H), 2.27 (s, 3H), 2.21 (s, 3H), 1.96 (s, 3H), 1.77 (dd, J_1=12.3 Hz, J_2=15.6 Hz, 1H), 0.90 (d, J=6.9 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$)): δ 174.8, 169.0, 146.8, 144.4, 142.8, 140.5, 140.2, 131.1, 128.8, 120.8, 120.5, 117.1, 112.9, 111.6, 101.5, 60.3, 59.0, 56.5, 56.3, 55.6, 55.1, 50.2, 41.6, 39.5, 26.8, 26.3, 24.9, 20.2, 15.4, 9.2.

ESI-MS m/z: Calcd. for $C_{31}H_{37}N_5O_7$: 591.65. Found $(M+H)^+$: 592.3.

To a solution of 43 (154 mg, 0.26 mmol) in CH_2Cl_2 (1.3 ml), phenyl isothiocyanate (186 ml, 1.56 mmol) was added and the mixture was stirred at 23° C. for 2 h. The reaction was concentrated in vacuo and the residue was purified by flash column chromatography (SiO_2, gradient Hexane to hexane: ethyl acetate 1:1) to afford 44 (120 mg, 63%) as a white solid.

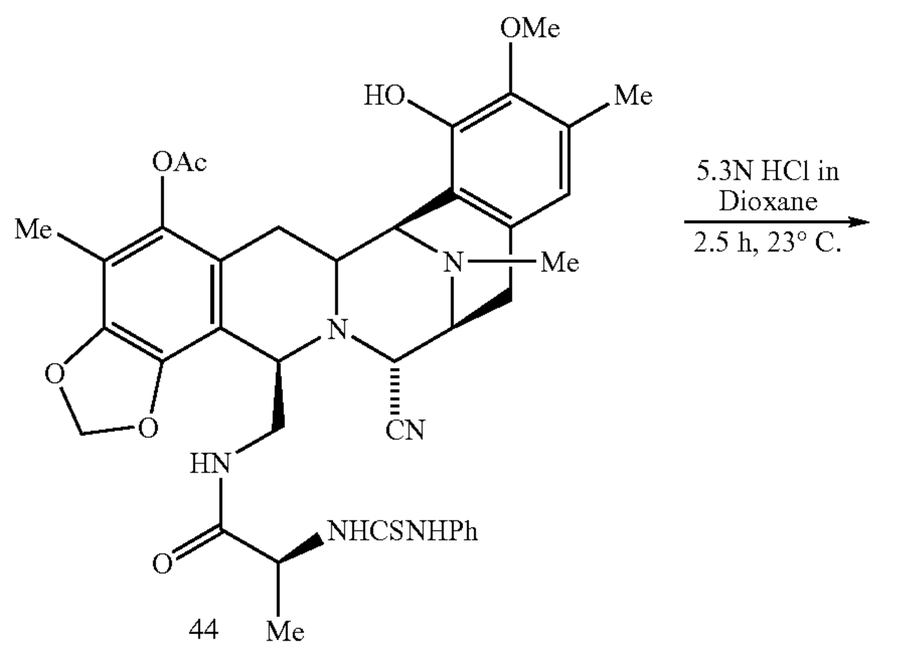
Rf: 0.41 (ethyl acetate:methanol 5:1).

^{1}H NMR (300 MHz, $CDCl_3$). δ 8.17 (s, 1H), 7.49-7.44 (m, 3H), 7.31-7.24 (m, 3H), 7.05 (d, J=6.9 Hz, 1H), 5.98 (d, J=1.2 Hz, 1H), 5.87 (d, J=1.2 Hz, 1H), 5.52 (bs, 1H), 4.54 (t, J=6.6 Hz, 1H), 4.15 (d, J=2.1 Hz, 1H), 4.03 (d, J=2.7 Hz, 2H), 3.80 (bs, 1H), 3.66 (s, 3H), 3.40 (bs, 1H), 3.32 (d, J=7.8 Hz, 1H), 3.16 (d, J=11.7 Hz, 1H), 2.82-2.61 (m, 3H), 2.29 (s, 3H), 2.20 (s, 3H), 2.01 (s, 3H), 1.99 (s, 3H), 1.80 (dd, J_1=12.0 Hz, J_2=15.9 Hz, 1H), 0.62 (d, J=6.0 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 178.5, 171.9, 168.7, 146.7, 144.5, 142.6, 140.6, 140.3, 136.3, 131.0, 129.9, 128.9, 126.7, 124.4, 120.9, 120.6, 117.7, 116.6, 112.7, 111.9, 101.4, 60.4, 58.7, 57.5, 56.1, 55.7, 55.1, 53.3, 41.4, 38.8, 26.3, 24.4, 20.2, 18.1, 15.3, 9.2.

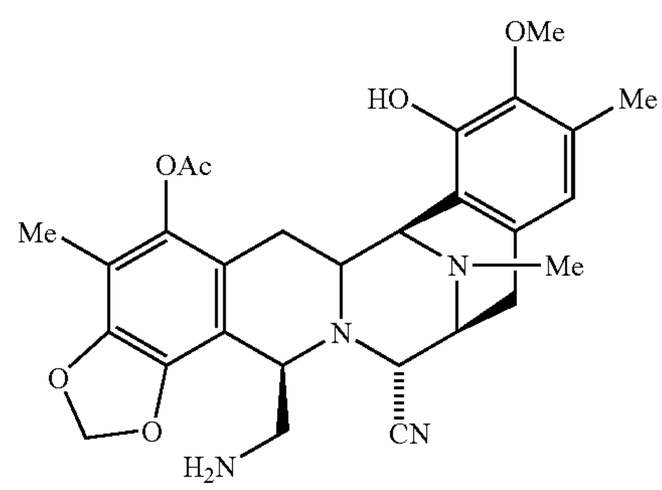
ESI-MS m/z: Calcd. for $C_{38}H_{42}N_6O_7S$: 726.3. Found $(M+H)^+$: 727.3.

Example 28



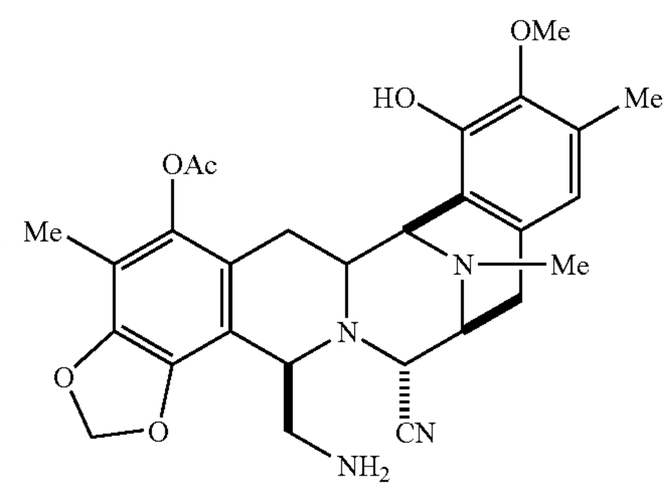
44

5.3N HCl in Dioxane
2.5 h, 23° C.



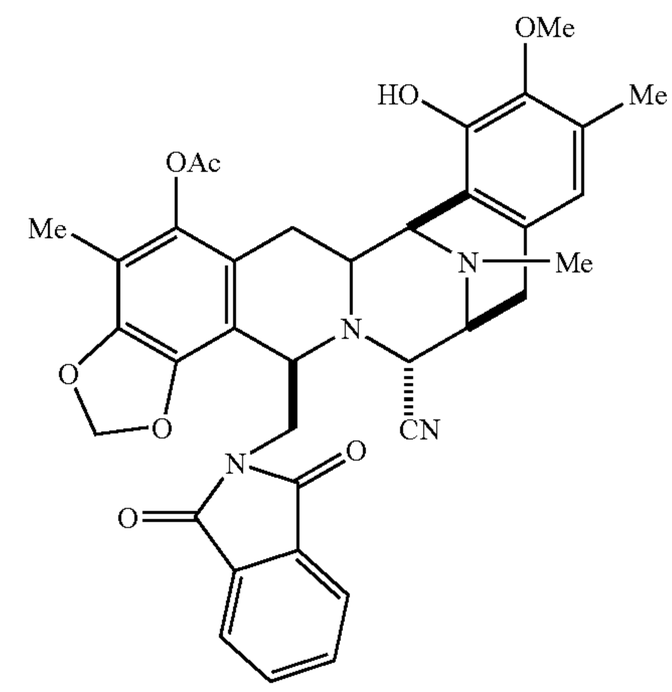
45

Example 29



45

Pht Anh.,
CH_2Cl_2, CDI
17h, 23° C.



Phtalascidin

To a solution of 44 (120 mg, 0.165 mmol) in dioxane (0.9 ml), 5.3N HCl/dioxane (1.8 ml) was added and the reaction was stirred at 23° C. for 2.5 h. Then, CH_2Cl_2 (10 ml) and H_2O (5 ml) were added to this reaction and the organic layer was decanted. The aqueous phase was basified with saturated aq sodium bicarbonate (20 ml) (pH=8) at 0° C. and then, extracted with CH_2Cl_2 (2×15 ml). The combined organic extracts were dried (sodium sulphate), and concentrated in vacuo to afford 45 (75 mg, 87%) as a white solid that was used in subsequent reactions with no further purification.

Rf: 0.23 (ethyl acetate:methanol 5:1).

^{1}H NMR (300 MHz, $CDCl_3$): δ 6.43 (s, 1H), 5.94 (d, J=1.2 Hz, 1H), 5.87 (d, J=1.2 Hz, 1H), 4.10 (d, J=2.1 Hz, 1H), 3.98 (d, J=2.4 Hz, 1H), 3.91 (bs, 1H), 3.69 (s, 3H), 3.34-3.25 (m, 2H), 3.05 (dd, J_1=1.8 Hz, J_2=8.1 Hz, 1H), 2.80-2.73 (m, 3H), 2.46 (d, J=18 Hz, 1H), 2.30 (s, 3H), 2.28 (s, 3H), 2.20 (s, 3H), 1.98 (s, 3H), 1.79 (dd, J_1=12.6 Hz, J_2=16.2 Hz, 1H);

^{13}C NMR (75 MHz, $CDCl_3$)): δ 168.7, 146.7, 144.4, 142.9, 140.4, 130.4, 128.9, 121.1, 120.8, 117.8, 116.8, 113.6, 111.5, 101.4, 67.6, 60.5, 59.8, 58.4, 56.6, 55.8, 55.3, 43.6, 41.8, 31.3, 25.6, 20.2, 15.6, 9.2.

ESI-MS m/z: Calcd. for $C_{28}H_{32}N_4O_6$: 520.58. Found $(M+H)^+$: 521.3.

To a solution of 45 (10 mg, 0.02 mmol) in CH_2Cl_2 (0.4 ml) was added phthalic anhydride (2.84 mg, 0.02 mmol) and the reaction mixture was stirred for 2 h at 23° C. Then, carbonyldiimidazole (0.5 mg, 0.003 mmol) was added and the mixture was stirred at 23° C. for 7 h. Then, carbonyldiimidazole (2.61 mg, 0.016 mmol) was added and the reaction was stirred at 23° C. for an additional 17 h. The solution was diluted with CH_2Cl_2 (10 ml) and washed with 0.1 N HCl (5 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography (RP-18, CH_3CN:H_2O 60:40) to afford phthalascidin (11.7 mg, 93%) as a white solid.

Rf: 0.37 (CH_3CN:H_2O 7:3, RP-18).

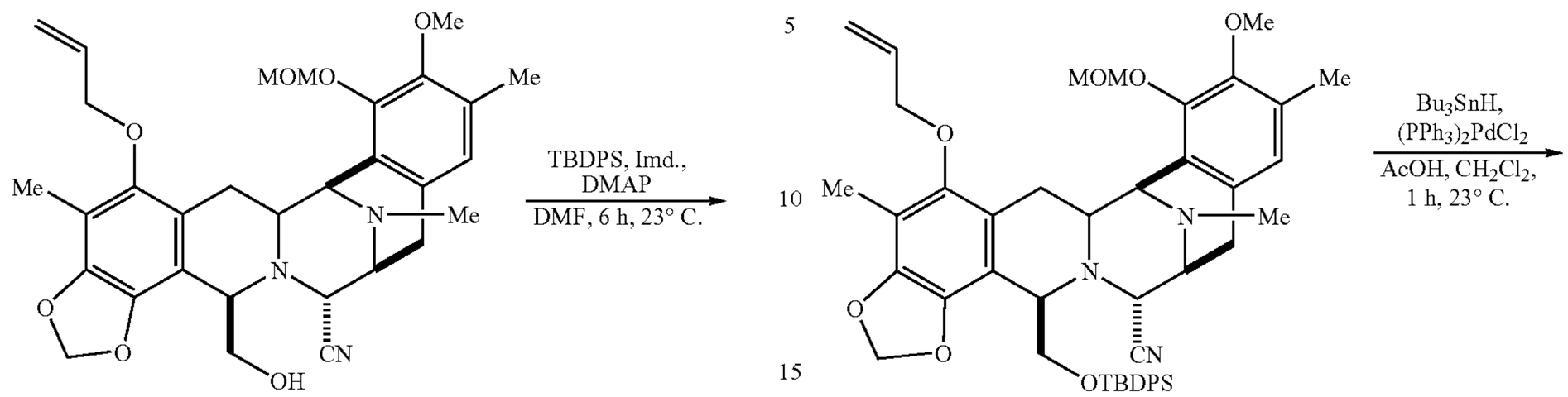
^{1}H NMR (300 MHz, $CDCl_3$): δ 7.72-7.68 (m, 2H), 7.67-7.63 (m, 2H), 6.38 (s, 1H), 5.69 (d, J=1.2 Hz, 1H), 5.64 (d, J=1.2 Hz, 1H), 5.30 (bs, 1H), 4.25-4.21 (m, 2 h), 4.02 (d, J=2.1 Hz, 1H), 3.64-3.62 (m, 5H), 3.33 (d, J=8.4 Hz, 1H), 3.21-3.16 (m, 1H), 3.02 (dd, J_1=8.1 Hz, J_2=18 Hz, 1H), 2.76 (dd, J_1=1.8 Hz, J_2=15.6 Hz, 1H), 2.63 (d, J=17.7 Hz, 1H), 2.29 (s, 3H), 2.28 (s, 3H), 2.21 (s, 3H), 2.0 (s, 3H), 1.73 (dd, J_1=12.0 Hz, J_2=15.3 Hz, 1H)).

^{13}C NMR (75 MHz, $CDCl_3$)): δ 168.5, 167.6, 146.2, 144.2, 142.5, 141.0, 140.5, 133.4, 131.8, 130.7, 128.2, 120.9, 120.8, 117.9, 116.4, 113.6, 101.1, 60.4, 60.0, 57.0, 56.3, 55.6, 55.4, 41.6, 41.5, 26.5, 25.2, 20.2, 15.7, 9.4.

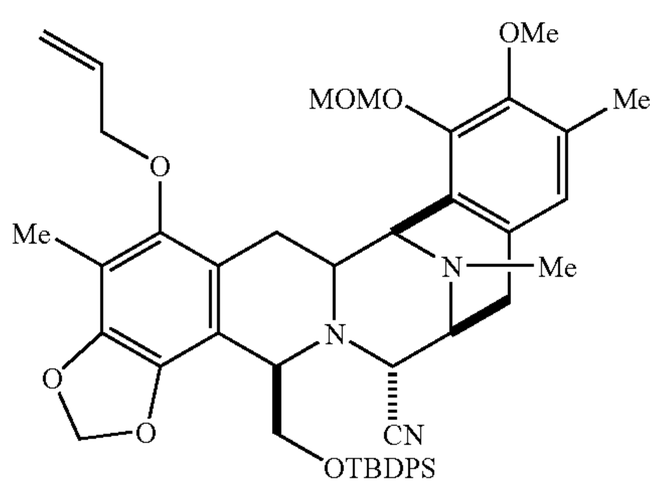
ESI-MS m/z: Calcd. for $C_{36}H_{34}N_4O_8$: 650. Found $(M+H)^+$: 651.2.

Example 30

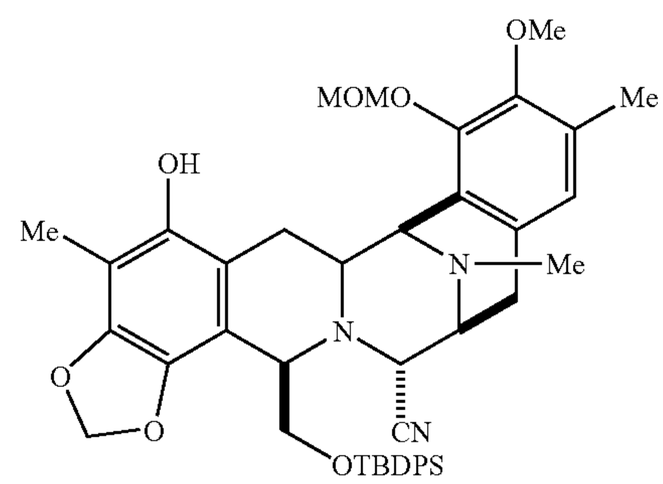
Exampe 31



25 26



26



ET-11

To a solution of 25 (18 mg, 0.032 mmol) in DMF (0.05 ml), cat. DMAP (0.5 mg, 0.004 mmol), imidazole (5 mg, 0.08 mmol) and tert-Butyldiphenylsilyl chloride (12.5 ml, 0.048 mmol) were added at 0° C. and the reaction mixture was stirred for 6 h at 23° C. Water (10 ml) was added at 0° C. and the aqueous phase was extracted with hexane:ethyl acetate 1:10 (2×10 mmol). The organic layer was dried (sodium sulphate), filtered, and the solvent was removed under reduced pressure. The crude was purified by flash column chromatography (SiO_2, hexane:ethyl acetate 3:1) to afford 26 (27 mg, 88%) as a white solid.

Rf: 0.29 (hexane:ethyl acetate 3:1).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.61-7.58 (m, 2 h), 7.42-7.28 (m, 8H), 6.71 (s, 1H), 6.19-6.02 (m, 1H), 5.78 (d, J=1.2 Hz, 1H), 5.64 (d, J=1.2 Hz, 1H), 5.40 (dd, J_1=1.2 Hz, J_2=17.1 Hz, 1H), 5.27 (dd, J_1=1.2 Hz, J_2=10.2 Hz, 1H), 5.13 (s, 2 h), 4.45 (d, J=2.4 Hz, 1H), 4.24 (d, J=2.1 Hz, 1H), 4.17-4.06 (m, 3H), 3.75 (s, 3H), 3.64 (dd, J_1=2.4 Hz, J_2=9.9 Hz, 1H), 3.59 (s, 3H), 3.42-3.21 (m, 4H), 3.10 (dd, J_1=8.1 Hz, J_2=17.7 Hz, 1H), 2.70 (d, J=17.7 Hz, 1H), 2.33 (s, 3H), 2.26 (s, 3H), 2.11 (s, 3H), 2.08-1.89 (m, 1H), 0.87 (s, 9H);

^{13}C NMR (75 MHz, $CDCl_3$): δ 148.5, 148.3, 148.1, 144.0, 139.0, 135.6, 135.4, 133.8, 133.1, 132.6, 130.5, 130.3, 129.6, 129.4, 127.5, 127.4, 125.1, 124.3, 121.6, 118.5, 117.5, 112.9, 111.7, 100.8, 99.2, 74.0, 67.7, 61.5, 59.6, 59.0, 57.7, 57.1, 55.4, 41.6, 29.6, 26.6, 25.5, 18.8, 15.8, 9.2.

ESI-MS m/z: Calcd. for $C_{47}H_{55}N_3O_7Si$: 801.3. Found $(M+H)^+$: 802.3.

To a solution of 26 (7 mg, 0.0087 mmol) in CH_2Cl_2 (0.15 ml), acetic acid (2.5 ml, 0.044 mmol), $(PPh_3)_2PdCl_2$ (0.5 mg, 6.96×10^{-4} mmol) and Bu_3SnH (3.5 ml, 0.013 mmol) were added at 23° C. The reaction mixture was stirred at that temperature for 1 h. The solution was diluted with a mixture of hexane:ethyl acetate 5:1 (0.5 ml) and poured into a pad of flash column (SiO_2, gradient 5:1 to 1:1 hexane:ethyl acetate) affording ET-11 (5 mg, 75%) as a white solid.

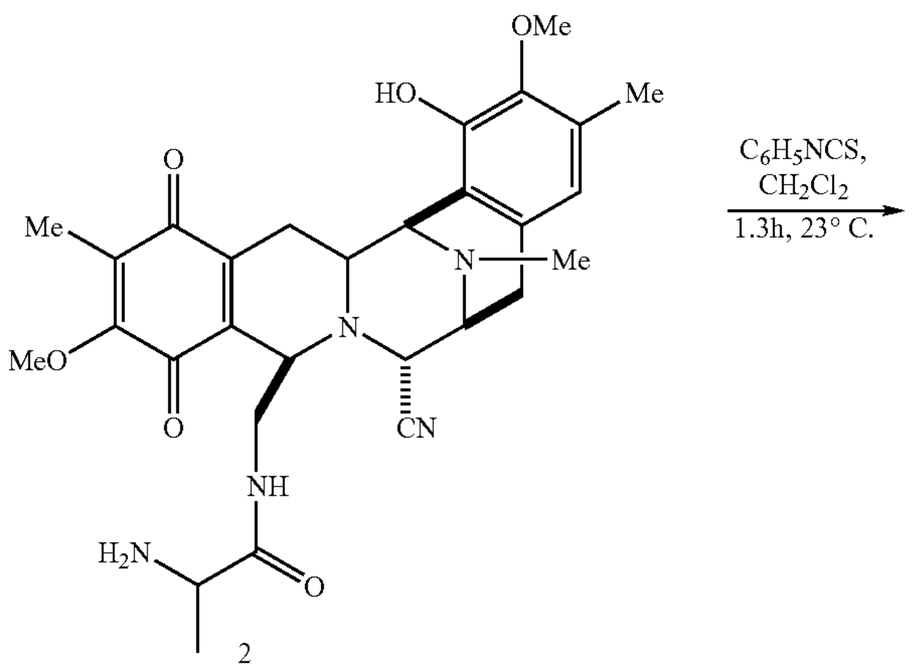
Rf: 0.36 (hexane:ethyl acetate 1:5, silica).

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.56 (m, 2 h), 7.41-7.25 (m, 8H), 6.67 (s, 1H), 5.72 (d, J=1.0 Hz, 1H), 5.58 (d, J=1.0 Hz, 1H), 5.51 (s, 1H), 5.38 (d, J=5.75 Hz, 1H), 5.16 (d, J=5.7 Hz, 1H), 4.57 (d, J=2.9 Hz, 1H), 4.21 (m, 1H), 4.09 (m, 1H), 3.72 (s, 3H), 3.71 (s, 3H), 3.68 (dd, J_1=2.1 Hz, J_2=10.4 Hz, 1H), 3.38-3.26 (m, 3H), 3.11 (dd, J_1=2.5 Hz, J_2=15.7 Hz, 1H), 3.01 (dd, J_1=8.9 Hz, J_2=17.9 Hz, 1H), 2.70 (d, J=17.9 Hz, 1H), 2.31 (s, 3H), 2.25 (s, 3H), 2.06 (s, 3H), 1.89 (dd, J_1=12.1 Hz, J_2=15.7 Hz, 1H), 0.9 (s, 9H).);

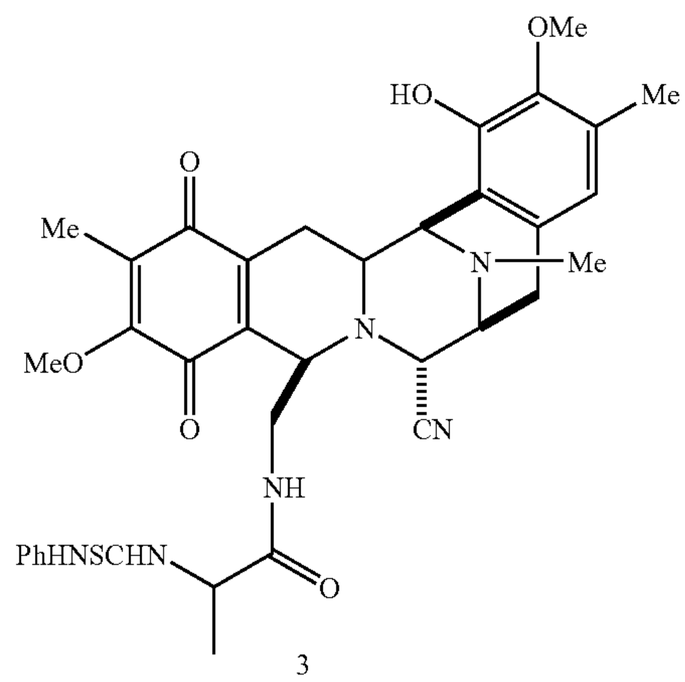
^{13}C NMR (75 MHz, $CDCl_3$): δ 149.0, 147.4, 145.3, 144.3, 136.3, 135.7, 135.4, 133.2, 130.9, 130.5, 129.6, 129.5, 127.5, 125.0, 118.6, 112.5, 112.1, 105.7, 100.5, 99.8, 68.5, 61.5, 59.7, 58.8, 57.7, 56.9, 56.5, 55.4, 41.7, 26.6, 26.2, 25.5, 18.9, 15.8, 14.2, 8.7.

ESI-MS m/z: Calcd. for $C_{44}H_{51}N_3O_7Si$: 761. Found $(M+H)^+$: 762.

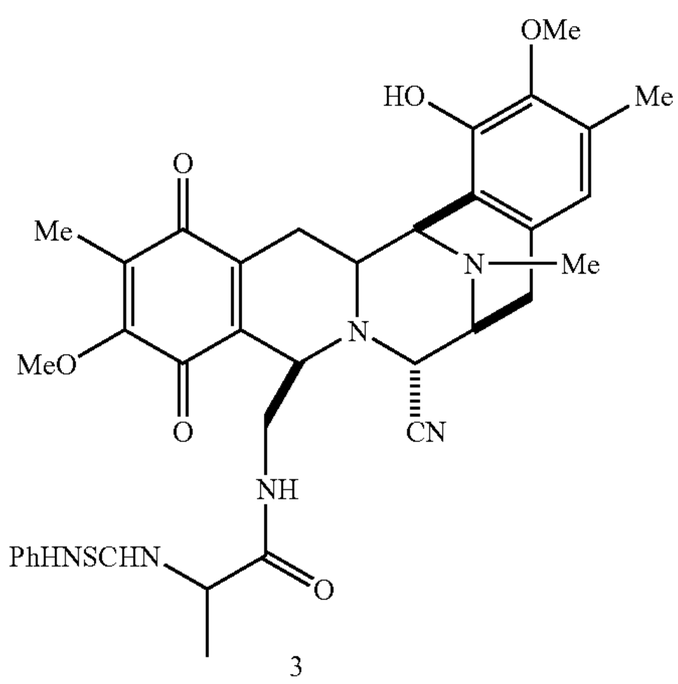
Example 32

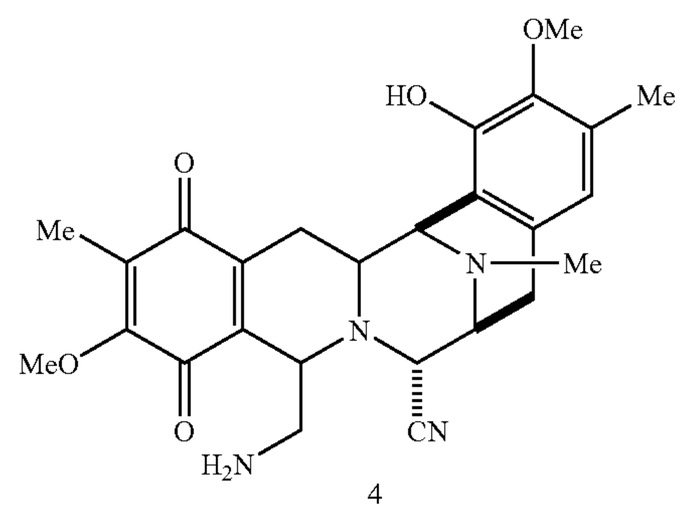


C_6H_5NCS, CH_2Cl_2 → 1.3h, 23° C.



1) HCl 6.5 M in dioxane → 2) $NaCO_3H$





A solution of 2 (3.0 g, 5.46 mmol) and phenyl isothiocyanate (3.92 mL, 32.76 mmol) in CH_2Cl_2 (27 ml) was stirred at 23° C. for 1.5 h. The reaction mixture was partitioned between CH_2Cl_2 (10 ml) and H_2O (5 ml). The organic layer was dried over sodium sulphate, filtered and concentrated. The residue was purified by flash column chromatography (SiO_2, gradient Hex to 2:3 hexane:ethyl acetate) to give 3 (3.29 g, 88%) as a yellow solid.

Rf: 0.27 (ACN:H_2O 3:2, RP-C18);

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.77 (bs, 1H), 7.42-7.11 (m, 5H), 6.65 (d, 1H), 6.29 (s, 1H), 5.6-5.5 (m, 1H), 4.19-4.14 (m, 2 h), 4.08 (d, 1H), 3.92 (s, 3H), 3.87-3.65 (m, 6H), 3.77 (s, 3H), 3.37-2.98 (m, 8H), 2.50 (d, 1H), 2.31 (s, 3H), 2.20 (s, 3H), 1.96 (d, 1H), 1.87 (s, 3H), 1.81-1.75 (m, 1H), 0.96 (d, 3H);

^{13}C NMR (75 MHz, $CDCl_3$): δ 185.7, 180.9, 178.9, 172.0, 155.7, 147.1, 143.2, 142.4, 136.0, 135.1, 130.5, 129.9, 129.3, 128.5, 126.9, 124.4, 120.2, 117.4, 116.3, 77.1, 60.9, 58.6, 56.2, 55.8, 55.0, 54.6, 53.5, 41.7, 40.3, 25.1, 24.5, 18.4, 15.8, 8.7

ESI-MS m/z: Calcd. for $C_{36}H_{40}N_6O_6S$: 684.8. Found $(M+H)^+$: 685.2.

Example 33

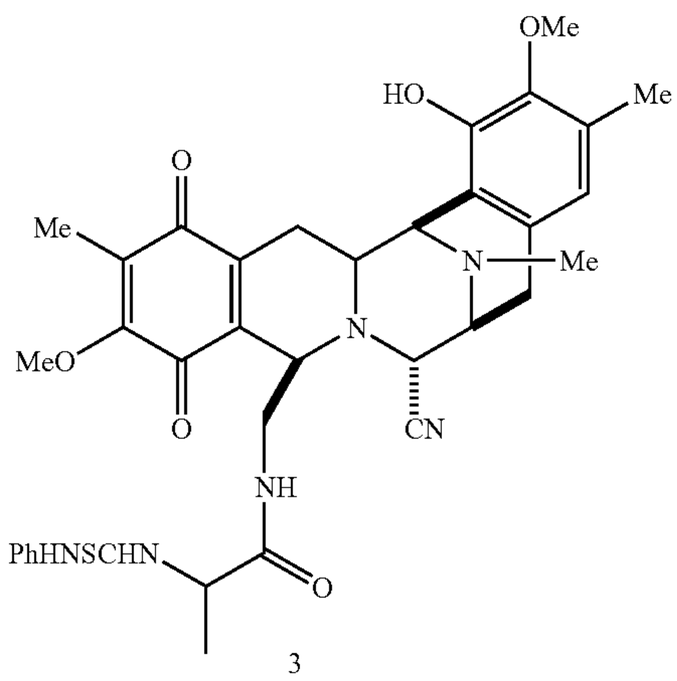
A solution of 3 (0.143 g, 0.208 mmol) in 6.5 M HCl/dioxane (150 ml) was stirred at 23° C. for 6 h. Then, toluene (3 ml) was added to this reaction and the organic layer was decanted. The residue was partitioned between saturated aqueous sodium bicarbonate (3 ml) and $CHCl_3$ (3×3 ml) The organic layers were dried and concentrated to afford title compound as a mixture of 4 and 6 (4:6 90:10) which slowly cyclizes to 6 on standing.

Rf: 0.4 (ethyl acetate:methanol 5:1, silica);

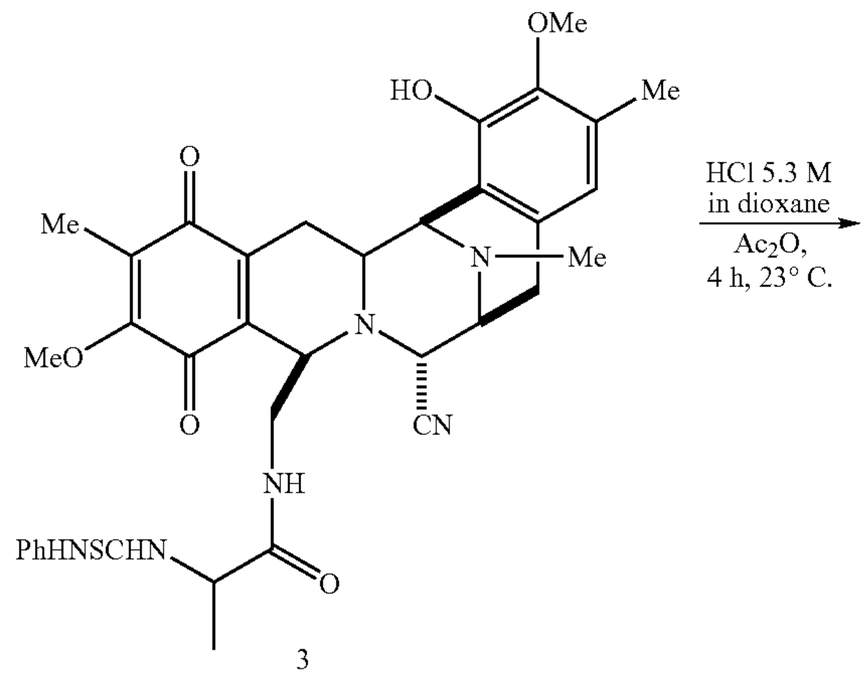
^{1}H NMR (300 MHz, $CDCl_3$): δ 6.45 (s, 1H), 4.16 (m, 1H), 4.02 (d, 1H), 3.96 (s, 3H), 3.79 (m, 2 h), 3.75 (s, 3H), 3.35 (m, 1H), 3.20-3.00 (m, 3H), 2.87 (d, 1H), 2.75 (d, 1H), 2.43 (d, 1H), 2.34 (s, 3H), 2.30 (s, 3H), 1.93 (s, 3H), 1.72-1.5 (m, 3H).

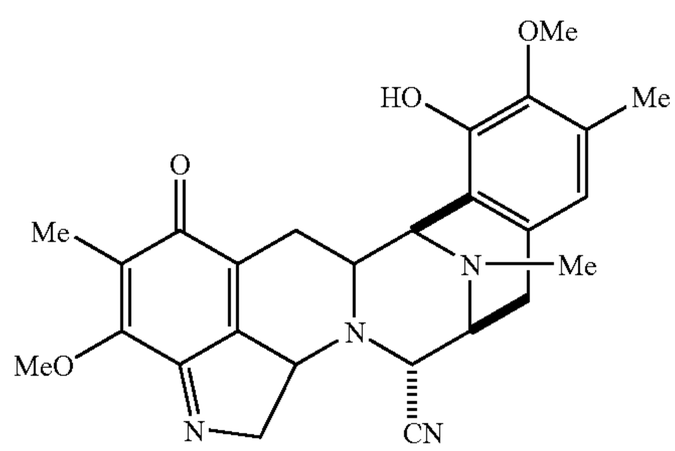
ESI-MS m/z: Calcd. for $C_{26}H_{30}N_4O_5$: 478.5. Found $(M+H)^+$: 479.2

Example 34



3





6

A solution of 3 (0.143 g, 0.208 mmol) in 6.5M HCl/dioxane (150 ml) was stirred at 23° C. for 1 h. Evaporation of the solvent gave a residue which was purified by flash column chromatography (ethyl acetate/methanol/triethylamine 100:25:0.1) to give 6 (80 mg, 83%) as a yellow solid.

Rf: 0.26 (ACN:H_2O 3:2, RP-C18);

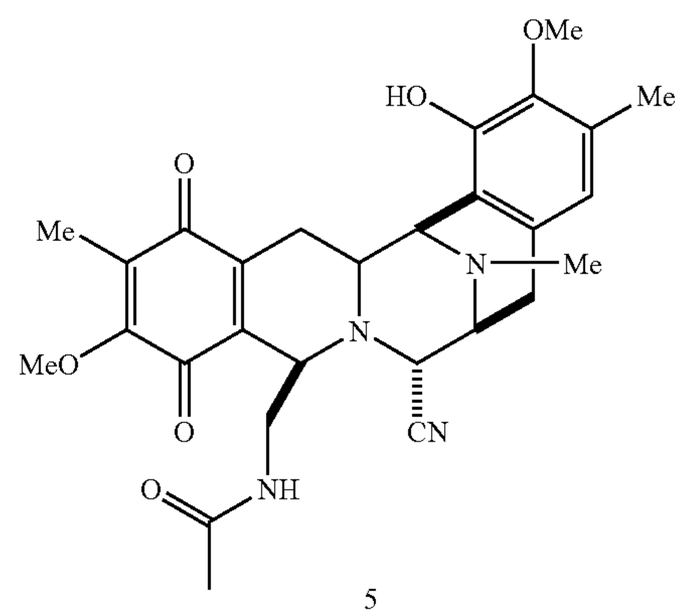
^{1}H NMR (500 MHz, $CDCl_3$): δ 6.46 (s, 1H), 5.9 (bs, 1H) 4.67 (dd, J=18.3 Hz, J=7.8 Hz, 1H), 4.24 (d, 1H), 4.16 (s, 3H), 3.93 (d, J=2.7 Hz, 1H), 3.8 (m, 2 h), 3.77 (s, 3H), 3.45 (m, 2 h), 3.08 (dd, J=17.9 Hz, J=3.6 Hz, 1H), 2.78 (m, 1H), 2.55 (d, 1H), 2.3 (m, 1H), 2.3 (s, 3H), 2.28 (s, 3H), 1.90 (s, 3H);

^{13}C NMR (75 MHz, $CDCl_3$): δ 186.2, 162.1, 154.9, 146.9, 145.3, 143.0, 130.1, 129.4, 128.1, 125.0, 121.4, 116.4, 116.2, 66.6, 60.7, 60.7, 60.1, 59.6, 58.8, 55.6, 54.9, 41.9, 25.3, 24.7, 15.7, 8.9.

ESI-MS m/z: Calcd. for $C_{26}H_{28}N_4O_4$: 460.5. Found $(M+H)^+$: 461.1

Example 35

3



5

To a solution of 3 (2.38 g, 3.47 mmol) in dioxane (5 ml) 5.3M HCl in dioxane (34 ml) was added and the reaction was stirred at 23° C. for 45 minutes. Then Ac_2O (51 ml, 539.5 mmol) was added and the mixture was stirred for 4 h. The reaction was cooled at 0° C. and partitioned between aqueous saturated Na_2CO_3 (300 ml) and ethyl acetate (300 ml) at this temperature. The organic phase was dried over sodium sulphate, filtered and concentrated. The residue was purified by flash column chromatography (SiO_2, gradient CH_2Cl_2 to CH_2Cl_2:ethyl acetate 1:2) to give 5 (1.75 g, 97%) as a yellow solid.

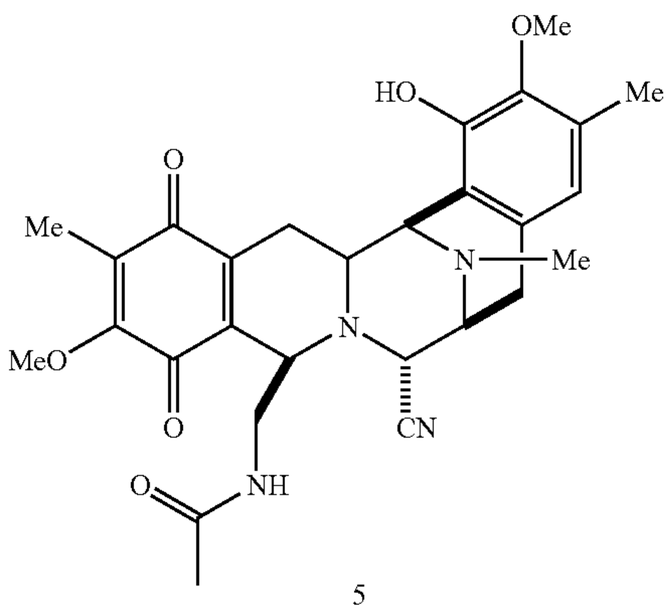
Rf: 0.53 (ACN:H_2O 3:2, RP-C18);

^{1}H NMR (300 MHz, $CDCl_3$): δ 6.51 (s, 1H), 5.98 (bs, 1H), 4.84 (dd, 1H), 4.17 (d, 1H), 4.00 (d, 1H), 3.99 (s, 3H), 3.85 (bs, 1H), 3.81 (m, 1H), 3.74 (s, 3H), 3.70 (d, 1H), 3.23 (m, 1H), 3.11 (dd, 1H), 3.09 (m, 1H), 2.93 (m, 2 h), 2.44 (d, 1H), 3.67 (s, 3H), 2.25 (s, 3H), 1.70 (s, 3H), 1.60-1.50 (m, 2 h), 1.29 (s, 3H);

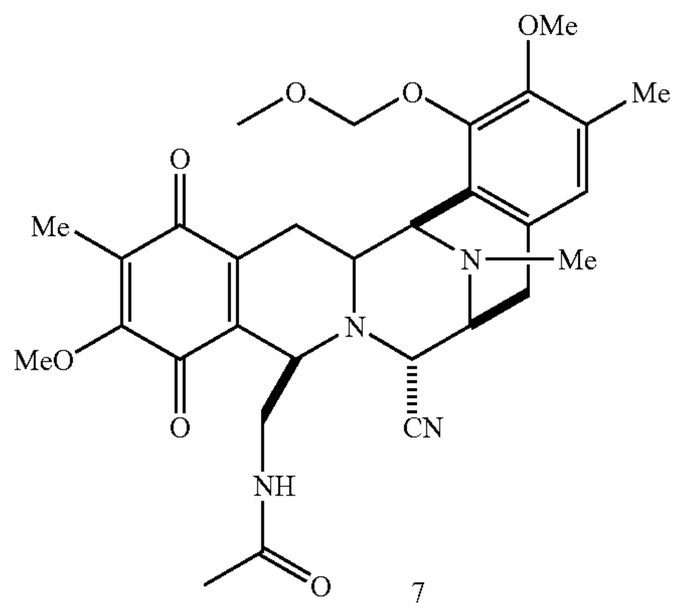
^{13}C NMR (75 MHz, $CDCl_3$): δ 185.9, 180.8, 169.9, 160.2, 156.2, 147.0, 143.1, 140.4, 136.1, 130.6, 129.6, 127.9, 120.4, 117.2, 61.0, 60.7, 58.6, 56.1, 55.7, 55.1, 54.3, 41.8, 41.1, 25.7, 23.9, 22.2, 15.7, 8.7.

ESI-MS m/z: Calcd. for $C_{28}H_{32}N_4O_6$: 520.6. Found $(M+H)^+$: 521.1

Example 36

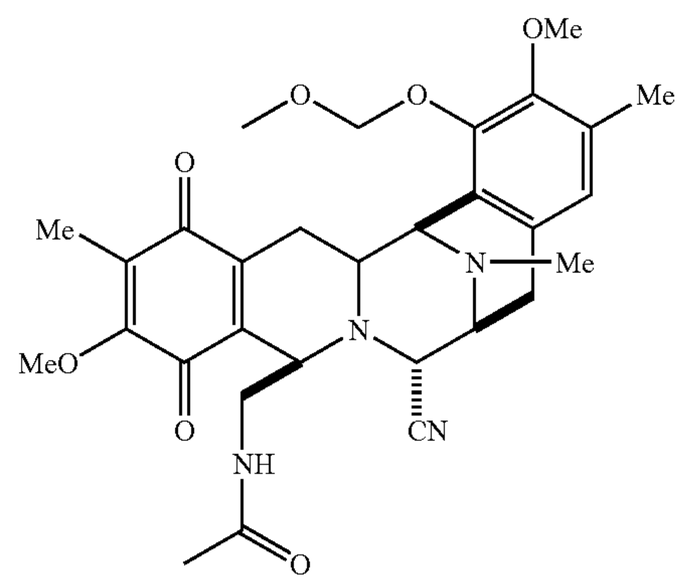


5

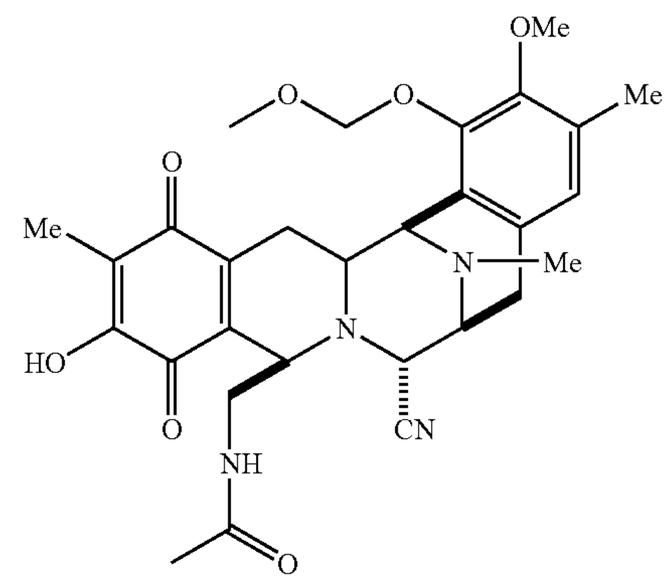


7

Example 37



7



8

To a solution of 5 (1.75 g, 3.36 mmol) in CH_2Cl_2 (17 ml) diisopropylethylamine (11.71 ml, 67.23 mmol), DMAP (20 mg, 0.17 mmol) and bromomethyl methyl ether (4.11 ml, 50.42 mmol) were added at 0° C. After 6 h at 23° C. the reaction was partitioned between CH_2Cl_2 (50 ml) and aqueous saturated sodium bicarbonate (25 ml). The organic layer was dried over sodium sulphate and the solvent was eliminated under reduced pressure. The crude was purified by flash column chromatography (RP-18, CH_3CN/H_2O 1/1) to give 7 (1.32 g, 70%) as a yellow solid.

Rf: 0.34 (ACN:H_2O 2:3, RP-C18);

^{1}H NMR (300 MHz, $CDCl_3$): δ 6.74 (s, 1H), 5.14 (s, 2 h), 4.82 (m, 1H), 4.22 (d, 1H), 4.00 (s, 3H), 4.0 (m, 1H), 3.83 (m, 2 h), 3.7 (s, 3H), 3.58 (s, 3H), 3.4 (m, 1H), 3.2-2.95 (m, 6H), 2.43 (d, 1H), 2.37 (s, 3H), 2.22 (s, 3H), 1.89 (s, 3H), 1.5-1.4 (m, 2 h), 1.31 (s, 3H);

^{13}C NMR (75 MHz, $CDCl_3$): δ 185.9, 180.7, 169.6, 156.2, 148.9, 148.5, 140.3, 136.2, 131.3, 130.1, 127.7, 124.6, 123.7, 117.3, 99.5, 99.2, 60.9, 59.7, 58.8, 57.7, 56.4, 55.7, 55.0, 54.2, 51.0, 41.6, 41.0, 40.5, 25.5, 23.9, 22.3, 19.3, 15.6, 14.6, 8.6.

ESI-MS m/z: Calcd. for $C_{30}H_{36}N_4O_7$: 564.6. Found $(M+H)^+$: 565.3

To a solution of 7 (0.37 g, 0.65 mmol) in methanol (74 ml) at 0° C. was added 1M sodium hydroxide (130 ml). The reaction was stirred for 15 minutes and then, quenched at 0° C. with 6M HCl to pH=5. The mixture was extracted with ethyl acetate (3×50 ml) and the combined organic layers were dried over sodium sulphate and concentrated in vacuo. The residue was purified by flash column chromatography (RP-C18 CH_3CN:H_2O 1/:1) to afford 8 (232 mg, 65%) as a yellow oil.

Rf: 0.5 (ACN:H_2O 3:2, RP-C18);

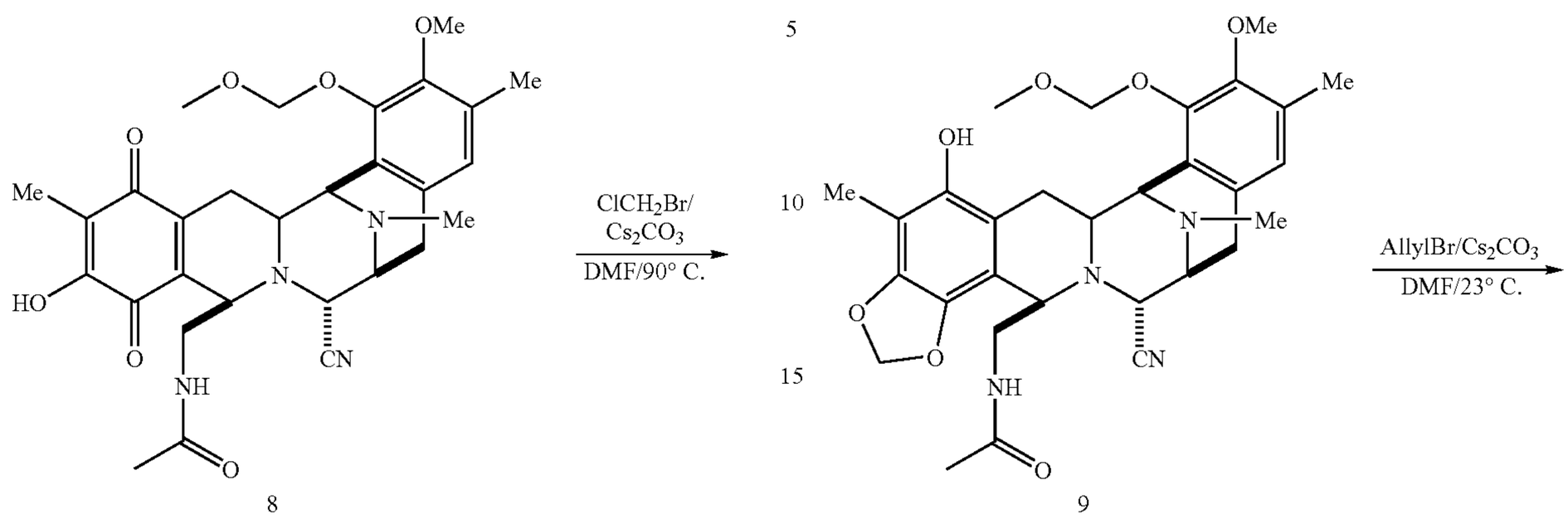
^{1}H NMR (300 MHz, $CDCl_3$): δ 6.75 (s, 1H), 5.15 (s, 2 h), 4.86 (m, 1H), 4.26 (d, 1H),), 4.01 (d, 1H), 3.88-3.81 (m, 2 h), 3.70 (s, 3H), 3.58 (s, 3H), 3.39 (m, 1H), 3.27-3.21 (m, 1H), 3.18-3.08 (m, 2 h), 3.03-2.97 (m, 1H) 2.47 (d, 1H), 2.37 (s, 3H), 2.22 (s, 3H), 1.90 (s, 3H), 1.57-1.46 (m, 2 h), 1.33 (s, 3H);

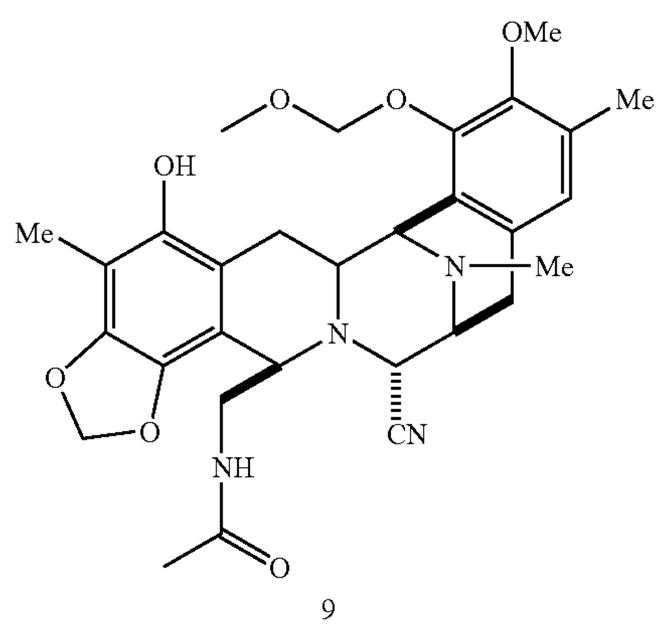
^{13}C NMR (75 MHz, □$CDCl_3$): 185.3, 180.6, 175.9, 170.1, 151.5, 148.9, 148.6, 143.3, 133.7, 131.5, 129.9, 124.7, 123.5, 117.1, 117.0, 99.2, 59.8, 58.7, 57.8, 56.3, 55.3, 54.9, 54.3, 41.5, 40.7, 29.6, 25.5, 24.4, 22.2, 20.7, 15.7, 8.0.

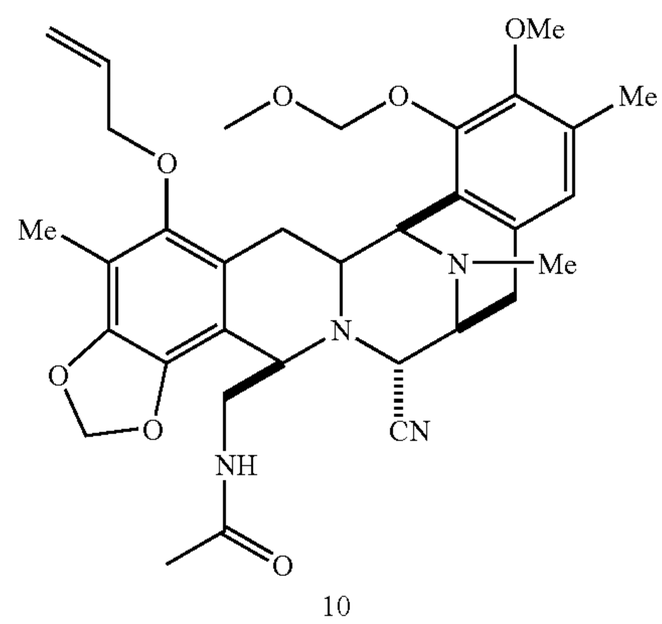
ESI-MS m/z: Calcd. for $C_{29}H_{34}N_4O_7$: 550.6. Found $(M+H)^+$: 551.2

Example 38

Example 39







To a degassed solution of compound 8 (240 mg, 0.435 mmol) in DMF (30 ml) 10% Pd/C (48 mg) was added and the reaction was stirred under H_2 (atmospheric pressure.) for 1 h. The reaction was filtered through a pad of celite under Argon to a Schlenk tube, as a colourless solution, containing anhydrous Cs_2CO_3 (240 mg, 0.739 mmol). Then, bromochloromethane (0.566 ml, 8.71 mmol) was added. The tube was sealed and stirred at 90° C. for 3 h. The reaction was cooled and filtrated through celite and washed with CH_2Cl_2. The organic layer was concentrated and dried (sodium sulphate) to afford 9 as a brown oil that was used in the next step with no further purification.

Rf: 0.36 (SiO_2, hexane:ethyl acetate 1:5)

^{1}H NMR (300 MHz, $CDCl_3$): δ 6.71 (s, 3H), 5.89 (d, 1H), 5.81 (d, 1H), 5.63 (bs, 1H), 5.33 (d, 1H), 5.17 (d, 1H), 4.97 (m, 1H), 4.20 (d, 1H), 4.09 (m, 1H), 3.99 (m, 1H), 3.68 (m, 1H), 3.65 (s, 6H), 3.59-3.47 (m, 4H), 3.37-3.27 (m, 2 h), 3.14-2.97 (m, 2 h), 2.62 (d, 1H), 2.32 (s, 3H), 2.20 (s, 3H), 2.08 (s, 3H), 1.72 (m, 1H), 1.36 (s, 3H);

^{13}C NMR (75 MHz, $CDCl_3$): δ 169.8, 149.1, 147.4, 145.5, 136.2, 130.9, 130.8, 125.0, 122.9, 117.7, 112.6, 111.8, 106.4, 100.8, 99.8, 59.8, 58.9, 57.7, 56.6, 56.4, 55.5, 55.2, 41.6, 40.1, 29.6, 25.9, 25.0, 22.6, 15.6, 8.8.

ESI-MS m/z: Calcd. for $C_{30}H_{36}SiN_4O_7$: 564.6. Found $(M+H)^+$: 565.3.

To a flask containing 9 (245 mg, 0.435 mmol) in DMF, (4 ml), cesium carbonate (425 mg, 1.30 mmol) and allyl bromide (376 ml, 4.35 mmol) were added at 0° C. and the mixture was stirred at 23° C. for 1 h. The reaction was filtered though a pad of celite and partitioned between CH_2Cl_2 (25 ml) and H_2O (10 ml). The organic phase was dried (sodium sulphate) and concentrated at reduced pressure to afford a residue that was purified by flash column chromatography (SiO_2, $CHCl_3$: ethyl acetate 1:2) to give 10 as a yellow oil. (113 mg, 43%).

Rf: 0.36 (hexane:ethyl acetate 1:5)

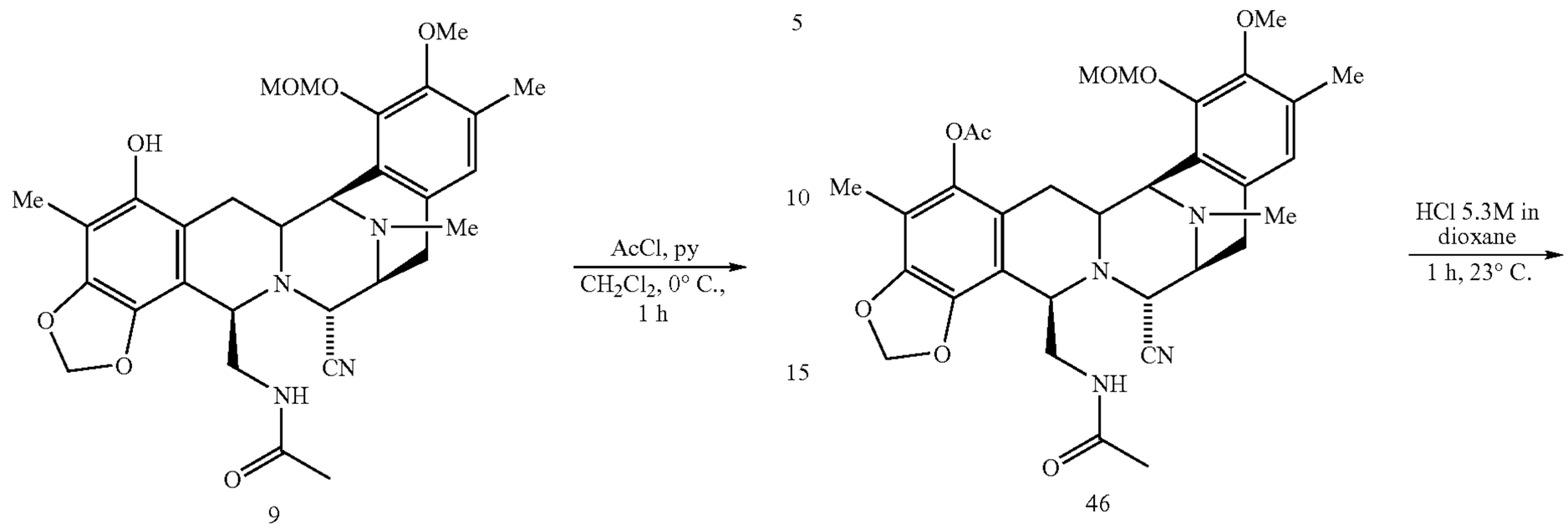
^{1}H NMR (300 MHz, $CDCl_3$): δ 6.74 (s, 1H), 6.3-6.0 (m, 1H), 5.94 (d, 1H), 5.87 (d, 1H), 5.43-5.36 (m, 2 h), 5.22 (s, 2 h), 5.00 (m, 1H), 4.22 (m, 1H), 4.17-4.01 (m, 1H), 3.98 (m, 2 h), 3.71-3.67 (m, 1H), 3.69 (s, 3H), 3.62-3.51 (m, 3H), 3.58 (s, 3H), 3.39-3.37 (m, 1H), 3.31-3.26 (m, 3H), 3.09 (dd, 1H), 2.56 (d, 1H), 2.36 (s, 3H), 2.21 (s, 3H), 2.11 (s, 3H), 2.24-2.10 (m, 1H), 1.82-1.73 (m, 1H), 1.24 (bs, 3H)

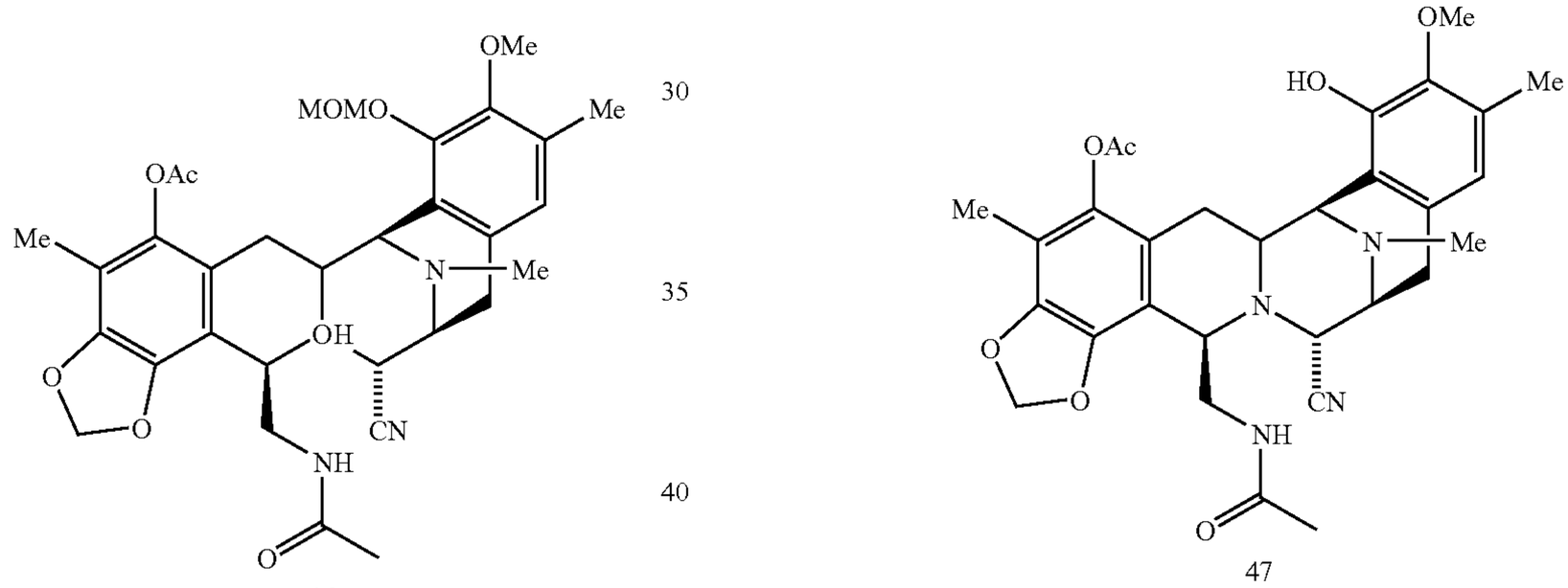
^{13}C NMR (75 MHz, $CDCl_3$): δ 169.4, 148.8, 148.3, 139.1, 133.7, 130.9, 130.3, 125.2, 120.2, 117.7, 113.1, 112.6, 101.3, 99.3, 74.1, 59.7, 59.3, 57.8, 57.0, 56.1, 56.1, 55.2, 41.6, 41.0, 40.9, 29.7, 26.3, 22.5, 15.6, 9.3

ESI-MS m/z: Calcd. for $C_{33}H_{40}N_4O_7$: 604.7. Found $(M+H)^+$: 605.3.

Example 40

Example 41





To a solution of 9 (22 mg, 0.039 mmol) in CH_2Cl_2 (0.2 ml), acetyl chloride (2.79 ml, 0.039 mmol) and pyridine (3.2 ml, 0.039 mmol) were added at 0° C. The reaction mixture was stirred for 1 h and then, the solution was diluted with CH_2Cl_2 (10 ml) and washed with 0.1 N HCl (5 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure to afford 46 (22 mg, 93%) as a white solid.

Rf: 0.4 (hexane:ethyl acetate 1:5).

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.74 (s, 1H), 5.97 (d, J=0.9 Hz, 1H), 5.91 (d, J=0.9 Hz, 1H), 5.12 (d, J=5.7 Hz, 2 h), 5.04 (d, J=5.7 Hz, 1H) 4.90 (t, J=6 Hz, 1H), 4.17 (d, J=2.7 Hz, 1H), 4.05 (d, J=2.7 Hz, 1H), 4.01 (bs, 1H), 3.71 (s, 3H), 3.57 (s, 3H), 3.50-3.44 (m, 2 h), 3.38-3.36 (m, 1H), 3.30-3.26 (m, 1H), 3.00 (dd, J_1=7.8 Hz, J_2=18.0 Hz, 1H), 2.79 (d, J=12.9 Hz, 1H), 2.60 (d, J=18.0 Hz, 1H), 2.35 (s, 3H), 2.32 (s, 3H), 2.21 (s, 3H), 2.00 (s, 3H), 1.68 (dd, J_1=11.7 Hz, J_2=15.6 Hz, 1H).

ESI-MS m/z: Calcd. for $C_{32}H_{38}N_4O_8$: 606.67. Found $(M+H)^+$: 607.3.

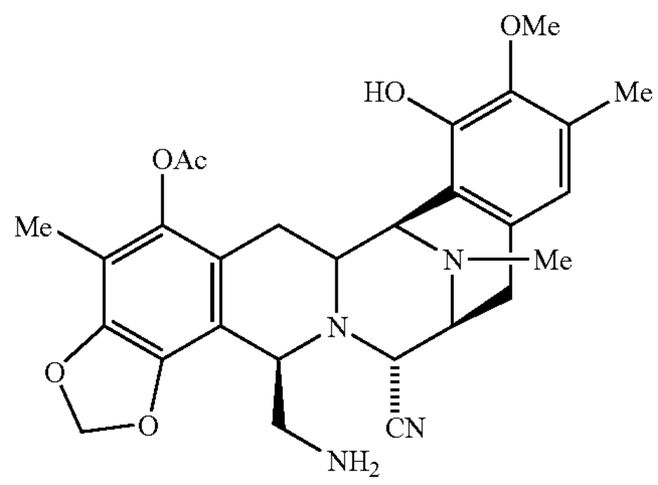
To a solution of 46 (8 mg, 0.013 mmol) in dioxane (0.1 ml), 5.3N HCl/dioxane (0.5 ml) was added and the reaction was stirred at 23° C. for 1 h. Then, the solution was diluted with CH_2Cl_2 (5 ml) and washed with 0.1 N HCl (3 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure to afford 47 (5 mg, 70%) as a white solid.

Rf: 0.4 (hexane:ethyl acetate 1:5).

^{1}H NMR (300 MHz, $CDCl_3$). δ 6.51 (s, 1H), 5.97 (d, J=1.2 Hz, 1H), 5.91 (d, J=1.2 Hz, 1H), 4.97 (bs, 1H), 4.11 (bs, 1H), 4.04-4.02 (m, 2 h), 3.75 (s, 3H),), 3.65 (d, J=2.1 Hz, 2 h), 3.56-3.30 (m, 2 h), 3.04 (dd, J_1=7.5 Hz, J_2=18 Hz, 1H), 2.80 (d, J=14.4 Hz, 1H), 2.59 (d, J=18.3 Hz, 1H), 2.33 (s, 3H), 2.24 (s, 3H), 2.00 (s, 3H), 1.76 (dd, J_1=12.0 Hz, J_2=15.9 Hz, 1H), 1.33 (s, 3H), 1.25 (s, 3H).

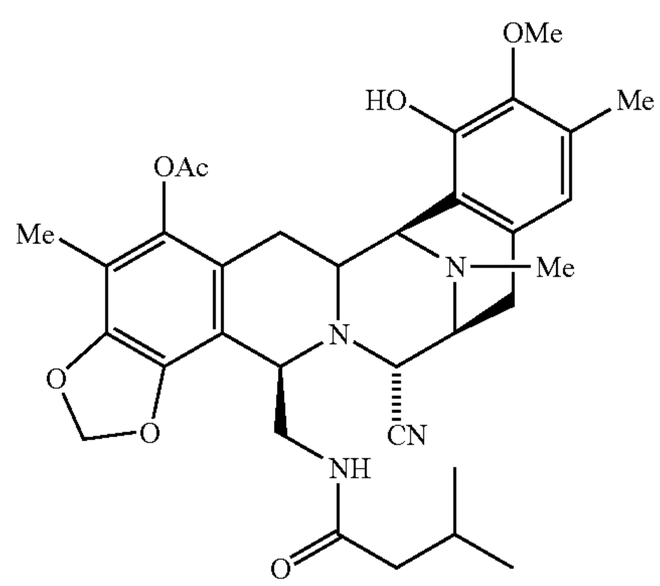
ESI-MS m/z: Calcd. for $C_{30}H_{34}N_4O_7$: 562.61. Found $(M+H)^+$: 563.3.

Example 42



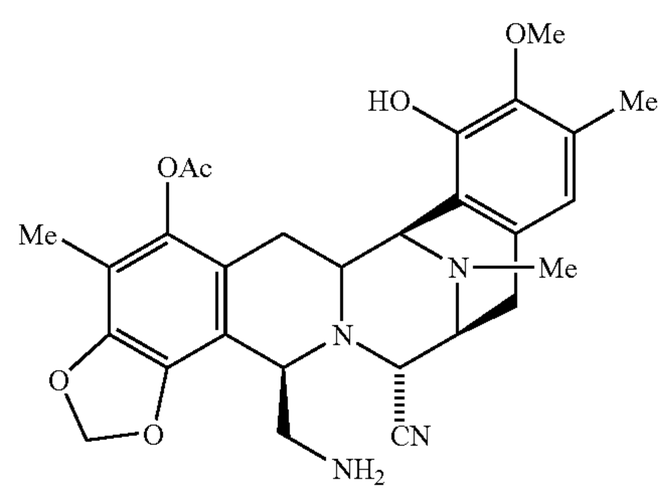
45

Isovaleryl chloride, py.
CH_2Cl_2, 0° C., 1 h



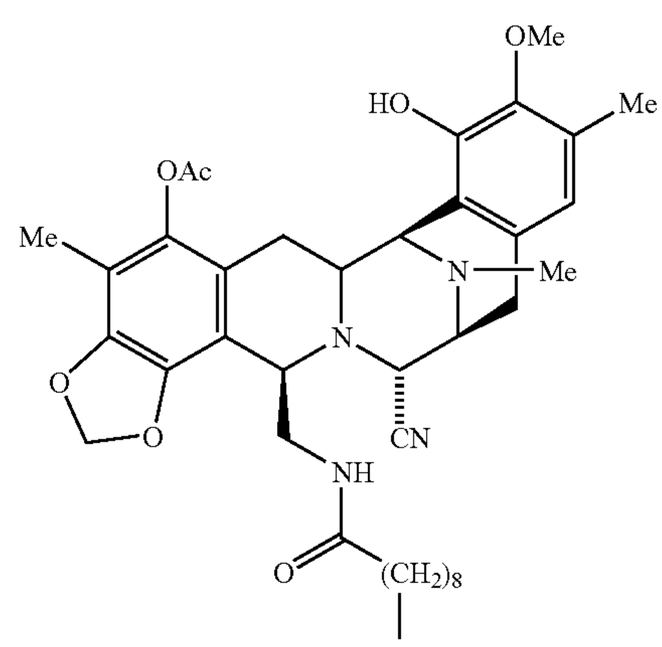
48

Example 43



45

decanoyl chloride, py.
CH_2Cl_2, 0° C., 1 h



49

To a solution of 45 (10 mg, 0.0192 mmol) in CH_2Cl_2 (0.3 ml), isovaleryl chloride (2.34 ml, 0.0192 mmol) and pyridine (1.55 ml, 0.0192 mmol) were added at 0° C. The reaction mixture was stirred for 1 h and then, the solution was diluted with CH_2Cl_2 (5 ml) and washed with 0.1 N HCl (3 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, Hex: ethyl acetate 1:2) to afford 48 (11 mg, 95%) as a white solid.

Rf: 0.12 (Hex:ethyl acetate 1:2).

^{1}H NMR (300 MHz, $CDCl_3$): δ 6.50 (s, 1H), 5.98 (d, J=1.5 Hz, 1H), 5.91(d, J=1.5 Hz, 1H), 5.75 (s, 1H), 5.02 (t, J=5.4 Hz, 1H), 4.10 (d, J=1.5 Hz, 1H), 4.06 (d, J=2.7 Hz, 1H), 4.02 (d, J=2.7 Hz, 1H), 3.77 (s, 3H), 3.76-3.71 (m, 1H), 3.86-3.28 (m, 3H), 3.04 (dd, J_1=8.1 Hz, J_2=18.3 Hz, 1H), 2.78 (d, J=15.9 Hz, 1H), 2.55 (d, J=18 Hz, 1H), 2.32 (s, 6H), 2.26 (s, 3H), 1.98 (s, 3H), 1.84-1.68 (m, 2 h), 1.36 (d, J=7.2 Hz, 2 h), 0.69 (d, J=6.6 Hz, 3H), 0.62 (d, J=6.6 Hz, 3H).

ESI-MS m/z: Calcd. for $C_{33}H_{40}N_4O_7$: 604.69. Found $(M+H)^+$: 605.3.

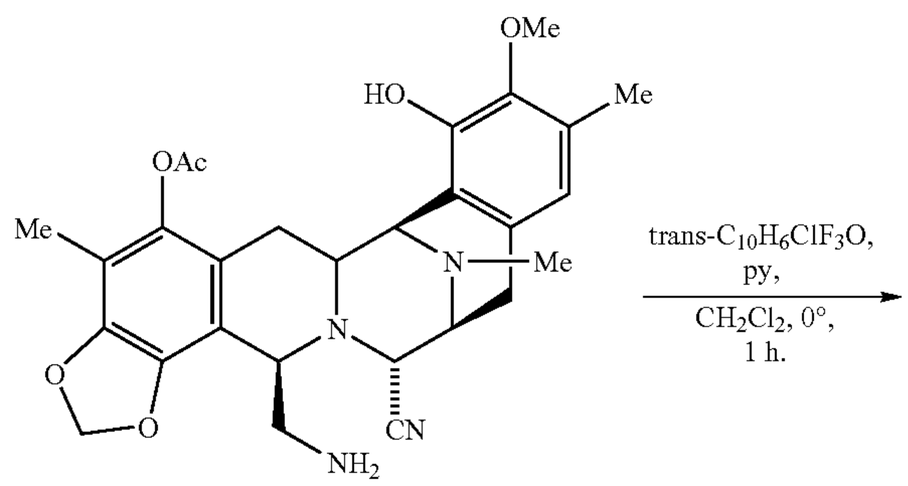
To a solution of 45 (10 mg, 0.0192 mmol) in CH_2Cl_2 (0.3 ml), isovaleryl chloride (3.98 ml, 0.0192 mmol) and pyridine (1.55 ml, 0.0192 mmol) were added at 0° C. The reaction mixture was stirred for 1 h and then, the solution was diluted with CH_2Cl_2 (5 ml) and washed with 0.1 N HCl (3 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, Hex: ethyl acetate 1:2) to afford 49 (12.4 mg, 96%) as a white solid.

Rf: 0.7 (ethyl acetate:methanol 10:1).

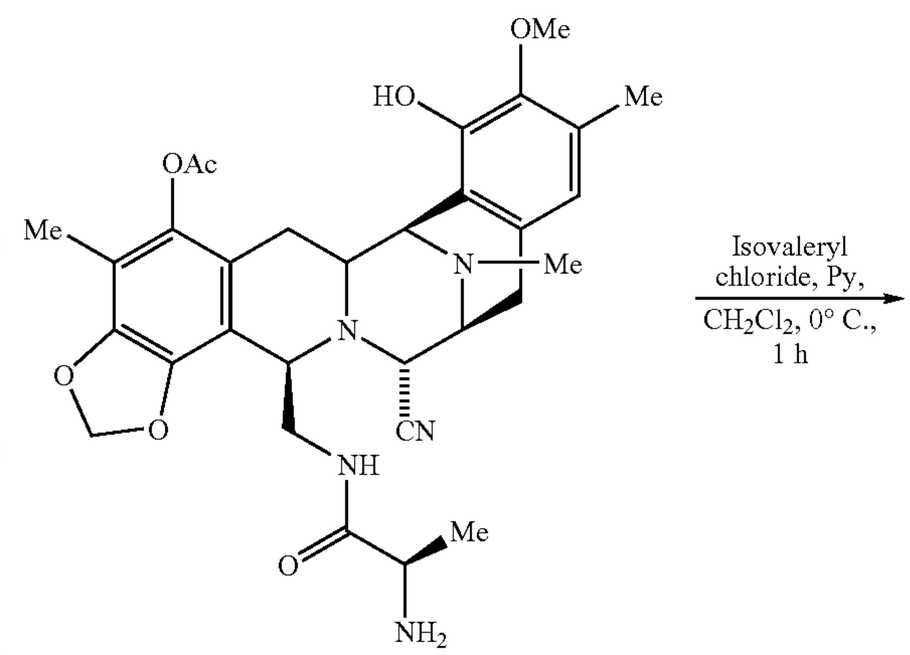
^{1}H NMR (300 MHz, $CDCl_3$): δ 6.50 (s, 1H), 5.98 (d, J=1.5 Hz, 1H), 5.91 (d, J=1.5 Hz, 1H), 5.73 (s, 1H), 5.08 (t, J=5.4 Hz, 1H), 4.10 (d, J=1.5 Hz, 1H), 4.05 (m., 1H), 4.01 (m, 1H), 3.76 (s, 3H), 3.65-3.61 (m, 1H), 3.40-3.27 (m, 3H), 3.03 (dd, J_1=8.1 Hz, J_2=18.6 Hz, 1H), 2.78 (d, J=13.2 Hz, 1H), 2.57 (d, J=18.3 Hz, 1H), 2.32 (s, 3H), 2.31 (s, 3H), 2.25 (s, 3H), 1.99 (s, 3H), 1.79 (dd, J_1=12.0 Hz, J_2=16.5 Hz, 1H), 1.73-1.42 (m, 4H), 1.33-1.18 (m, 10H), 1.03 (m, 2 h), 0.87 (t, J=6.6 Hz, 3H).

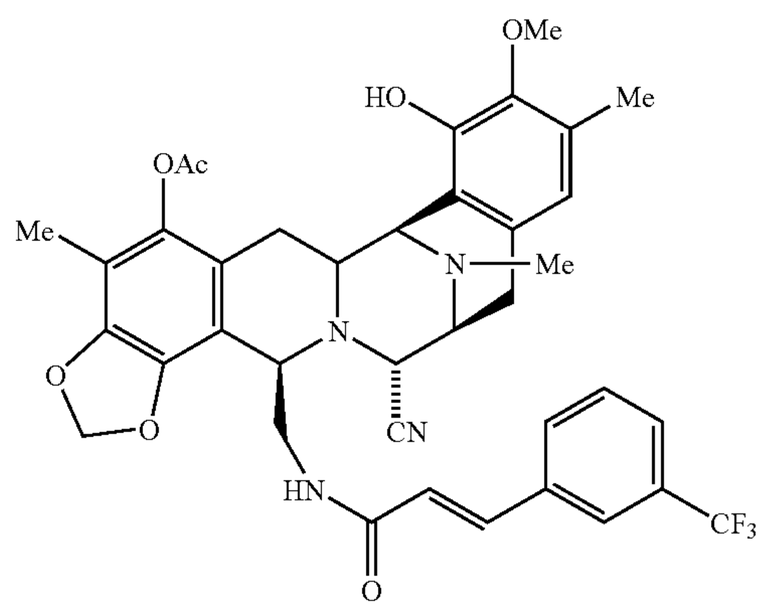
ESI-MS m/z: Calcd. for $C_{38}H_{50}N_4O_7$: 674.83. Found $(M+H)^+$: 675.5.

Example 44



45

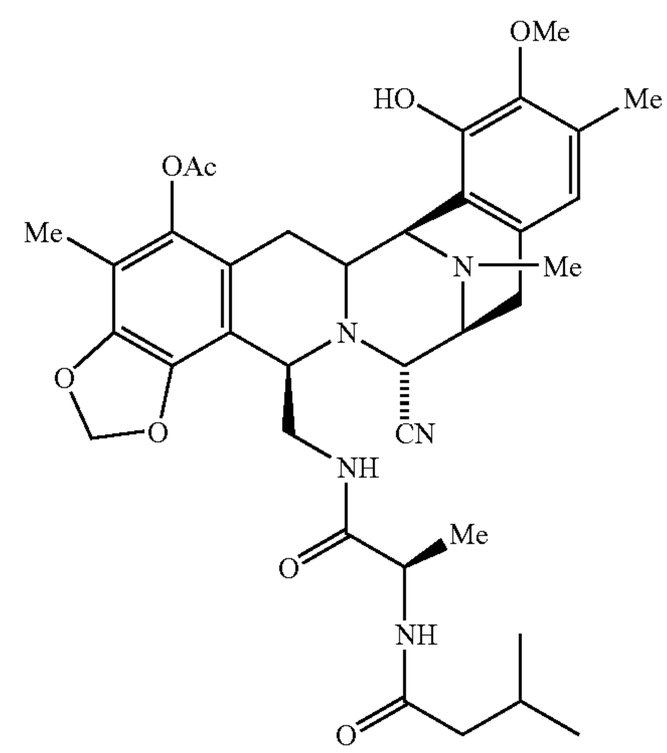




50

Example 45

43



51

To a solution of 45 (14.5 mg, 0.0278 mmol) in CH_2Cl_2 (0.3 ml), trans-3-trifluoromethyl cinnamoyl chloride (4.76 ml, 0.0278 mmol) and pyridine (2.25 ml, 0.0278 mmol) were added at 0° C. The reaction mixture was stirred for 1 h and then, the solution was diluted with CH_2Cl_2 (5 ml) and washed with 0.1 N HCl (3 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, Hex:ethyl acetate 1:1) to afford 50 (18.7 mg, 94%) as a white solid.

Rf: 0.64 (ethyl acetate:methanol 5:1).

^{1}H NMR (300 MHz, CH_3OD). δ 7.74-7.55 (m, 4H), 7.23 (d, J=16.0 Hz, 1H), 6.34 (s, 1H), 6.12 (d, J=16.0 Hz, 1H), 6.07 (d, J=0.9 Hz, 1H), 5.96 (d, J=0.9 Hz, 1H), 4.39 (d, J=2.4 Hz, 1H), 4.07-4.05 (m, 1H), 3.81 (bs, 1H), 3.46-3.51 (m, 3H), 3.42 (s, 3H), 3.09 (br d, J=12.0 Hz, 1H), 2.94-2.85 (m, 2 h), 2.74 (d, J=18.3 Hz, 1H), 2.38 (s, 3H), 2.23 (s, 3H), 2.02 (s, 3H), 1.80 (s, 3H), 1.84-1.75 (m, 1H).

^{13}C NMR (75 MHz, $CDCl_3$)): δ 168.7, 165.3, 146.5, 144.7, 142.6, 140.6, 138.0, 135.9, 131.0, 130.9, 129.1, 128.6, 125.8, 125.7, 124.5, 124.4, 122.7, 121.2, 117.8, 116.5, 113.0, 112.0, 101.7, 60.4, 59.1, 56.5, 56.4, 55.6, 55.3, 41.8, 40.3, 26.6, 25.1, 20.3, 15.4, 9.3.

ESI-MS m/z: Calcd. for $C_{38}H_{37}F_3N_4O_7$: 718.72. Found $(M+H)^+$: 719.3.

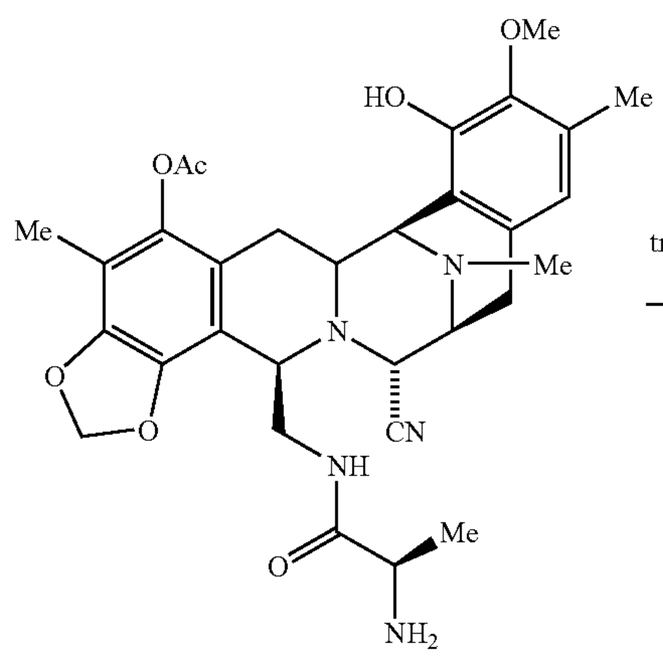
To a solution of 43 (33 mg, 0.0557 mmol) in CH_2Cl_2 (0.4 ml), isovaleryl chloride (6.79 ml, 0.0557 mmol) and pyridine (4.5 ml, 0.0557 mmol) were added at 0° C. The reaction mixture was stirred for 1 h and then, the solution was diluted with CH_2Cl_2 (5 ml) and washed with 0.1 N HCl (3 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, Hex: ethyl acetate 1:2) to afford 51 (34 mg, 91%) as a white solid.

Rf: 0.09 (Hex:ethyl acetate 1:2).

^{1}H NMR (300 MHz, $CDCl_3$): δ 6.46 (s, 1H), 6.10 (bs, 1H), 5.99 (d, J=0.9 Hz, 1H), 5.90 (d, J=0.9 Hz, 1H), 5.30 (t, J=6.0 Hz, 1H), 4.10-4.05 (m, 3H), 3.81 (bs, 1H), 3.74 (s, 3H), 3.54 (bs, 1H), 3.38-3.36 (m, 1H), 3.29-3.21 (m, 1H), 3.00 (dd, J_1=8.0 Hz, J_2=18.0 Hz, 1H), 2.25 (s, 3H), 2.20 (s, 3H), 2.00 (s, 3H), 1.95-1.90 (m, 3H), 0.87 (d, J=6.6 Hz, 6H), 0.76 (d, J=6.0 Hz, 3H).

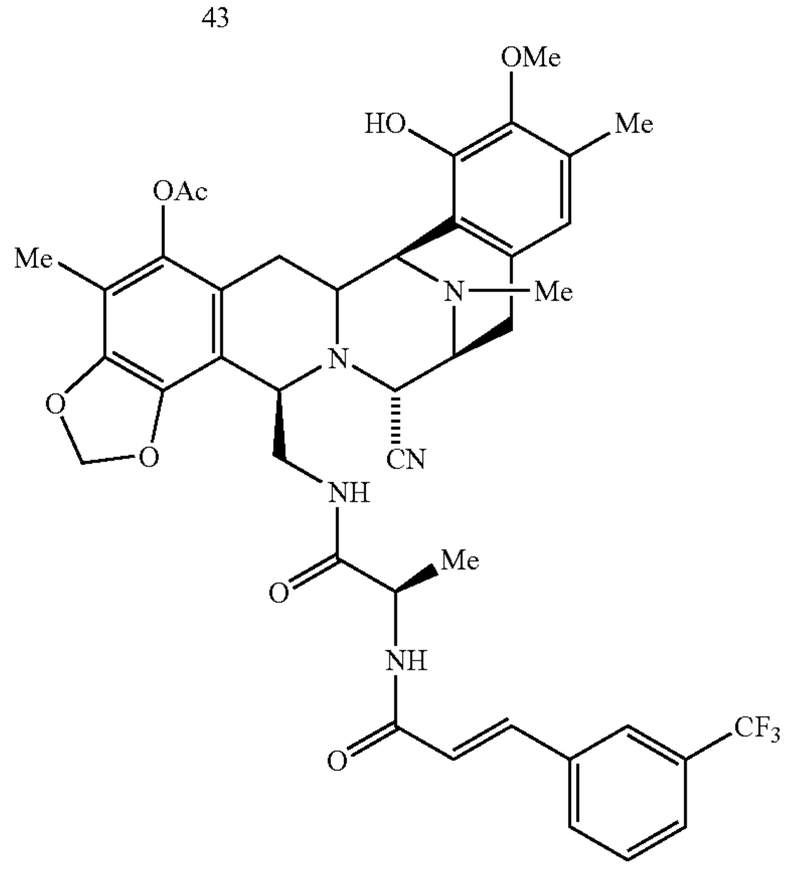
ESI-MS m/z: Calcd. for $C_{36}H_{45}N_5O_8$: 675.77. Found $(M+H)^+$: 676.3.

Example 46



43

trans-$C_{10}H_6ClF_3O$, Py, CH_2Cl_2, 0°, 1 h.



52

To a solution of 43 (33 mg, 0.0557 mmol) in CH_2Cl_2 (0.4 ml), trans-3-trifluoromethyl cinnamoyl chloride (9.52 ml, 0.0557 mmol) and pyridine (4.5 ml, 0.0557 mmol) were added at 0° C. The reaction mixture was stirred for 1 h and then, the solution was diluted with CH_2Cl_2 (5 ml) and washed with 0.1 N HCl (3 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, Hex:ethyl acetate 1:2) to afford 52 (40 mg, 92%) as a white solid.

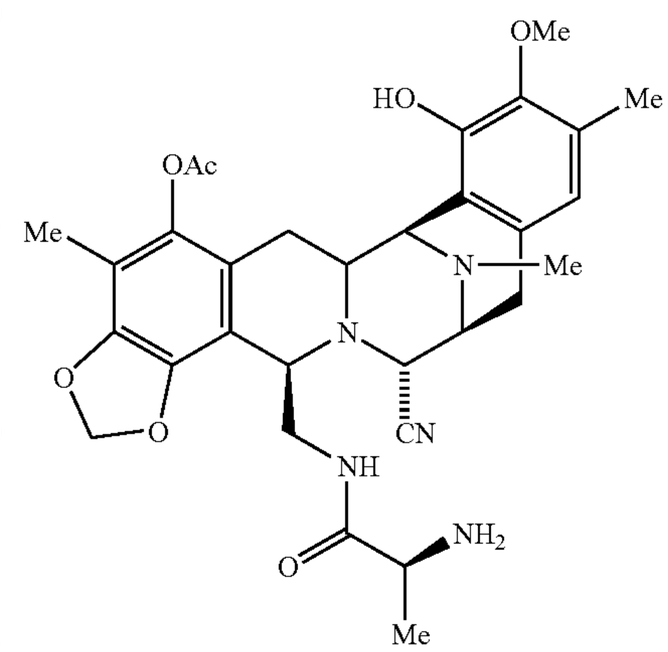
Rf: 0.21 (hexane:ethyl acetate 1:2).

^{1}H NMR (300 MHz, CD_3OD). δ 7.74-7.47 (m, 4H), 6.49 (s, 1H), 6.40 (d, J=15.6 Hz, 1H), 6.00 (d, J=1.5 Hz, 1H), 5.90 (d, J=1.5 Hz, 1H), 5.47 (t, J=6 Hz, 1H), 4.12-4.09 (m, 3H), 3.93 (bs, 1H), 3.71 (s, 3H), 3.59-3.58 (m, 1H), 3.38 (d, J=7.8 Hz, 1H), 3.29 (d, J=12.0 Hz, 1H), 3.00 (dd, J_1=8.1 Hz, J_2=18.3 Hz, 1H), 2.79-2.78 (m, 1H), 2.65 (d, J=18.3 Hz, 1H) 2.29 (s, 6H), 2.28 (s, 3H), 2.22 (s, 3H), 1.84-1.80 (m, 1H), 0.85-0.84 (m, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 171.9, 168.8, 164.4, 146.9, 144.6, 143.0, 140.5, 140.5, 139.3, 135.7, 131.1, 131.0, 129.4, 129.1, 126.0, 124.1, 124.0, 122.4, 121.1, 120.7, 120.6, 117.7, 116.9, 112.8, 112.0, 101.6, 60.6, 59.3, 57.1, 56.3, 55.9, 55.2, 49.0, 41.7, 49.9, 26.5, 25.1, 20.2, 18.4, 15.7, 9.3.

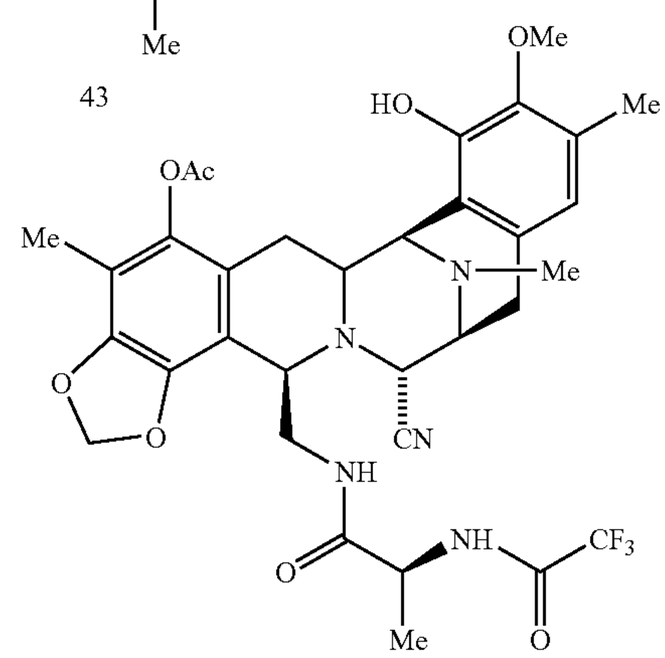
ESI-MS m/z: Calcd. for $C_{41}H_{42}F_3N_5O_8$: 789.8. Found $(M+H)^+$: 790.3.

Example 47



43

Trifluoroacetic anhydride, CH_2Cl_2, 5 h, 23° C.



53

To a solution of 43 (10 mg, 0.0169 mmol) in CH_2Cl_2 (0.2 ml) trifluoroacetic anhydride (2.38 μl, 0.0169 mmol) was added at 23° C. The reaction mixture was stirred for 5 h and then, the solution was diluted with CH_2Cl_2 (5 ml) and washed with 0.1 N HCl (3 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, Hex:ethyl acetate 3:2) to afford 53 (10.7 mg, 93%) as a white solid.

Rf: 0.57 (ethyl acetate:methanol 5:1).

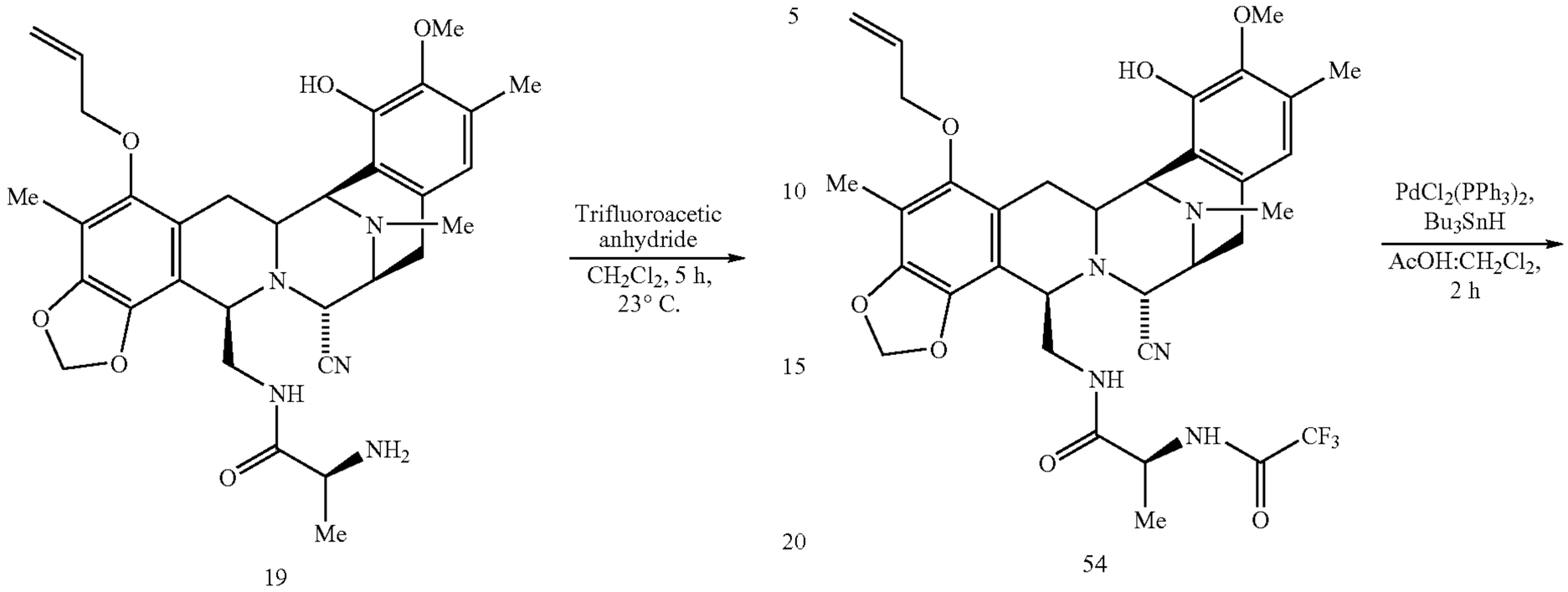
^{1}H NMR (300 MHz, $CDCl_3$) δ 6.45 (s, 1H), 6.00 (d, J=1.2 Hz, 1H), 5.90 (d, J=1.2 Hz, 1H), 5.87 (bs, 1H), 5.32 (bs, 1H), 4.12(d, J=2.1 Hz, 1H), 4.08 (d, J=1.8 Hz, 1H), 3.78-3.56 (m, 3H), 3.72 (s, 3H), 3.40 (d, J=8.1 Hz, 1H), 3.25 (d, J=9.3 Hz, 1H), 3.00 (dd, J_1=8.4 Hz, J_2=18.0 Hz, 1H), 2.77 (dd, J_1=2.1 Hz, J_2=15.9 Hz, 1H), 2.68 (d, J=18.6 Hz, 1H), 2.30 (s, 3H), 2.28 (s, 3H), 2.22 (s, 3H), 2.00 (s, 3H), 1.75 (dd, J_1=11.4 Hz, J_2=15.9 Hz, 1H), 0.69 (d, J=6.3 Hz, 3H).

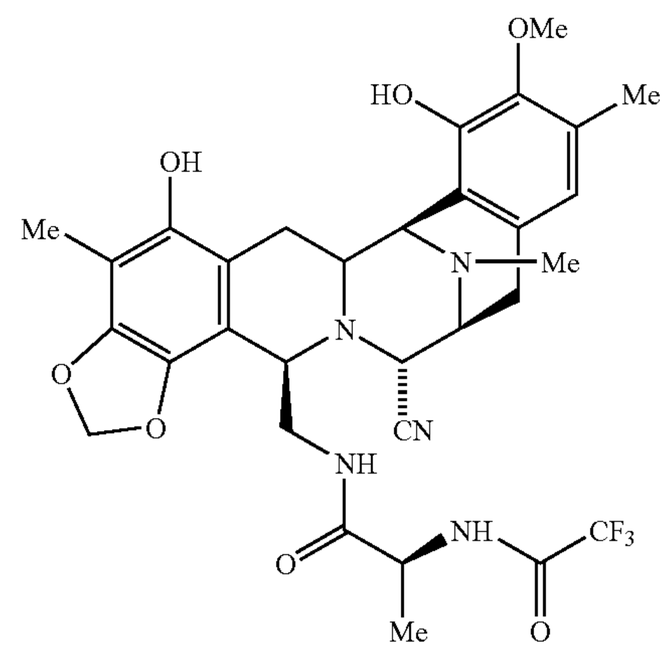
^{13}C NMR (75 MHz, $CDCl_3$) δ 170.1, 168.6, 156.0, 147.0, 144.6, 143.0, 140.6, 140.4, 131.0, 129.4, 120.9, 120.7, 117.6, 116.8, 112.4, 112.1, 101.6, 60.5, 59.0, 57.1, 56.3, 55.6, 55.2, 48.7, 41.6, 39.4, 26.5, 24.9, 20.2, 17.8, 15.4, 9.2.

ESI-MS m/z: Calcd. for $C_{33}H_{36}F_3N_5O_8$: 687.63. Found $(M+H)^+$: 688.66.

Example 48

Example 49





To a solution of 19 (11 mg, 0.0169 mmol) in CH_2Cl_2 (0.2 ml) trifluoroacetic anhydride (2.38 ml, 0.0169 mmol) was added at 23° C. The reaction mixture was stirred for 5 h and then, the solution was diluted with CH_2Cl_2 (5 ml) and washed with 0.1 N HCl (3 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, Hex:ethyl acetate 3:2) to afford 54 (10.7 mg, 93%) as a white solid.

Rf: 0.6 (ethyl acetate:methanol5:1).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33 (d, J=6.3 Hz, 1H), 6.45 (s, 1H), 6.04 (m, 1H), 5.95 (d, J=1.5 Hz, 1H), 5.84 (d, J=1.5 Hz, 1H), 5.32 (m, 2 h), 5.21 (m, 1H), 4.11 (m, 4H), 3.73 (s, 3H), 3.64 (m, 2 h), 3.51 (m, 1H), 3.37 (d, J=7.8 Hz, 1H), 3.22 (m, 2 h), 3.03 (dd, 1H, J_1=8.1 Hz, J_2=18.3 Hz, 1H), 2.60 (d, J=18.3 Hz, 1H), 2.29 (s, 3H), 2.24 (s, 3H), 2.08 (s, 3H), 1.86 (dd, J_1=12 Hz, J_2=16.2 Hz, 1H), 0.82 (d, J=7.2 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 170.0, 156.0, 148.4, 147.1, 144.3, 143.0, 138.7, 133.8, 130.5, 129.4, 120.6, 120.4, 117.6, 117.5, 117.0, 113.5, 112.5, 112.4, 101.1, 74.1, 66.8, 60.4, 59.3, 56.9, 56.6, 56.3, 55.4, 48.7, 41.6, 40.1, 26.2, 25.0, 17.6, 15.4, 9.1.

ESI-MS m/z: Calcd. for $C_{35}H_{39}F_3N_5O_7$: 685.69. Found $(M+H)^+$: 686.3.

To a solution of 54 (100 mg, 0.415 mmol) in CH_2Cl_2 (4 ml), acetic acid (40 ml), $(PPh_3)_2PdCl_2$ (8.4 mg, 0.012 mmol) and Bu_3SnH (157 ml, 0.56 mmol) were added at 23° C. After stirring at that temperature for 2 h the reaction was poured into a pad of flash column (SiO_2, gradient Hex to hexane:ethyl acetate 2:1) to afford 55 (90 mg, 96%) as a white solid.

Rf: 0.6 (hexane:ethyl acetate 1:2).

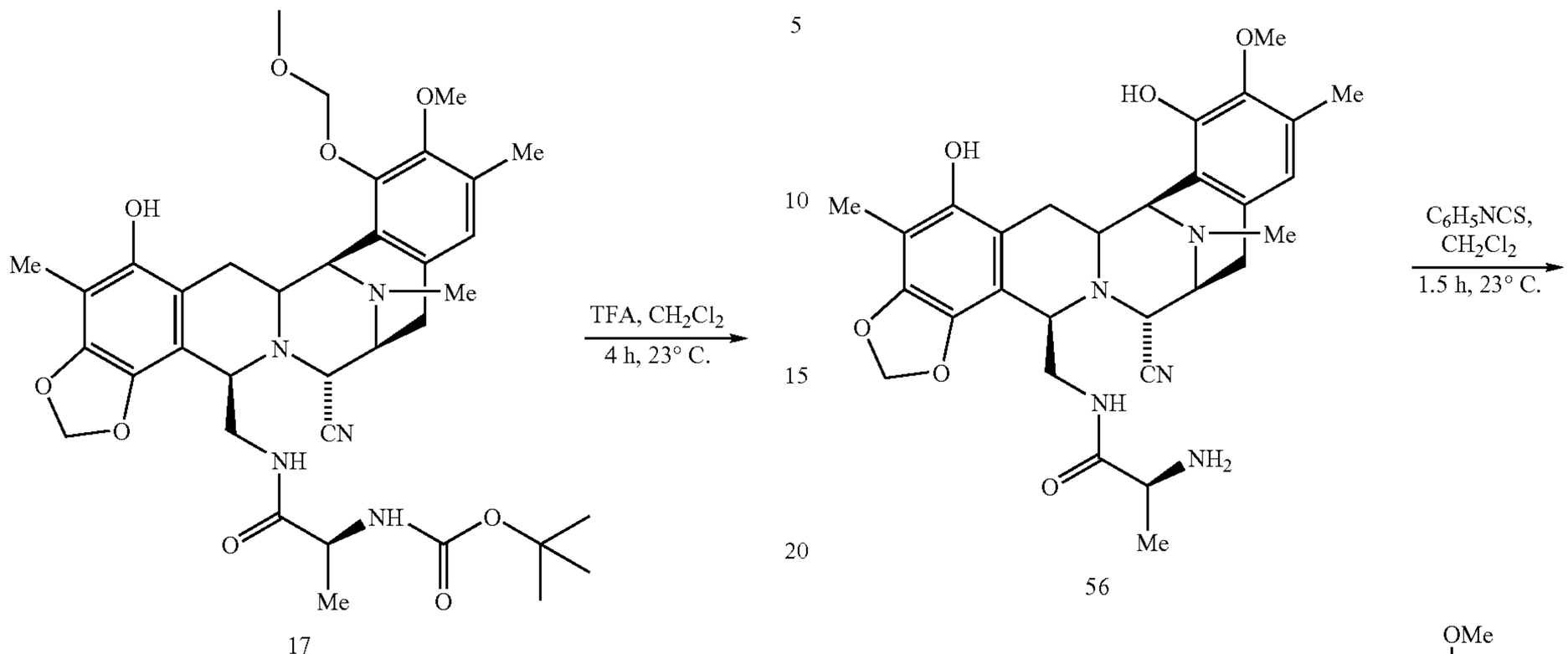
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55 (d, J=7.2 Hz, 1H), 6.45 (s, 1H), 5.90 (d, J=1.2 Hz, 1H), 5.82 (d, J=1.2 Hz, 1H), 5.37 (t, J=6.0 Hz, 1H), 4.15 (d, J=2.1 Hz, 1H), 4.04 (d, J=1.8 Hz, 1H), 3.70 (s, 3H), 3.66-3.53 (m, 2 h), 3.37-3.31 (m, 2 h), 3.19-3.15 (d, J=11.7 Hz, 1H), 3.08-3.00 (m, 2 h), 2.56 (d, J=18.3 Hz, 1H), 2.30 (s, 3H), 2.24 (s, 3H), 2.04 (s, 3H), 1.91 (dd, J_1=12.0 Hz, J_2=15.6 Hz, 1H), 0.84 (d, J=6.9 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 170.1, 156.3, 147.3, 144.9, 144.4, 143.3, 136.7, 130.7, 129.3, 120.6, 117.6, 117.4, 114.4, 112.1, 107.7, 101.0, 85.8, 60.5, 59.3, 56.5, 56.4, 56.2, 55.2, 48.9, 41.6, 40.9, 25.7, 25.3, 18.0, 15.6, 8.7.

ESI-MS m/z: Calcd. for $C_{32}H_{35}F_3N_5O_7$: 645.63. Found $(M+H)^+$: 646.2.

Example 50

Example 51



17

56

56

57

To a solution of 17 (200 mg, 0.288 mmol) in CH_2Cl_2 (1.44 ml), trifluoroacetic acid (888 ml, 11.53 mmol) was added and the reaction mixture was stirred for 4 h at 23° C. The reaction was quenched at 0° C. with saturated aqueous sodium bicarbonate (60 ml) and extracted with ethyl acetate (2×70 ml). The combined organic layers were dried (sodium sulphate) and concentrated in vacuo to afford 56 (147 mg, 93%) as a white solid that was used in subsequent reactions with no further purification.

Rf: 0.19 (ethyl acetate:methanol5:1).

^{1}H NMR (300 MHz, CD_3OD). δ 6.48 (s, 1H), 5.88, d, J=0.9 Hz, 1H), 5.81 (d, J=0.9 Hz, 1H), 4.35 (d, J=2.4 Hz, 1H), 4.15 (d, J=1.8 Hz, 1H), 3.99-3.98 (m, 1H), 3.70 (s, 3H), 3.52-2.96 (m, 7H), 2.68 (d, J=18.3 Hz, 1H), 2.24 (s, 3H), 2.23 (s, 3H), 2.06 (s, 3H), 1.85 (dd, J_1=11.7 Hz, J_2=15.6 Hz, 1H), 0.91 (d, J=6.6 Hz, 3H).

^{13}C NMR (75 MHz, CD_3OD): δ 173.2, 149.1, 145.6, 144.9, 138.0, 132.2, 130.6, 121.4, 119.6, 117.4, 114.3, 109.2, 102.5, 82.3, 60.4, 58.4, 58.3, 57.8, 56.6, 50.1, 42.3, 41.6, 27.8, 26.2, 19.5, 15.5, 9.8.

ESI-MS m/z: Calcd. for $C_{29}H_{35}N_5O_6$: 549.62. Found $(M+H)^+$: 550.3.

To a solution of 56 (10 mg, 0.018 mmol) in CH_2Cl_2 (0.4 ml), phenyl isothiocyanate (13 ml, 0.109 mmol) was added and the reaction was stirred at 23° C. for 1.5 h. The mixture was concentrated in vacuo and the residue was purified by flash column chromatography (SiO_2, gradient Hexane to 1:1 hexane:ethyl acetate) to afford 57 (8 mg, 65%) as a white solid.

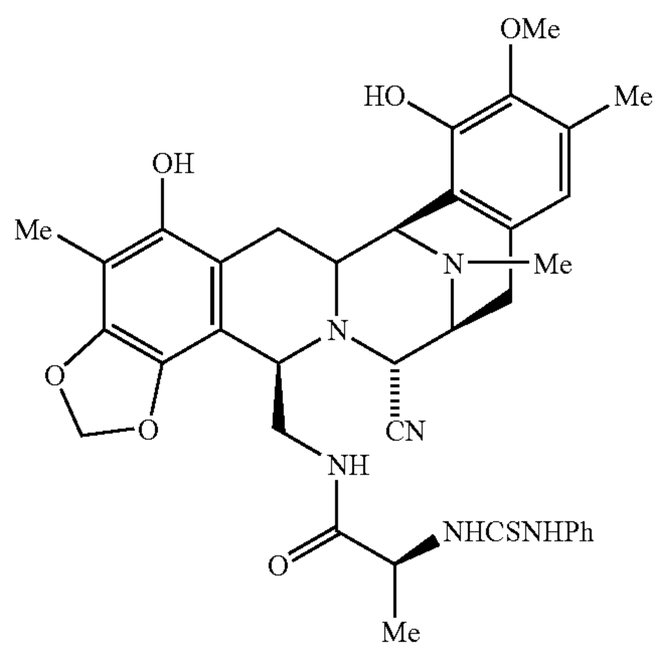
Rf: 0.57 (ethyl acetate:methanol 10:1).

^{1}H NMR (300 MHz, $CDCl_3$): δ 7.88 (bs, 1H), 7.41-7.36 (m, 2 h), 7.27-7.22 (m, 1H), 7.02-7.00 (d, J=7.8 Hz, 2 h), 6.71 (d, J=7.2 Hz, 1H), 6.31 (s, 1H), 6.17 (bs, 1H), 5.93 (d, J=1.2 Hz, 1H), 5.83 (d, J=1.2 Hz, 1H), 5.55 (bs, 1H), 5.20-5.17 (m, 1H), 4.16 (d, J=1.8 Hz, 1H), 4.05 (bs, 1H), 4.02 (d, J=2.4 Hz, 1H), 3.79 (s, 3H), 3.75-3.71 (m, 1H), 3.35 (d, J=7.8 Hz, 1H), 3.28-3.19 (m, 2 h), 3.12-2.97 (m, 2 h), 2.50 (d, J=18.3 Hz, 1H), 2.32 (s, 3H), 2.21 (s, 3H), 2.15-2.09 (dd, J_1=11.4 Hz, J_2=15.9 Hz, 1H), 1.95 (s, 3H), 0.88 (d, J=6.9 Hz; 3H).

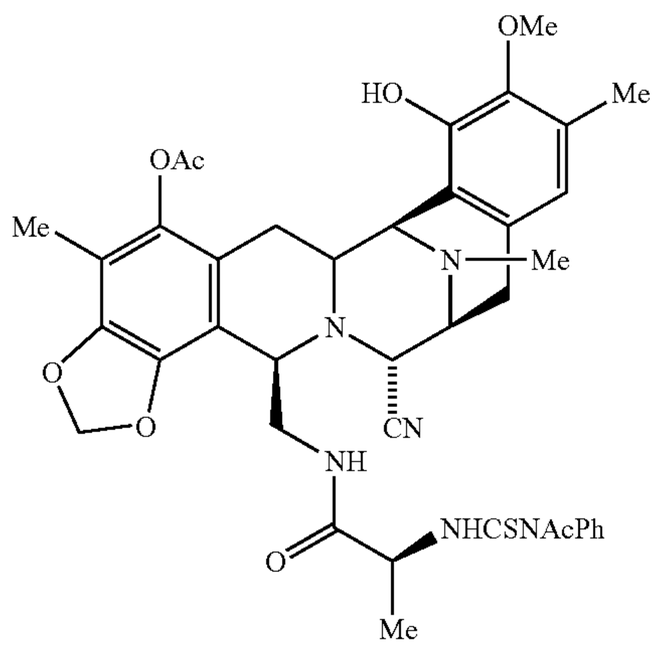
^{13}C NMR (75 MHz, $CDCl_3$): δ 178.5, 171.7, 147.2, 145.0, 144.3, 143.3, 137.0, 135.7, 130.6, 130.4, 129.6, 127.5, 124.3, 120.6, 117.7, 117.2, 115.3, 112.1, 108.3, 100.9, 60.9, 59.5, 56.7, 56.5, 56.2, 55.2, 54.1, 41.7, 41.1, 26.3, 25.4, 18.5, 15.8, 9.0.

ESI-MS m/z: Calcd. for $C_{36}H_{40}N_6O_6S$: 684.81. Found $(M+H)^+$: 685.3.

Example 52



57



58

To a solution of 57 (45 mg, 0.065 mmol) in CH_2Cl_2 (0.5 ml), acetyl chloride (4.67 ml, 0.065 mmol) and pyridine (5.3 ml, 0.065 mmol) were added at 0° C. The reaction mixture was stirred for 3 h and then, the solution was diluted with CH_2Cl_2 (10 ml) and washed with 0.1 N HCl (5 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography (RP-18, CH_3CN: H_2O 40:60) to afford 58 (14 mg, 28%) as a white solid.

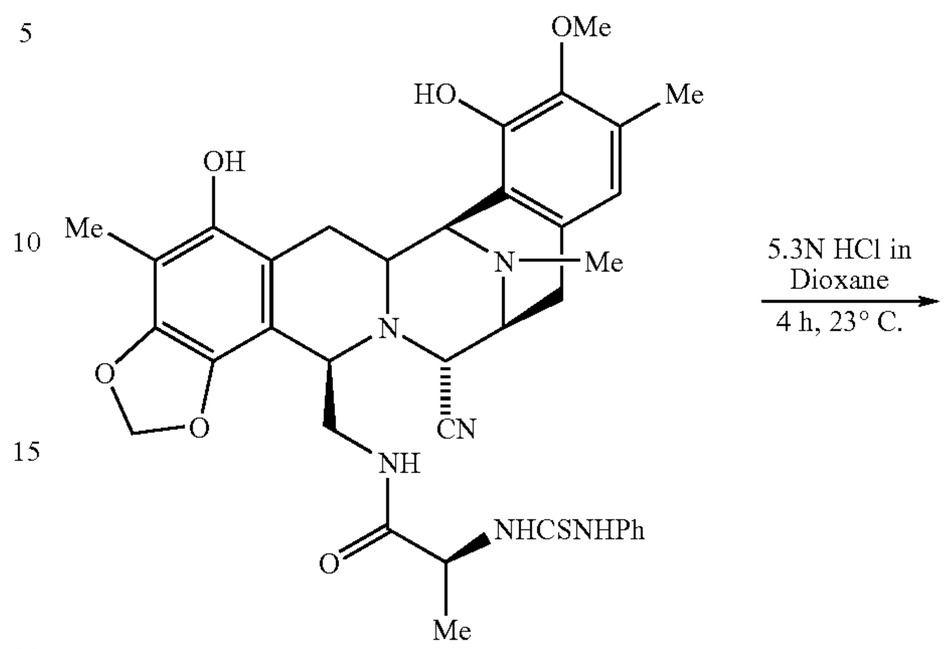
Rf: 0.34 (CH_3CN: H_2O 7:15).

^{1}H NMR (300 MHz, $CDCl_3$). δ 11.90 (d, J=6.6 Hz, 1H), 7.45-7.40 (m, 3H), 7.18-7.15 (m, 2 h), 6.58 (s, 1H), 6.00 (d, J=1.2 Hz, 1H), 5.89 (d, J=1.2 Hz, 1H), 5.70 (s, 1H), 5.37 (t, J=4.8 Hz, 1H), 4.48 (m, 1H), 4.23 (bs, 1H), 4.07 (bs, 2 h), 3.85-3.75 (m, 1H), 3.70 (s, 3H), 3.46-3.41 (m, 2 h), 3.24-3.20 (m, 1H), 3.00-2.95 (m, 1H), 2.87-2.75 (m, 1H), 2.31 (s, 3H), 2.28 (s, 3H), 2.24 (s, 3H), 2.00 (s, 3H), 1.85 (dd, J_1=11.4 Hz, J_2=15.6 Hz, 1H), 1.66 (s, 3H), 0.82 (d, J=6.0 Hz, 3H).

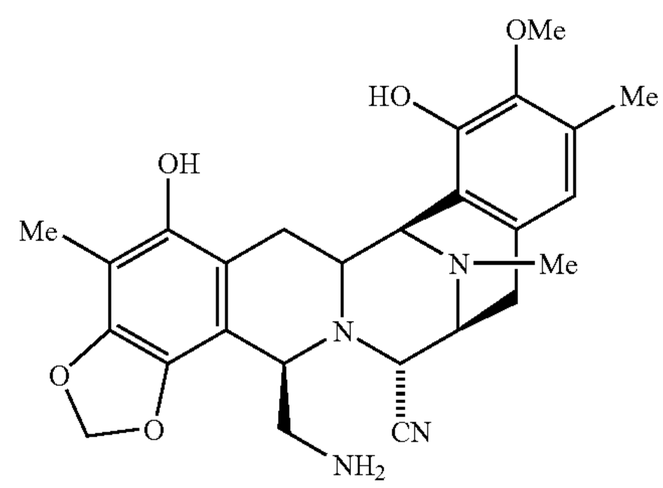
^{13}C NMR (75 MHz, $CDCl_3$)): δ 182.6, 174.3, 171.0, 146.6, 144.6, 142.7, 142.3, 140.7, 140.2, 131.3, 129.8, 129.3, 128.9, 128.8, 121.5, 120.4, 117.3, 116.6, 112.8, 112.0, 111.3, 101.5, 60.5, 59.0, 57.6, 56.2, 55.9, 55.3, 55.1, 41.6, 39.4, 27.8, 26.5, 24.8, 20.2, 17.1, 15.5, 9.3.

ESI-MS m/z: Calcd. for $C_{40}H_{44}N_6O_8S$: 768.88. Found $(M+H)^+$: 769.2.

Example 53



57



59

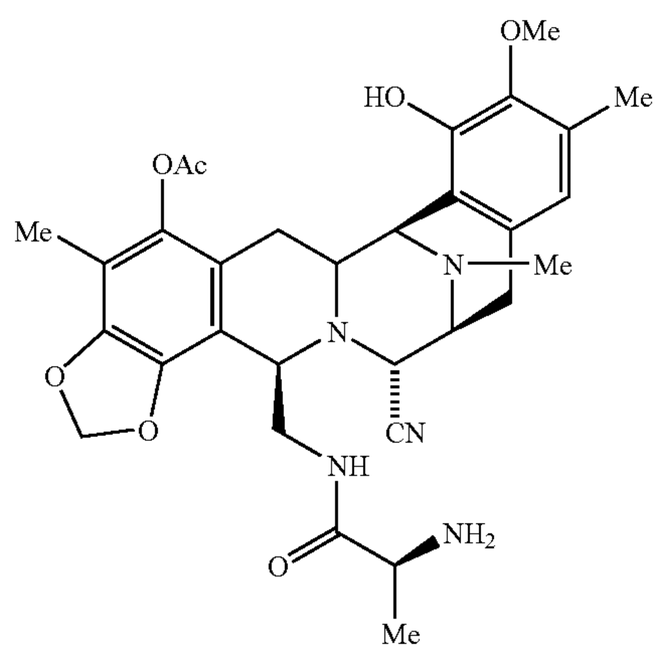
A solution of 57 (130 mg, 0.189 mmol) in dioxane (1 ml), 5.3N HCl/dioxane (1.87 ml) was added and the reaction was stirred at 23° C. for 4 h. Then, CH_2Cl_2 (15 ml) and H_2O (10 ml) were added to this reaction and the organic layer was decanted. The aqueous phase was basified with saturated aq sodium bicarbonate (60 ml) (pH=8) at 0° C. and then, extracted with ethyl acetate (2×50 ml). The combined organic extracts were dried (sodium sulphate), and concentrated in vacuo to afford 59 (63 mg, 70%) as a white solid.

Rf: 0.15 (ethyl acetate:methanol5:1).

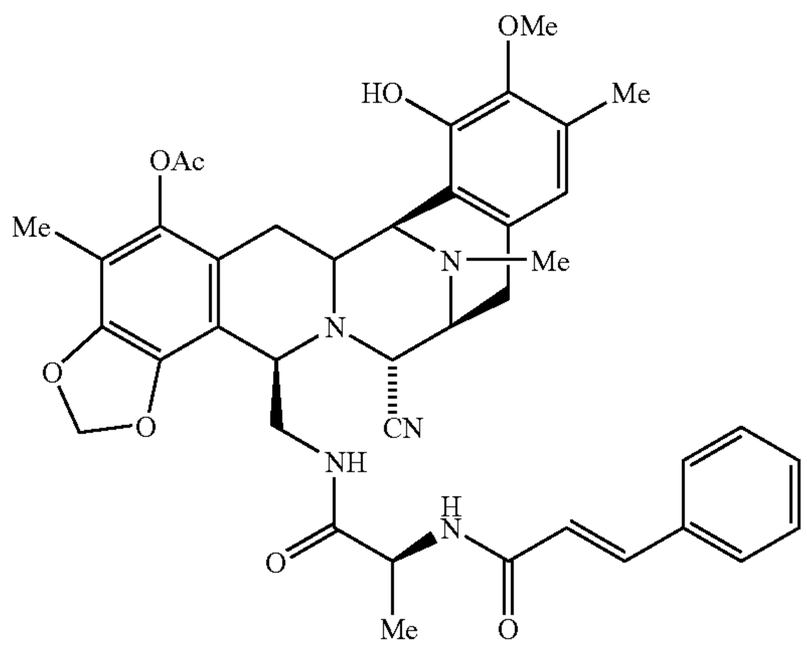
^{1}H NMR (300 MHz, $CDCl_3$). δ 6.67 (s, 1H), 5.99 (d, J=0.9 Hz, 1H), 5.91 (d, J=1.2 Hz, 1H), 5.10 (bs, 1H), 4.32 (d, J=7.2 Hz, 1H), 4.25 (dd, J_1=3.6 Hz, J_2=9.3 Hz, 1H), 3.7 (s, 3H), 3.71-3.64 (m, 2 h), 3.50 (dd, J_1=2.4 Hz, J_2=15.9 Hz, 1H), 3.42-3.37 (m, 2 h), 3.16 (dd, J_1=3.6 Hz, J_2=12.9 Hz, 1H), 2.57 (dd, J_1=9.3 Hz, J_2=12.9 Hz, 1H), 2.27 (s, 3H), 2.11 (s, 3H), 1.91 (dd, J_1=12.0 Hz, J_2=15.9 Hz, 1H).

ESI-MS m/z: Calcd. for $C_{26}H_{30}N_4O_5$: 478.5. Found $(M+H)^+$: 479.3.

Example 54



43



60

A solution of 43 (20 mg, 0.0338 mmol) in CH_2Cl_2 (0.3 ml), cinnamoyl chloride (5.63 mg, 0.0338 mmol) and pyridine (2.73 ml, 0.0338 mmol) were added at 0° C. The reaction mixture was stirred for 1 h and then, the solution was diluted with CH_2Cl_2 (10 ml) and washed with 0.1 N HCl (5 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, EtOAc: MeOH 20:1) to afford 60 (22 mg, 90%) as a white solid.

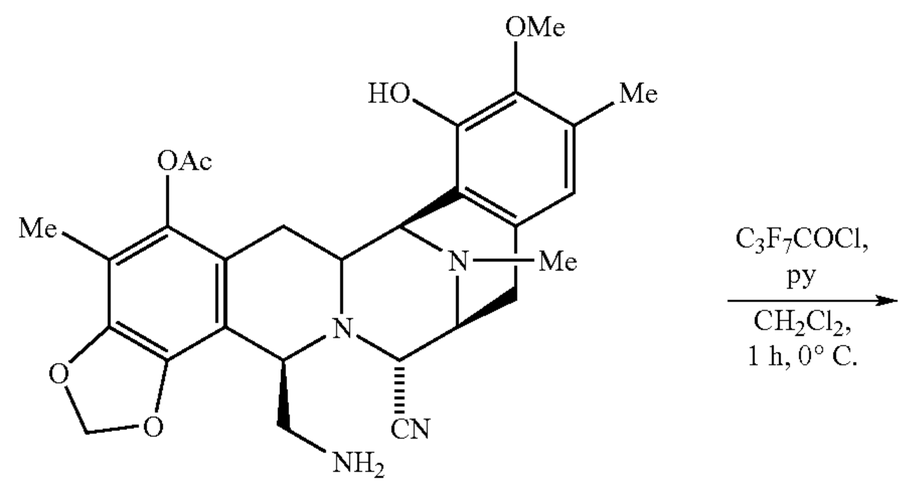
Rf: 0.56 (EtOAc:MeOH 5:1).

^{1}H NMR (300 MHz, $CDCl_3$). δ 7.51 (s, 1H), 7.50-7.47 (m, 2H), 7.36-7.35 (m, 2H), 6.43 (s, 1H), 6.36 (brd, J=15.9 Hz, 2H), 6.01 (d, J=1.5 Hz, 1H), 5.90 (brd, J=1.5 Hz, 2H), 5.42 (t, J=6.0 Hz 1H), 4.12-4.07 (m, 3H), 3.96-3.95 (m, 1H), 3.73 (bs, 3H), 3.58 (bs, 2H), 3.39 (d, J=8.7 Hz, 1H), 3.25 (d, J=11.7 Hz, 1H), 3.0 (dd, J_1=7.5 Hz, J_2=17.7 Hz, 1H), 2.78 (d, J=15.9 Hz, 1H), 2.67 (d, J=16.5 Hz, 1H), 2.29 (s, 6H), 2.23 (s, 3H), 1.99 (s, 3H), 1.82 (dd, J_1=11.4 Hz, J_2=15.6 Hz, 1H), 0.83 (d, J=6.0 Hz, 3H).

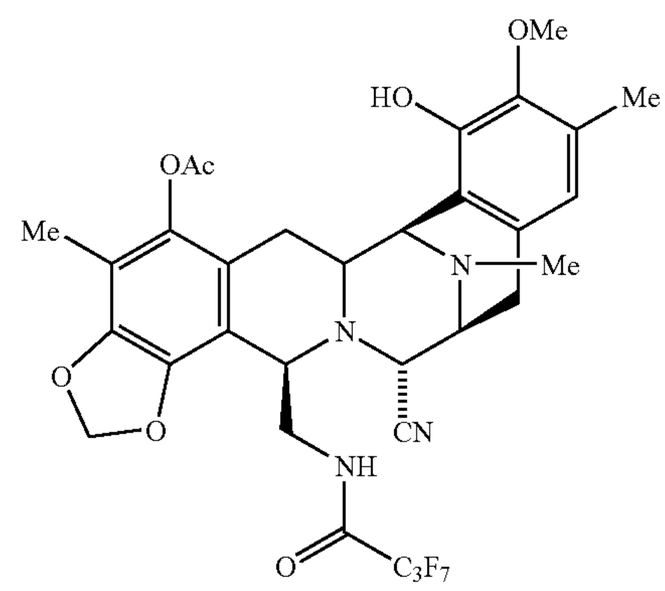
^{13}C NMR (75 MHz, $CDCl_3$)): δ 172.0, 165.0, 146.9, 144.6, 143.1, 141.0, 140.5, 134.8, 131.0, 129.7, 129.1, 128.8, 127.8, 125.5, 123.8, 123.0, 121.1, 120.5, 117.7, 116.9, 112.8, 112.0, 101.9, 60.6, 59.2, 57.1, 56.4, 55.9, 55.3, 48.8, 41.7, 40.0, 26.5, 25.1, 20.3, 18.5, 15.7, 9.3.

ESI-MS m/z: Calcd. for $C_{40}H_{43}N_5O_8$: 721.8. Found $(M+H)^+$: 722.3.

Example 55



45



61

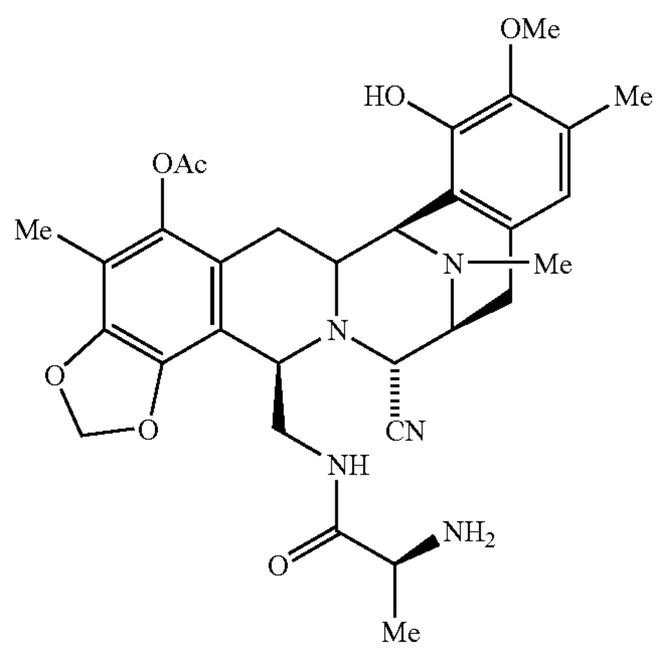
A solution of 45 (19 mg, 0.0364 mmol) in CH_2Cl_2 (0.3 ml), heptafluorobutyryl chloride (5.44 ml, 0.0364 mmol) and pyridine (2.95 ml, 0.0364 mmol) were added at 0° C. The reaction mixture was stirred for 1 h and then, the solution was diluted with CH_2Cl_2 (10 ml) and washed with 0.1 N HCl (5 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, EtOAc: MeOH 20:1) to afford 61 (11.7 mg, 45%) as a white solid.

Rf: 0.76 (EtOAc:MeOH 5:1).

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.46 (s, 1H), 6.12 (bs, 1H), 5.98 (d, J=1.2 Hz, 1H), 5.93 (d, J=1.2 Hz, 1H), 5.72 (bs, 1H), 4.13-4.11 (m, 2H), 4.0 (d, J=2.4 Hz, 1H), 3.98-3.96 (m, 1H), 3.73 (s, 3H), 3.39 (d, J=7.5 Hz, 1H), 3.39-3.28 (m, 2H), 3.09 (dd, J_1=8.1 Hz, J_2=18.0 Hz, 1H), 2.80 (d, J=16.2 Hz, 1H), 2.46 (d, J=18.3 Hz, 1H), 2.32 (s, 6H), 2.21 (s, 3H), 1.99 (s, 3H), 1.80 (dd, J_1=12.0 Hz, J_2=16.2 Hz, 1H).

ESI-MS m/z: Calcd. for $C_{32}H_{31}F_7N_4O_7$: 716.6. Found $(M+H)^+$: 717.2.

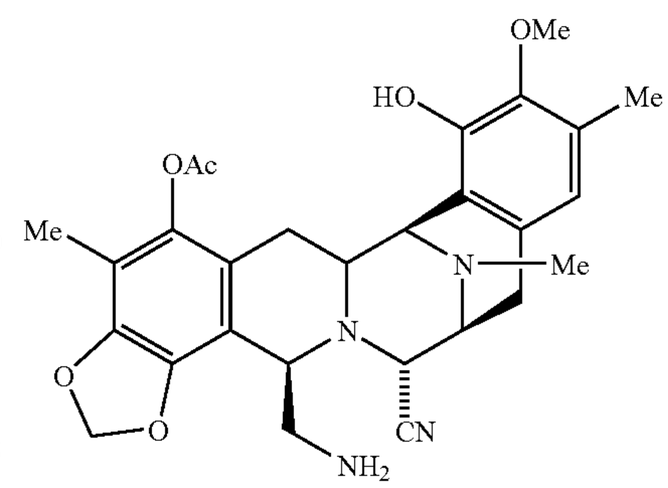
Example 56



43

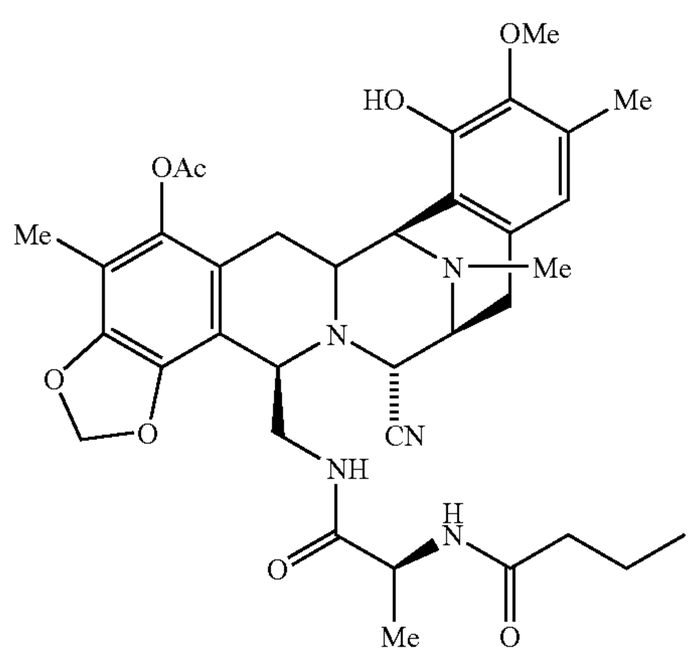
C_4H_7OCl, Py
CH_2Cl_2,
1 h, 0° C.

Example 57

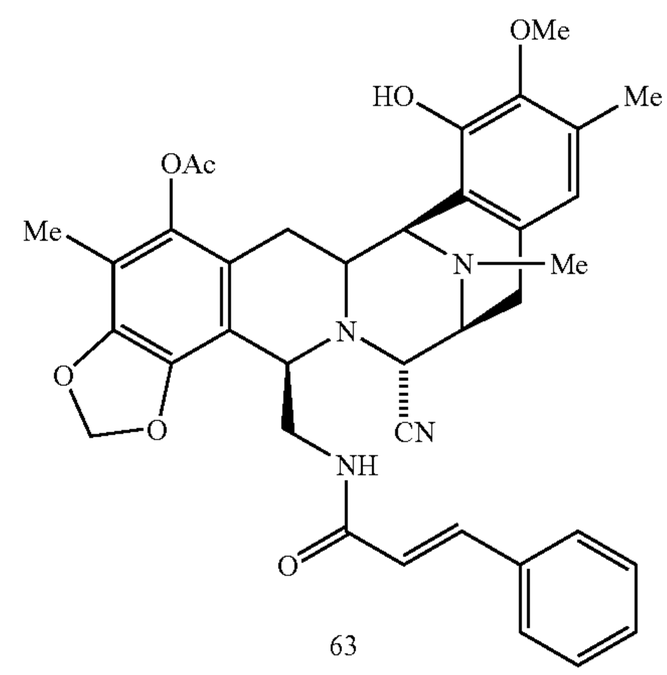


45

C_9H_7ClO, Py,
CH_2Cl_2, 0°, 1 h.



62



63

A solution of 43 (24 mg, 0.04 mmol) in CH_2Cl_2 (0.3 ml), butyryl chloride (4.15 ml, 0.04 mmol) and pyridine (3.28 ml, 0.04 mmol) were added at 0° C. The reaction mixture was stirred for 1 h and then, the solution was diluted with CH_2Cl_2 (10 ml) and washed with 0.1 N HCl (5 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, EtOAc:MeOH 20:1) to afford 62 (24 mg, 90%) as a white solid.

Rf: 0.35 (EtOAc:MeOH 5:1).

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.47 (s, 1H), 6.10 (d, J=6.5 Hz, 1H), 6.0 (d, J=1.5 Hz, 1H), 5.91 (d, J=1.5 Hz, 1H), 5.86 (bs, 1H), 5.31 (d, J=6.9 Hz, 1H), 4.11-4.06 (m, 3H), 3.85-3.81 (m, 1H), 3.75 (s, 3H), 3.59-3.53 (m, 2H), 3.38 (d, J=7.5 Hz, 1H), 3.27-3.22 (m, 1H), 3.0 (dd, J_1=7.8 Hz, J_2=17.4 Hz, 1H), 2.79 (d, J=15.3 Hz, 1H), 2.63 (d, J=17.7 Hz, 1H), 2.31 (s, 3H), 2.0 (s, 3H), 1.80 (dd, J_1=12.0 Hz, J_2=15.9 Hz, 1H), 1.58 (q, J=7.2 Hz, 2H), 0.89 (t, J=7.2 Hz, 3H), 0.76 (d, J=6.6 Hz, 3H).

ESI-MS m/z: Calcd. for $C_{35}H_{43}N_5O_8$: 661.64. Found $(M+H)^+$: 662.3

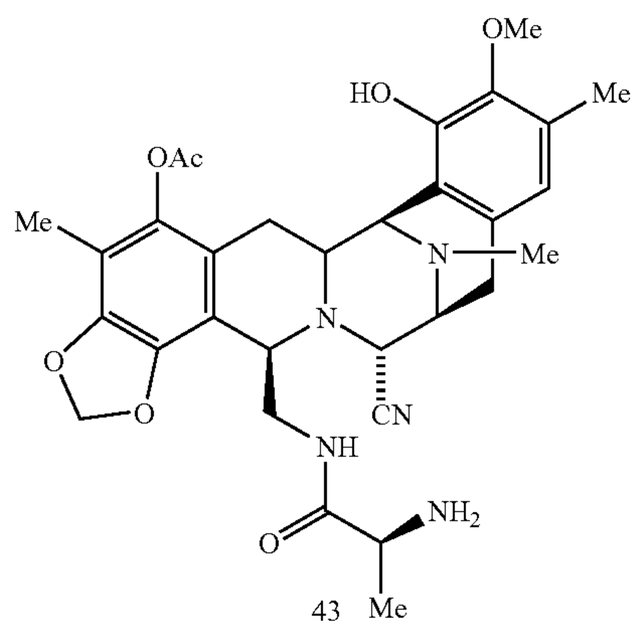
A solution of 43 (19 mg, 0.0364 mmol) in CH_2Cl_2 (0.3 ml), cinnamoyl chloride (6.06 mg, 0.0364 mmol) and pyridine (2.95 ml, 0.0364 mmol) were added at 0° C. The reaction mixture was stirred for 1h and then, the solution was diluted with CH_2Cl_2 (10 ml) and washed with 0.1 N HCl (5 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, EtOAc: MeOH 20:1) to afford 63 (20.1 mg, 85%) as a white solid.

Rf: 0.65 (EtOAc:MeOH 5:1).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39-7.29 (m, 5H), 6.42, (s, 1H), 6.01 (d, J=1.5 Hz, 1H), 5.92 (d, J=1.5 Hz, 1H), 5.73 (bs, 1H), 5.24 (t, J=6.8 Hz, 1H), 4.12-4.08 (m, 3H), 3.66-3.64 (m, 2H), 3.58 (bs, 3H), 3.36 (d, J=8.7 Hz, 1H), 3.29 (d, J=12.0 Hz, 1H), 2.98 (dd, J_1=8.1 Hz, J_2=18 Hz, 1H), 2.33 (s, 6H), 2.29 (s, 3H), 2.01 (s, 3H), 1.84 (dd, J_1=12.0 Hz, J_2=15.9 Hz, 1H).

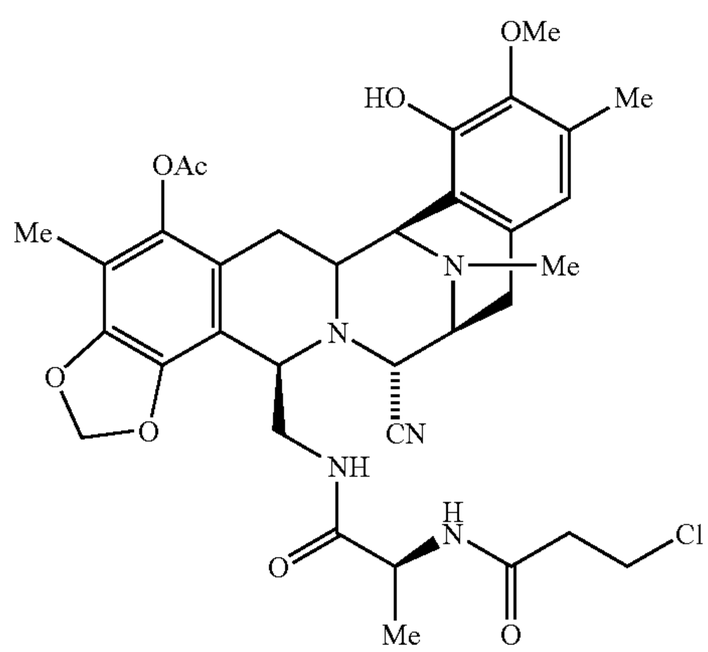
ESI-MS m/z: Calcd. for $C_{37}H_{38}N_4O_7$: 650.72. Found $(M+H)^+$: 651.2.

Example 58



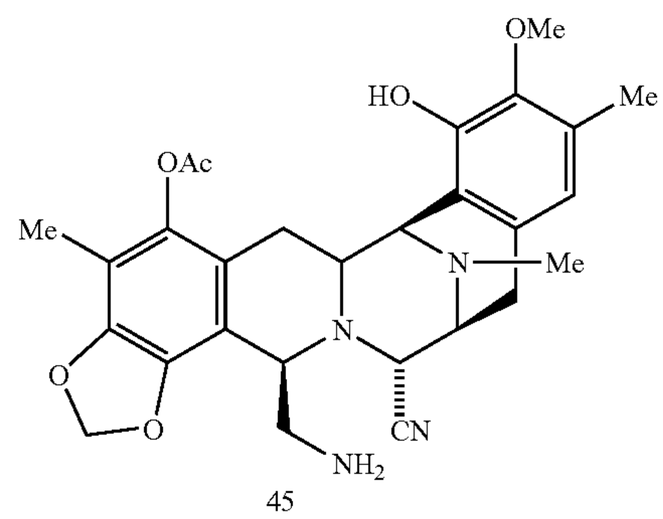
43

3-chloropropionyl chloride
Py, CH_2Cl_2,
1 h, 0° C.



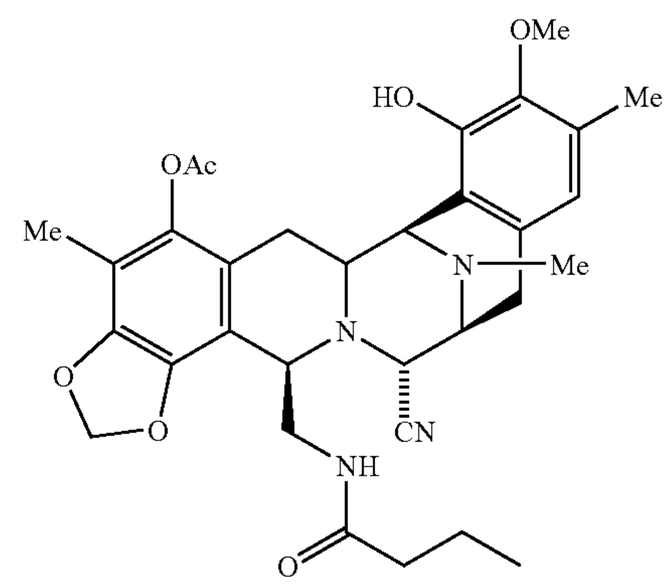
64

Example 59



45

butyryl chloride
Py, CH_2Cl_2,
1 h, 0° C.



65

A solution of 43 (20 mg, 0.0338 mmol) in CH_2Cl_2 (0.3 ml), 3-chloropropionyl chloride (3.22 ml, 0.0338 mmol) and pyridine (2.73 ml, 0.0338 mmol) were added at 0° C. The reaction mixture was stirred for 1 h and then, the solution was diluted with CH_2Cl_2 (10 ml) and washed with 0.1 N HCl (5 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, EtOAc:MeOH 20:1) to afford 64 (20.5 mg, 89%) as a white solid.

Rf: 0.32 (EtOAc:Hexane 5:1).

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.48 (s, 3H), 6.28 (m, 1H), 5.99 (d, J=1.2 Hz, 1H), 5.91 (d, J=1.2 Hz, 1H), 5.86 (bs, 1H), 5.31 (m, 1H), 4.08-4.07 (m, 3H), 3.75 (s, 3H), 3.72-3.53 (m, 5H), 3.39 (d, J=8.1 Hz, 1H), 3.24 (d, J=12.0 Hz, 1H), 3.00 (dd, J_1=8.1 Hz, J_2=18.0 Hz, 1H), 2.79 (d, J=13.5 Hz, 1H), 2.50 (t, J=6.3 Hz, 2H), 2.32 (s, 3H), 2.28 (s, 3H), 2.25 (s, 3H), 2.0 (s, 3H), 1.79 (dd, J_1=12.3 Hz, J_2=14.8 Hz, 1H), 0.81 (d, J=6.3 Hz, 3H).

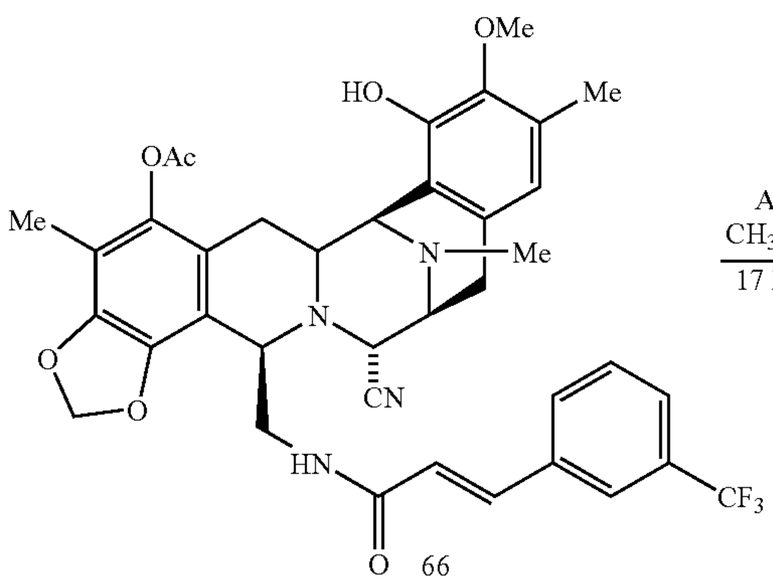
A solution of 43 (19 mg, 0.0364 mmol) in CH_2Cl_2 (0.3 ml), butyl chloride (3.78 ml, 0.0364 mmol) and pyridine (2.95 ml, 0.0364 mmol) were added at 0° C. The reaction mixture was stirred for 1 h and then, the solution was diluted with CH_2Cl_2 (10 ml) and washed with 0.1 N HCl (5 ml). The organic layer was dried over sodium sulphate, filtered, and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, EtOAc:MeOH 20:1) to afford 64 (19 mg, 87%) as a white solid.

Rf: 0.60 (EtOAc:MeOH 5:1).

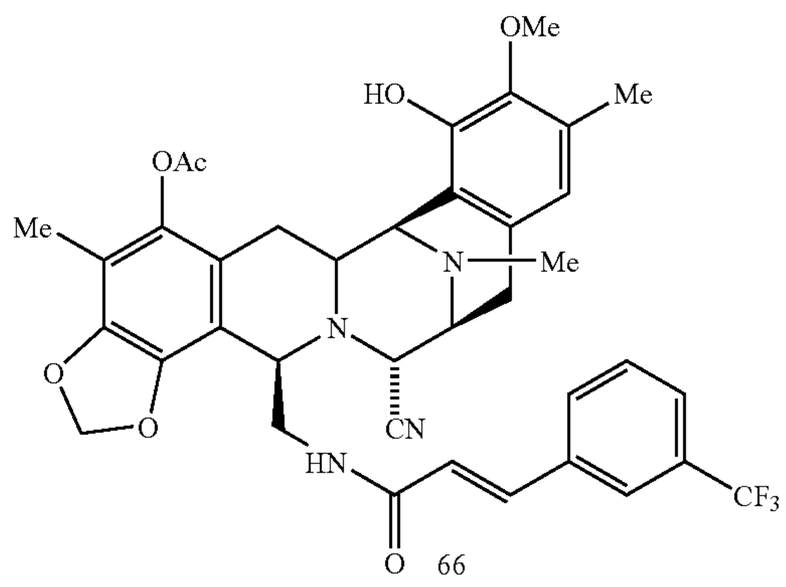
^{1}H NMR (300 MHz, $CDCl_3$) δ 6.50 (s, 1H), 5.98 (d, J=1.5 Hz, 1H), 5.91 (d, J=1.5 Hz, 1H), 5.75 (s, 1H), 5.01 (t, J=6.4 Hz, 1H), 4.10-4.09 (m, 1H), 4.06 (d, J=2.1 Hz, 1H), 4.03-4.02 (m, 1H), 3.76 (s, 3H), 3.67-3.60 (m, 1H), 3.42-3.35 (m, 2H), 3.29 (d, J=12.0 Hz, 1H), 3.02 (dd, J_1=7.8 Hz, J_2=17.7 Hz, 1H), 2.79 (d, J=14.1 Hz, 1H), 2.56 (d, J=18.3 Hz, 1H), 2.32 (s, 3H), 2.31 (s, 3H), 2.25 (s, 3H), 1.78 (dd, J_1=12.0 Hz, J_2=15.9 Hz, 1H), 1.63 (s, 3H), 1.53-1.46 (m, 2H), 1.28-1.16 (m, 2H), 0.68 (t, J=7.2 Hz, 3H).

ESI-MS m/z: Calcd. for $C_{32}H_{38}N_{4O7}$: 590.67. Found $(M+H)^+$: 591.2.

Example 60



$AgNO_3$, CH_3CN/H_2O — 17 h, 23° C.



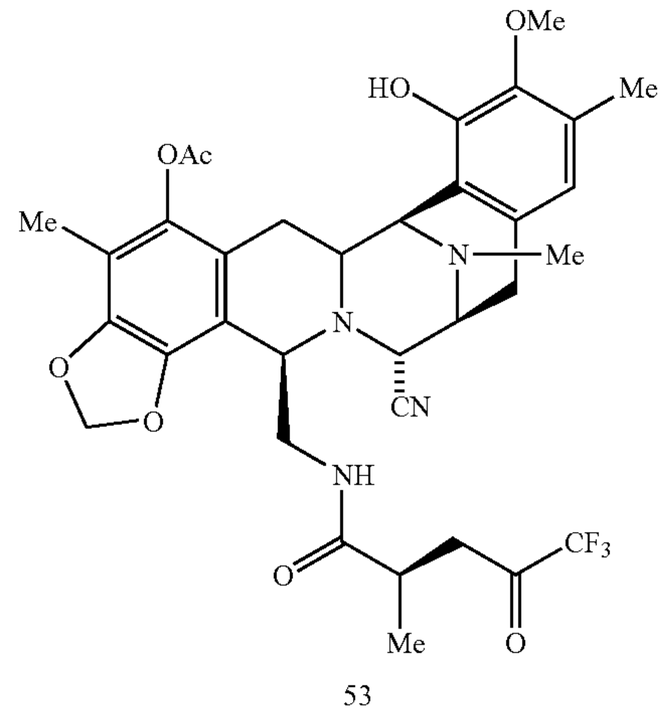
To a solution of 50 (31.7 mg, 0.044 mmol) in CH_3CN/H_2O (1.5 ml/0.5 ml), $AgNO_3$ (225 mg, 1.32 mmol) was added and the reaction was stirred at 23° C. for 17 h. Then brine (10 ml) and Aq sat $NaHCO_3$ (10 ml) were added at 0° C. and the mixture was stirred for 15 min, filtered through a pad of celite and washed with CH_2Cl_2 (20 ml). The solution was decanted and the organic layer was dried and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, EtOAc:MeOH 5:1) to afford 66 (16 mg, 51%) as a white solid.

Rf: 0.26 (EtOAc:MeOH 5:1).

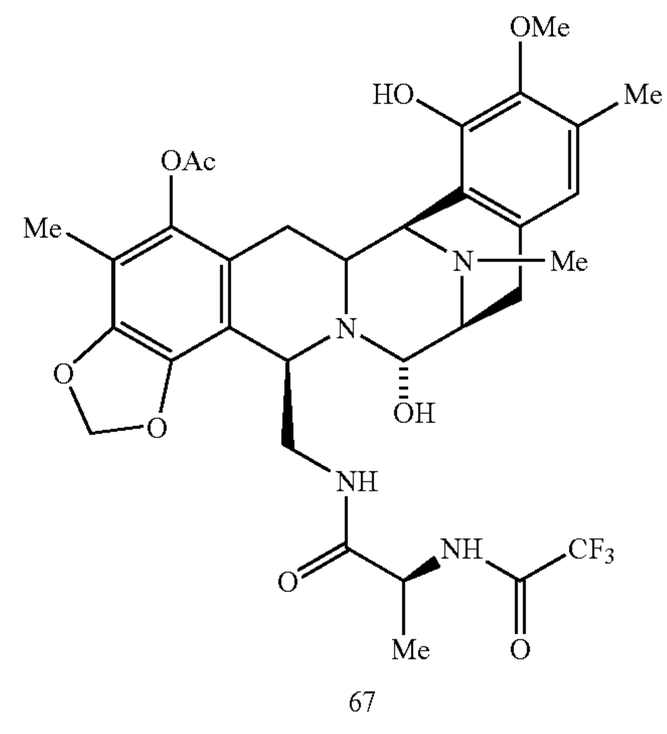
^{1}H NMR (300 MHz, $CDCl_3$) δ 7.66-7.42 (m, 4H), 7.20 (bs, 1H), 6.44 (s, 1H), 5.97 (b, J=1.2 Hz, 1H), 5.90 (d, J=1.2 Hz, 1H), 5.76 (bs, 1H), 5.28 (bs, 1H), 4.54 (bs, 1H), 4.43 (bs, 1H), 4.00 (bs, 1H), 3.68-3.57 (m, 4H), 3.47 (d, J=3.3 Hz, 1H), 3.40 (d, J=11.7 Hz, 1H), 3.17 (d, J=6.9 Hz, 1H), 2.92 (dd, J_1=8.1 Hz, J_2=17.7 Hz, 1H), 2.74 (d, J=17.1 Hz, 1H), 2.48 (d, J=18.6 Hz, 1H), 2.32 (s, 6H), 2.28 (s, 3H), 1.99 (s, 3H), 1.76 (dd, J_1=12.0 Hz, J_2=16.2 Hz, 1H).

ESI-MS m/z: Calcd. for $C_{37}H_{38}F_3N_3O_8$: 709. Found (M^+−17): 692.3.

Example 61



$AgNO_3$, CH_3CN/H_2O — 24 h, 23° C.



To a solution of 53 (57 mg, 0.0828 mmol) in CH_3CN/H_2O (1.5 mL/0.5 ml), $AgNO_3$ (650 mg, 3.81 mmol) was added and the reaction was stirred at 23° C. for 24 h. Then, brine (10 ml) and Aq sat $NaHCO_3$ (10 ml) were added at 0° C. and the mixture was stirred for 15 min, filtered through a pad of celite and washed with CH_2Cl_2 (20 ml). The solution was decanted and the organic layer was dried and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, EtOAc:MeOH 5:1) to afford 67 (28 mg, 50%) as a white solid.

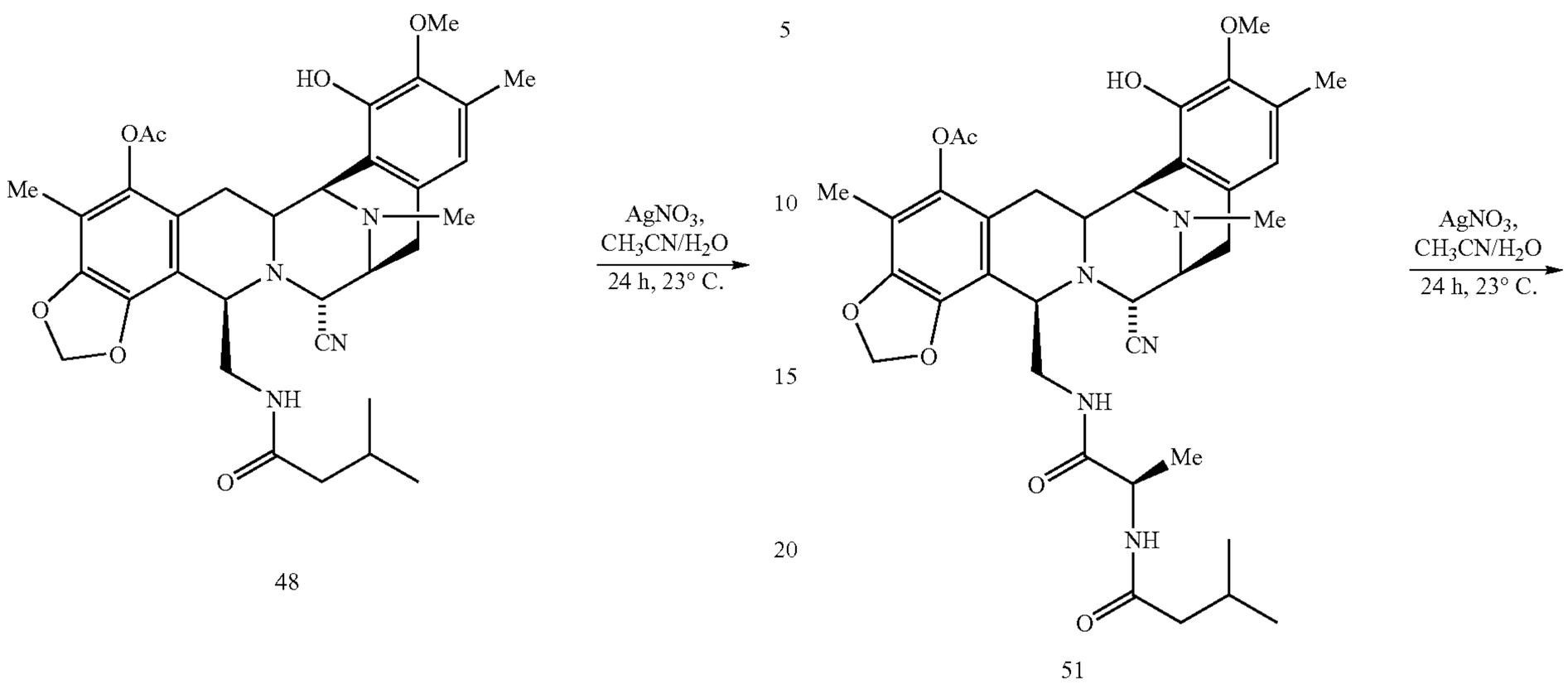
Rf: 0.28 (EtOAc:MeOH 10:1).

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.47 (s, 1H), 5.97 (s, 1H), 5.88 (s, 1H), 5.35 (bs, 1H), 4.51 (bs, 1H), 4.41 (bs, 1H), 4.12-4.05 (m, 1H), 4.00 (d, J=2.7 Hz, 1H), 3.77 (s, 3H), 3.64 (bs, 1H), 3.46 (d, J=3.3 Hz, 1H), 3.34 (d, J=11.4 Hz, 1H), 3.18 (d, J=7.5 Hz, 1H), 2.95 (dd, J_1=8.4 Hz, J_2=18.3 Hz, 1H), 2.70 (d, J=15.6 Hz, 1H), 2.48 (d, J=17.7 Hz, 1H), 2.28 (s, 3H), 2.27 (s, 3H), 2.26 (s, 3H), 1.98 (s, 3H), 1.68 (dd, J_1=12 Hz, J_2=15.6 Hz, 1H), 0.86 (d, J=6.3 Hz, 3H).

ESI-MS m/z: Calcd. for $C_{32}H_{37}F_3N_4O_9$: 678.66. Found (M^+−17): 661.2.

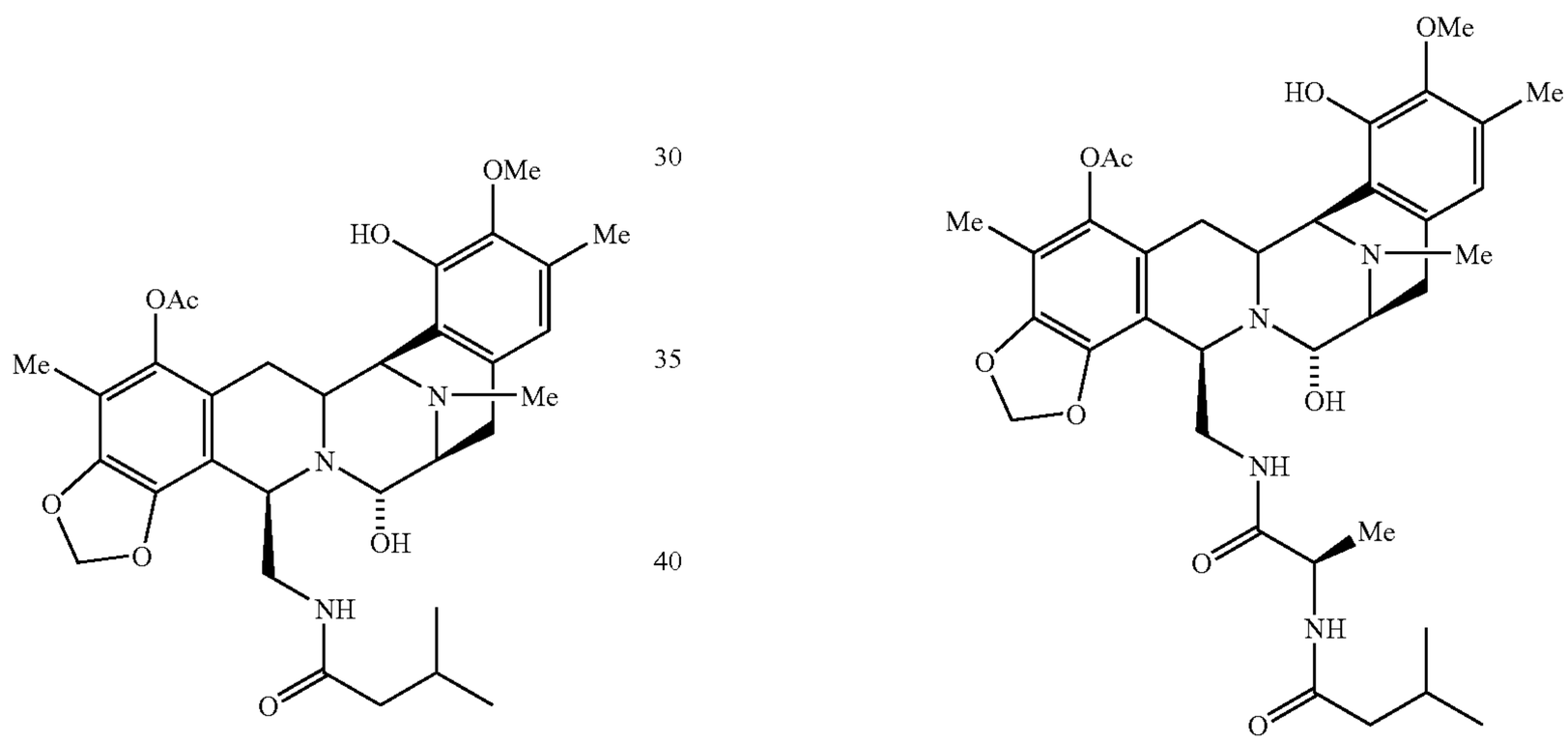
Example 62

Example 63



48

51



68

69

To a solution of 48 (32 mg, 0.0529 mmol) in CH_3CN/H_2O (1.5 ml/0.5 ml), $AgNO_3$ (270 mg, 1.58 mmol) was added and the reaction was stirred at 23° C. for 24 h. Then, brine (10 ml) and Aq sat $NaHCO_3$ (10 ml) were added at 0° C. and the mixture was stirred for 15 min, filtered through a pad of celite and washed with CH_2Cl_2 (20 ml). The solution was decanted and the organic layer was dried and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, EtOAc:MeOH 5:1) to afford 68 (18 mg, 56%) as a white solid.

Rf: 0.40 (EtOAc:MeOH 5:1).

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.50 (s, 1H), 5.95 (d, J=1.2 Hz, 1H), 5.88 (d, J=1.2 Hz, 1H), 5.23 (d, J=6.9 Hz, 1H), 4.45 (d, J=3.3 Hz, 1H), 4.38 (s, 1H), 4.01 (d, J=2.4 Hz, 1H), 3.78 (m, 1H), 3.77 (s, 3H), 3.41-3.37 (m, 1H), 3.17-3.15 (m, 1H), 2.96 (dd, J_1=7.8 Hz, J_2=18.0 Hz, 1H), 2.70 (d, J=15.3 Hz, 1H), 2.40 (d, J=18.0 Hz, 1H), 2.30 (s, 6H), 2.27 (s, 3H), 1.76-1.65 (m, 1H), 1.35-1.25 (m, 2H), 0.89-0.82 (m, 1H), 0.69 (d, J=6.6 Hz, 3H), 0.58 (d, J=6.6 Hz, 3H)

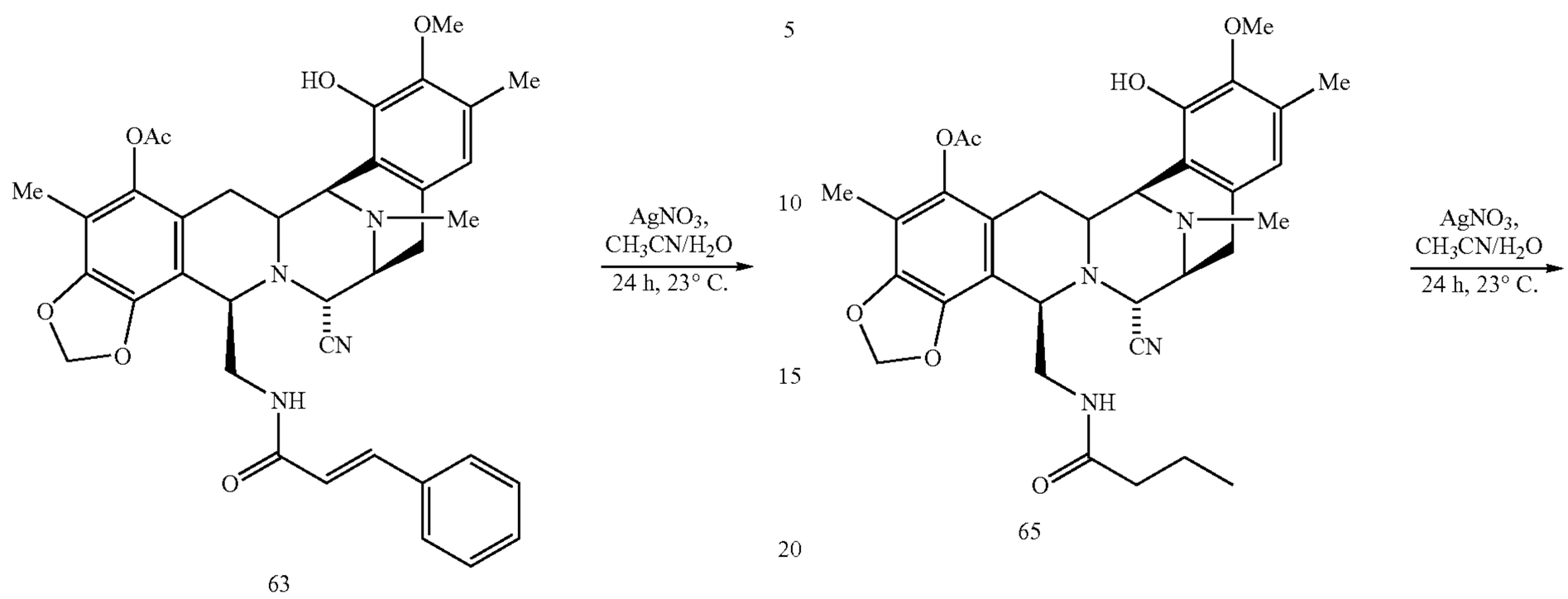
To a solution of 51 (27 mg, 0.04 mmol) in CH_3CN/H_2O (1.5 ml/0.5 ml), $AgNO_3$ (204 mg, 1.19 mmol) was added and the reaction was stirred at 23° C. for 24 h. Then, brine (10 ml) and Aq sat $NaHCO_3$ (10 ml) were added at 0° C. and the mixture was stirred for 15 min, filtered through a pad of celite and washed with CH_2Cl_2 (20 ml). The solution was decanted and the organic layer was dried and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, EtOAc:MeOH 5:1) to afford 69 (10 mg, 38%) as a white solid.

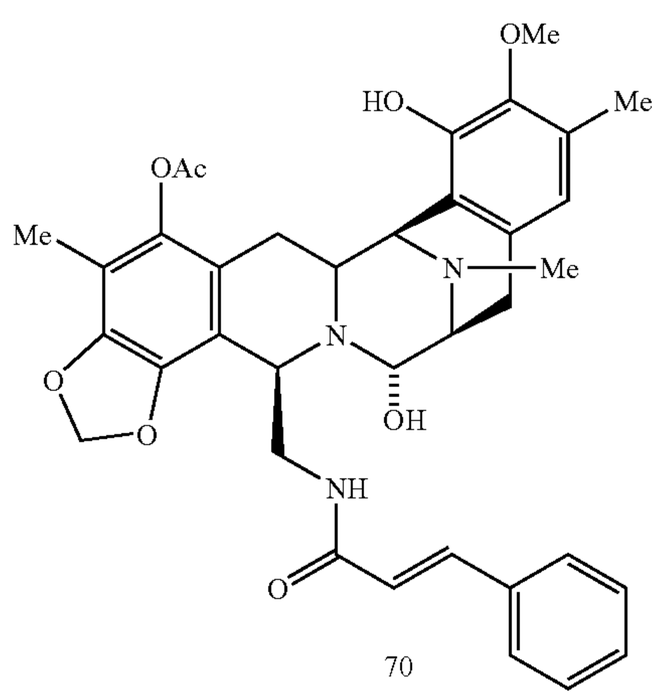
Rf: 0.38 (EtOAc:MeOH 5:1).

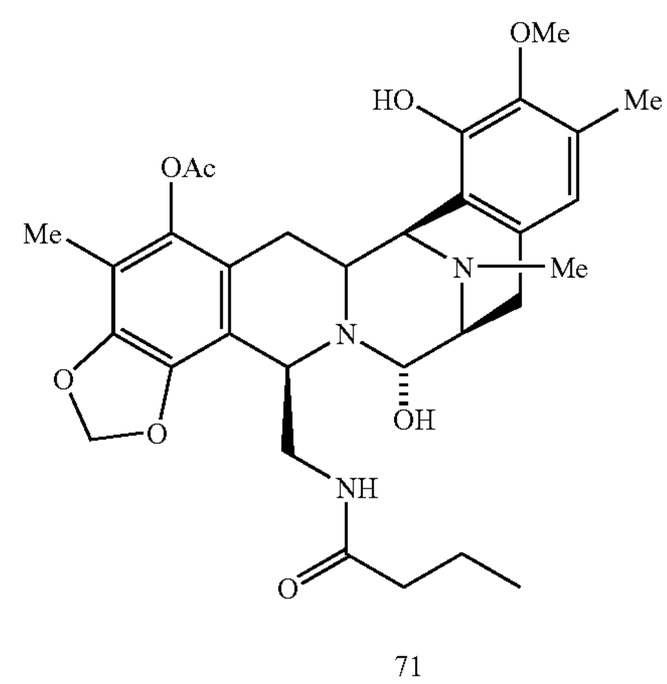
^{1}H NMR (300 MHz, $CDCl_3$) δ 6.48 s, 1H), 6.16 (bs, 1H), 5.98 (d, J=1.5 Hz, 1H), 5.89 (d, J=1.5 Hz, 1H), 5.33 (t, J=6.0 Hz, 1H), 4.50 (m, 1H), 4.40 (m, 1H), 4.11-4.09 (m, 1H), 4.00 (d, J=2.6 Hz, 1H), 3.78 (s, 3H), 3.41-3.32 (m, 3H), 3.18 (d, J=8.4 Hz, 1H), 2.94 (dd, J_1=8.4 Hz, J_2=18.3 Hz, 1H), 2.70 (d, J=14.4 Hz, 1H), 4.45 (d, J=18.3 Hz, 1H), 2.31 (s, 3H), 2.28 (s, 3H), 2.27 (s, 3H), 2.04 (s, 3H), 2.00-1.86 (m, 3H), 1.73 (m, 1H), 0.87 (d, J=6.3 Hz, 6H).

Example 64

Example 65







To a solution of 63 (15 mg, 0.023 mmol) in CH_3CN/H_2O (1.5 ml/0.5 ml), $AgNO_3$ (118 mg, 0.691 mmol) was added and the reaction was stirred at 23° C. for 24 h. Then, brine (10 ml) and Aq sat $NaHCO_3$ (10 ml) were added at 0° C. and the mixture was stirred for 15 min, filtered through a pad of celite and washed with CH_2Cl_2 (20 ml). The solution was decanted and the organic layer was dried and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, EtOAc:MeOH 5:1) to afford 70 (20.1 mg, 85%) as a white solid.

Rf: 0.43 (EtOAc:MeOH 5:1).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38-7.28 (m, 5H), 6.48 (s, 1H), 5.98 (d, J=1.5 Hz, 1H), 5.91 (d, J=1.5 Hz, 1H), 5.75 (bs, 1H), 5.38 (brd, 1H), 5.30 (bs, 1H), 4.53 (m, 1H), 4.42 (m, 1H), 4.02 (d, J=2.7 Hz, 1H), 3.78-3.65 (m, 5H), 3.46-3.40 (m, 2H), 3.17 (d, J=7.8 Hz, 1H), 2.94 (dd, J_1=7.8 Hz, J_2=17.7 Hz, 1H), 2.73 (d, J=16.8 Hz, 1H), 2.45 (d, J=18.0 Hz, 1H), 2.31 (s, 6H), 2.28 (s, 3H), 1.97 (s, 3H), 1.77 (dd, J_1=12.0 Hz, J_2=15.3 Hz, 1H).

To a solution of 65 (25 mg, 0.042 mmol) in CH_3CN/H_2O (1.5 ml/0.5 ml), $AgNO_3$ (215.56 mg, 1.269 mmol) was added and the reaction was stirred at 23° C. for 24 h. Then, brine (10 ml) and Aq sat $NaHCO_3$ (10 ml) were added at 0° C. and the mixture was stirred for 15 min, filtered through a pad of celite and washed with CH_2Cl_2 (20 ml). The solution was decanted and the organic layer was dried and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, EtOAc:MeOH 5:2) to afford 71 (16 mg, 65%) as a white solid.

Rf: 0.0.5 (EtOAc:MeOH 5:2).

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.50 (s, 1H), 5.95 (d, J=1.5 Hz, 1H), 5.78 (s, 1H), 5.19 (bs, 1H), 4.45 (d, J=3.3 Hz, 1H), 4.37 (bs, 1H), 4.11 (brd, J=4.8 Hz, 1H), 4.01 (d, J=2.1 Hz, 1H), 3.76 (s, 1H), 3.71-3.69 (m, 1H), 3.49-3.35 (m, 1H), 3.24 (d, J=13.5 Hz, 1H), 3.15 (d, J=9.3 Hz, 1H), 2.95 (dd, J_1=8.1 Hz, J_2=17.7 Hz, 1H), 2.70 (d, J=15.6 Hz, 1H), 2.40 (d, J=18.0 Hz, 1H), 2.31 (s, 3H), 2.29 (s, 3H), 2.26 (s, 3H), 1.96 (s, 3H), 1.75-1.66 (m, 1H), 1.52-1.17 (m, 2H), 0.66 (t, J=7.2 Hz, 3H).

Fermentation Procedures

Example A

Seed medium YMP3 containing 1% glucose; 0.25% beef extract; 0.5% bacto-peptone; 0.25% NaCl; 0.8% $CaCO_3$ was inoculated with 0.1% of a frozen vegetative stock of the microorganism, strain A2-2 of *Pseudomonas fluorescens*, and incubated on a rotary shaker (250 rpm) at 27° C. After 30 h of incubation, the seed culture was added to a agitated-vessel fermentor with a production medium composed of 2% dextrose; 4% mannitol, 2% dried brewer's yeast (Vitalevor® Biolux, Belgium); 1% $(NH_4)_2SO_4$; 0.04% K_2HPO_4; 0.8 KCl; 0.001% $FeCl_3$; 0.1% L-Tyr; 0.8% CO_3Ca; 0.05% PPG-2000; 0.2% anti-foam silicone (ASSAF-100, RHODIA UK). The sterilisation was carried out at 122° C. 30 minutes. The volume inoculated was a 2% (v/v). The temperature was 27° C. (0 to 16 h) and 24° C. from 16 h to final process (41 hours). The dissolve oxygen-pressure was upper to 25%. The pH was controlled at 6.0 with diluted sulphuric acid since 28 hours till final process. The overpressure was 0.5 bar. A 1% mannitol or sorbitol was added from 16 h to final process (for two days running) and 2% for three days fermentation-process.

After 41 or 64 hours, the fermentation broth must be extracted for recovery safracin B or KCN treatment in the clarified broth for recovery safracin B-cyano.

Example B

Obtention of Safracin B Cyano from the Crude Extract.

A clarification or filtration from the fermentation broth at pH 6 removes the solids. The clarified broth was adjusted a pH 9.5 with diluted sodium hydroxide and extracted twice with 2:1 (v/v) ethyl acetate, methylene chloride or butyl acetate. The extraction was carried out into an agitated-vessel during 20', the temperature of the mixture was maintained at 8 to 10° C. The two phases were separated by a liquid-liquid centrifuge. The organic phase was dried with sodium sulphate anhydrous or frozen and then filtered for removing ice. This organic phase (ethyl acetate layer) was evaporated until obtention of an oil-crude extract.

Example C

Obtention of Safracin B Cyano from the Clarified Broth.

A clarification or filtration from the fermentation broth at pH 6 removes the solids. The clarified broth was adjusted at pH 3.9 with concentrated acetic acid. 0.5 grams per litre of KCN are added to the clarified broth an incubated at 20° C. during 1 hour with agitation. Then, the temperature was decreased at 15° C. and the pH was adjusted at 9.5 with diluted sodium hydroxide and extracted with 2:1.5 (v/v) ethyl acetate. The extraction was carried out into an agitated-vessel during 20 minutes, the temperature of the mixture was maintained at 8 to 10° C. The two phases were separated by a liquid-liquid centrifuge. The organic phase was dried with sodium sulphate anhydrous. This organic phase (ethyl acetate layer) was evaporated until obtention of an oil-crude extract. This extract was purified by flash column chromatography (SiO_2, gradient 20:1 to 10: to 5:1 ethyl acetate:methanol) to afford quantitatively compound 2 as a light yellow solid.

Rf: 0.55 (ethyl acetate:methanol5:1); .t_R=19.9 min [HPLC, Delta Pack C4, 5 μm, 300 A, 150×3 mm, λ=215 nm, flow=0.7 ml/min, temp=50° C., grad.: CH_3CN-aq. NaOAc (10 mM) 85%-70% (20')];

^{1}H NMR (300 Mhz, $CDCl_3$): δ 6.54 (dd, J_1=4.4 Hz, J_2=8.4 Hz, 1H), 6.44 (s, 1H), 4.12 (d, J=2.4 Hz, 1H), 4.04 (d, J=2.4 Hz, 1H), 4.00 (s, 3H), 3.87 (bs, 1H), 3.65 (ddd, J_1=1.5 Hz, J_2=8.7 Hz, J_3=9.9 Hz, 1H), 3.35 (br. D, J=8.4 Hz, 1H), 3.15-2.96 (m, 4H), 2.92 (q, J=7.2 Hz, 1H), 2.47 (d, J=18.3 Hz, 1H), 2.29 (s, 3H), 2.18 (s, 3H) 1.83 (s, 3H), 1.64 (ddd, J_1=2.7 Hz, J_2=11.1 Hz, J_3=14.1 Hz, 1H), 0.79 (d, J=7.2 Hz, 3H);

^{13}C NMR (75 Mhz, $CDCl_3$): δ 186.0 (q), 175.9 (q), 156.2 (q), 146.8 (q), 142.8 (q), 140.7 (q), 136.6 (q), 130.5 (q), 128.8 (q), 127.0 (q), 120.5 (s), 117.4 (q), 116.5 (q), 60.8 (t), 60.4 (s), 58.7 (t), 56.2 (s), 55.7 (s), 54.8 (s), 54.8 (s), 54.4 (s), 50.0 (s), 41.6 (t), 39.8 (d), 25.2 (d), 24.4 (d), 21.2 (t), 15.5 (t), 8.4 (t).

ESI-MS m/z: Calcd for $C_{29}H_{35}N_5O_6$: 549.6. Found $(M+Na)^+$: 572.3.

Example D

A medium (50 l) composed of dextrose (2%), mannitol (4%), dry brewer's yeast (2%), ammonium sulphate (1%), potassium secondary phosphate (0.04%), potassium chloride (0.8%), iron (III) chloride 6-hydrate (0.001%), L-tyrosine (0.1%), calcium carbonate (0.8%), poly(propylene glycol) 2000 (0.05%) and antifoam ASSAF 1000 (0.2%) was poured into a jar-fermentor with 75 l total capacity and, after sterilisation, inoculated with seed culture (2%) of A2-2 strain (FERM. BP-14) and aerated cultivation under agitation was carried out at 27° C. to 24° C. for 64 hours (aeration of 75 l per minute and agitation from 350 to 500 rpm). The pH was controlled by automatic feeding of diluted sulphuric acid from 27 hours to final process. A 2% mannitol was added from 16 hours to final process. The cultured medium (45 l) thus obtained was, after removal of cells by centrifugation, adjusted to pH 9.5 with diluted sodium hydroxide, extracted with 25 litres of ethyl acetate twice. The mixture was carried out into an agitated-vessel at 8° C. for 20 minutes. The two phases were separated by a liquid-liquid centrifuge. The organic phases were frozen at −20° C. and filtered for removing ice and evaporated ice and evaporated until obtention of a 40 g oil-dark-crude extract. After introduction of the cyanide group and purification, 3.0 grams of safracin B cyano were obtained.

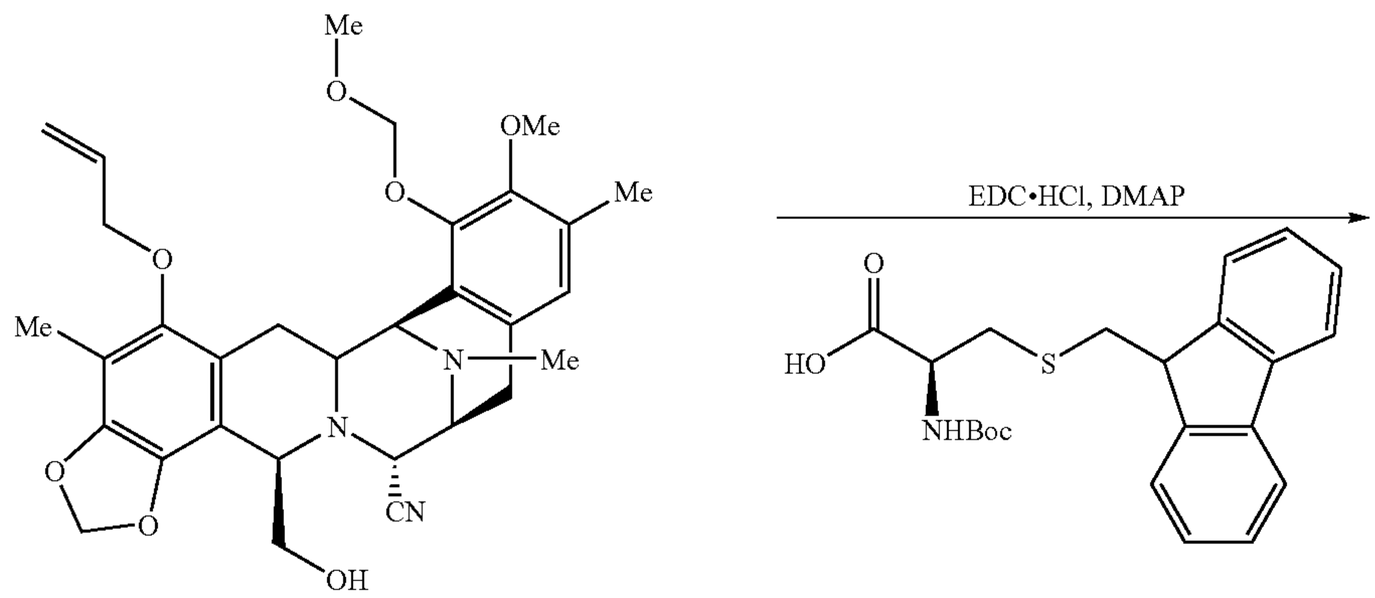
Example E

A medium (50 l) composed of dextrose (2%), mannitol (4%), dry brewer's yeast (2%), ammonium sulphate (1%), potassium secondary phosphate (0.02%, potassium chloride (0.2%), Iron (III) chloride 6-hydrate (0.001%, L-tyrosine (0.1%), calcium carbonate (0.8%, poly(propylene glycol) 2000 (0.05%) and antifoam ASSAF 1000 (0.2%) was poured into a jar-fermentor with 75 l total capacity and, after sterilisation, inoculated with seed culture (2%) of A2-2 strain (FERM BP-14) and aerated cultivation under agitation was carried out at 27° C. to 24° C. for 41 hours (aeration of 75 l per minute and agitation from 350 to 500 rpm). The pH was controlled by automatic feeding of diluted sulphuric acid from 28 hours to final process. A 1% mannitol was added from 16 hours to final process. The cultured medium (45 l) thus obtained was, after removal of cells by centrifugation, adjusted to pH 3.9 with 200 ml of conc. acetic acid. 25 grams of potassium cyanide 97% were added and after 1 hour of agitation at 20° C., the pH was adjusted to 9.5 with 1500 ml of a solution 10% sodium hydroxide. Then, extracted with 35 litres of ethyl acetate. The mixture was carried out into an agitated-vessel at 8° C. for 20 minutes. The two phases were separated by a liquid-liquid centrifuge. The organic phase

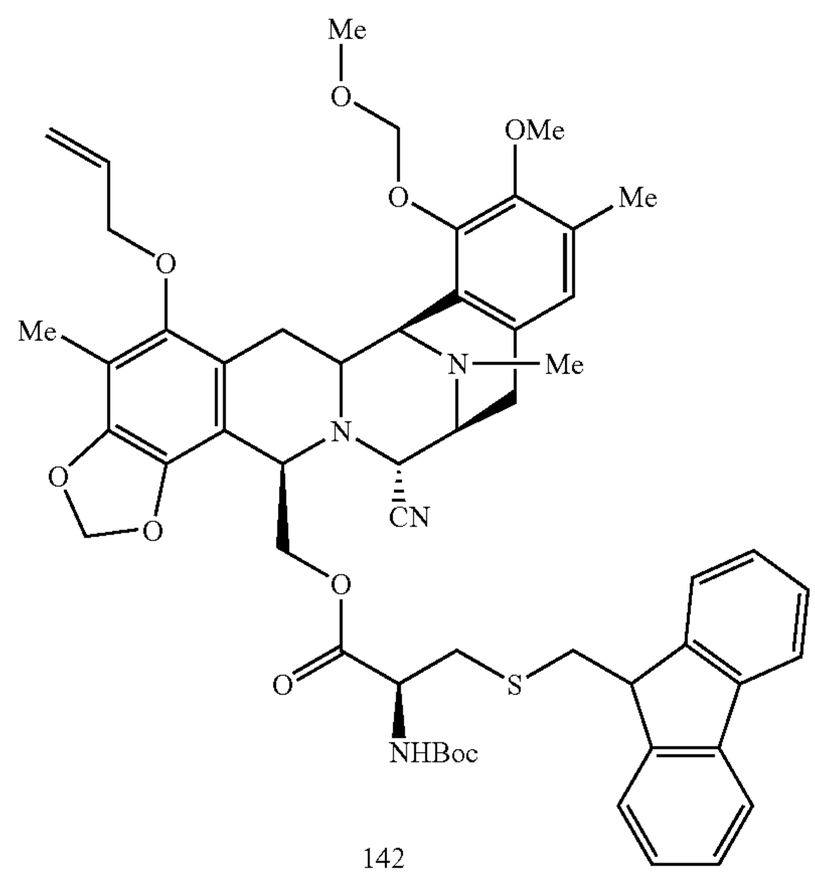
was dried by sodium sulphate anhydrous and evaporated until obtention of a 60 g oil-dark-crude extract.

After chromatography, 4.9 grams of safracin B cyano were obtained.

Example 66



25



142

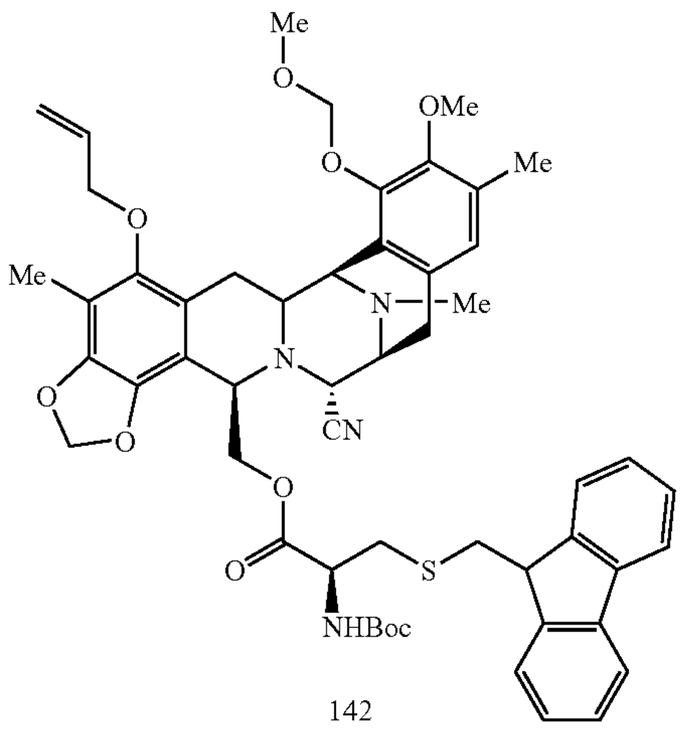
To a stirred solution of 25 (7.83 g, 0.0139 mol) and the commercial available Boc-Cys (Fm) derivative (Bachem) (8.33 g, 35.04 mmol) in dichloromethane (535 mL) under argon, dimethylaminopyridine (4.28 g, 35.04 mmol) and 1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (6.66 g, 35.04 mmol) were added at 23° C. The mixture was then stirred at 23° C. for 2.5 hours. The reaction was quenched by addition of a saturated aqueous sodium bicarbonate solution (500 mL), the organic phase separated and the aqueous layer back-extracted with dichloromethane (250 mL). The combined organic extracts were dried over sodium sulphate, filtrated and evaporated to dryness under reduced pressure. The crude product was purified by flash column chromatography eluting with mixtures of ethyl acetate and hexane in a gradient manner, from 1:4 to 2:1 to yield 142 (12.21 g, 93%) as a light yellow solid. Rf=0.35 Hex:EtOAc 1:1.

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.72 (d, J=7.3, 2.7 Hz 2H), 7.55 (dd, J_1=14.6, J_2=7.6 2H), 7.40-7.34 (m, 2H), 7.30-7.24 (m, 2H), 6.63 (s, 1H), 6.08-5.99 (m, 1H), 5.91 (d, J=1.5 Hz, 1H), 5.80 (d, J=1.5 Hz, 1H), 5.39 (dd, J_1=17.3, J_2=1.7 Hz 1H), 5.24 (dd, J_1=10.5, J_2=1.7 Hz, 1H), 5.09 (AB, J=4.48 Hz, 2H), 5.07 (t, J=7.8 Hz, 1H), 4.34-4.29 (m, 2H), 4.17 (d, J=1.9 Hz, 1H), 4.16-4.04 (m, 4H), 4.02-3.96 (m, 2H), 3.93 (t, J=5.3 Hz, 1H), 3.70 (s, 3H), 3.56 (s, 3H), 3.32 (d, J=8.0, 1H), 3.23-3.17 (m, 2H), 3.0-2.89 (m, 3H), 2.65-2.57 (m, 2H), 2.29 (s, 3H), 2.20 (s, 3H), 2.03 (s, 3H), 1.76 (dd, J_1=16.3, J_2=12.7 Hz, 1H), 1.45, 1.44 (s, 9H).

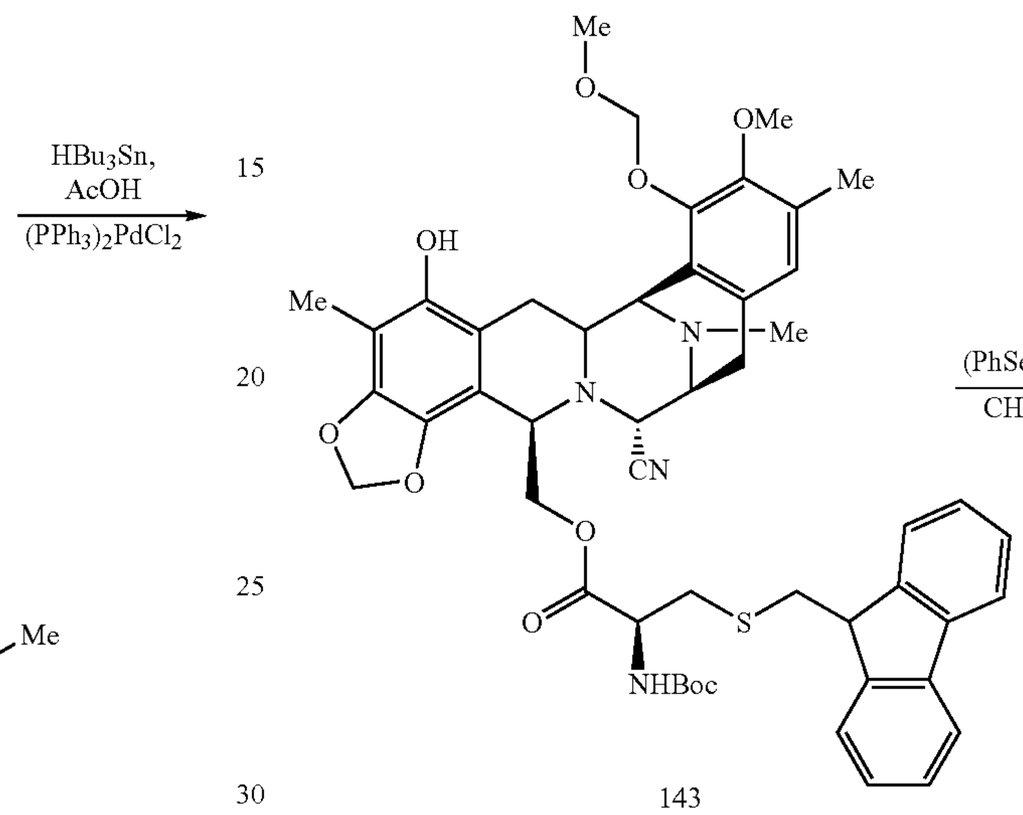
^{13}C-NMR (75 MHz, $CDCl_3$): δ 170.9, 155.3, 148.9, 148.6, 146.1, 146.0, 144.7, 141.2, 141.1, 139.4, 134.0, 131.0, 130.1, 127.8, 127.2, 125.2, 125.0, 124.3, 121.3, 121.2, 120.1, 118.1, 117.6, 112.9, 101.4, 99.5, 80.3, 74.2, 65.6, 60.4, 60.1, 57.9, 57.4, 57.2, 57.1, 56.9, 55.6, 53.2, 47.0, 41.8, 41.7, 36.7, 35.3, 28.5, 26.6, 25.3, 15.9, 9.4.

ESI-MS m/z: Calcd. For $C_{53}H_{60}N_4O_{10}S$: 945.13. Found $(M+1)^+$: 946.3.

Example 67



142



143

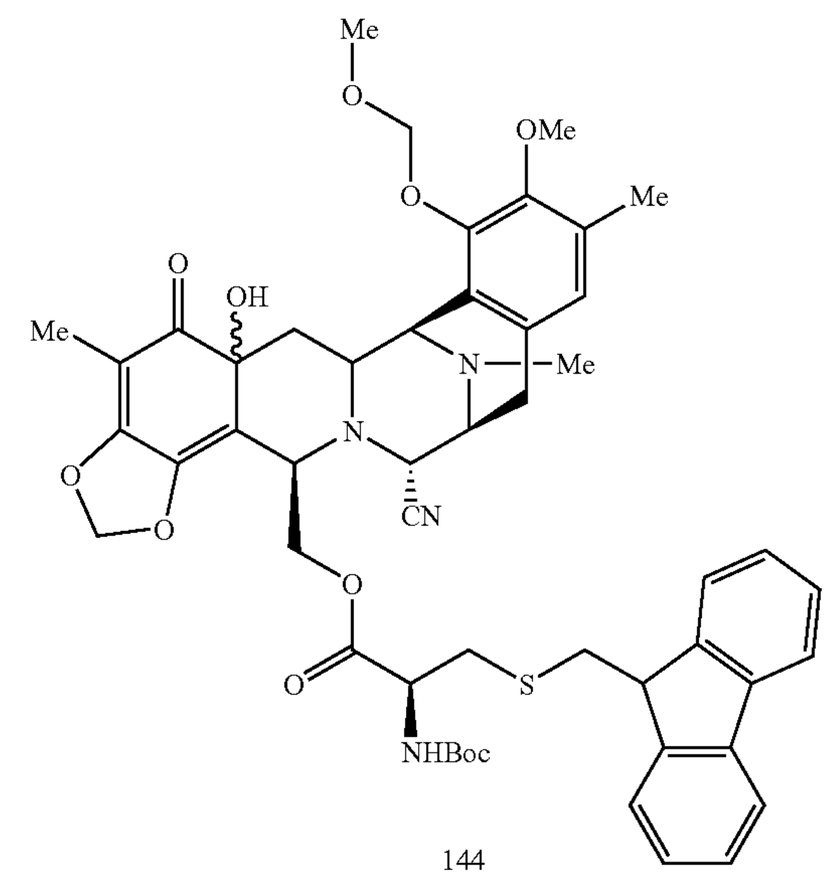
To a stirred solution of 142 (12.01 g, 0.0127 mol) in dichloromethane (318 mL), dichlorobis(triphenylphosphine) palladium (II) (0.71 g, 1.015 mmol) and acetic acid (3.6 mL, 0.176 mol) were added under argon at 23° C. Then, tributyl tin hydride (10.27 mL, 0.037 mol) was added in a dropwise manner. The mixture was stirred at 23° C. for 10 minutes. The reaction was then filtered through a silica gel column compacted with hexane. 143 (10.89 g, 95%) was obtained as a yellow solid by subsequent elution with mixtures of ethyl acetate and hexane in a gradient manner, from 1:4, 1:1 to 7:3. Rf=0.25 Hex:EtOAc 2:1.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72 (d, J=7.6 Hz, 2H), 7.61 (d, J=6.6 Hz, 1H), 7.52 (d, J=7.3 Hz, 1H), 7.37 (t, J=7.8 Hz, 2H), 7.28 (m, 2H), 6.63 (s, 1H), 5.87 (d, J=1.5 Hz, 1H), 5.76 (d, J=1.5 Hz, 1H), 5.58 (bs, 1H), 5.31 (d, J=5.8 Hz, 1H), 5.17 (d, J=5.6 Hz, 1H), 4.91 (d, J=8.3 Hz, 1H), 4.17-4.06 (m, 4-6H), 3.85 (t, J=5.7 Hz, 1H), 3.70 (s, 3H), 3.68 (s, 3H), 3.34 (brd, J=6.6 Hz, 1H), 3.23 (brd, J=11.2 Hz, 1H), 3.06 (brd, J=12.9 Hz, 1H), 3.04-2.86 (m, 3H), 2.65-2.54 (m, 2H), 2.28 (s, 3H), 2.21 (s, 3H), 1.94 (s, 3H), 1.80 (dd, J_1=11.5 Hz, J_2=15.8 Hz, 1H), 1.45 (s, 9H).

^{13}C NMR (75 MHz, $CDCl_3$) 8175.3, 170.5, 154.9, 149.1, 147.6, 145.9, 145.8, 145.7, 144.5, 140.9, 140.8, 136.1, 130.9, 127.4, 126.9, 124.3, 124.7, 122.9, 119.7, 117.6, 112.3, 111.4, 106.6, 100.7, 99.7, 80.0, 64.2, 60.3, 59.8, 57.6, 57.0, 56.5, 56.4, 55.2, 52.7, 46.7, 46.5, 41.4, 41.3, 36.9, 36.6, 34.9, 28.2, 26.0, 24.9, 20.9, 20.7, 15.7, 14.1, 8.5.

ESI-MS m/z: Calcd. For $C_{50}H_{56}N_4O_{10}S$: 905.5. Found $(M+1)^+$: 906.3.

Example 68



144

To a solution of 143 (10 g, 0.011 mol) in anhydrous dichloromethane (185 mL) at −10° C. (bath temperature −15° C.), a solution of benzeneseleninic anhydride (5.7 g, 0.011 mol) was added in anhydrous dichloromethane (185 mL), discarding any white solid present in the solution. The mixture was stirred for 10 minutes at the same temperature. The reaction was diluted with dichloromethane (200 mL) and a saturated aqueous sodium bicarbonate solution (500 mL) was added at −10° C. The organic phase was separated, dried over sodium sulphate, filtered and concentrated to dryness at reduced pressure. The residue was purified by flash column chromatography, eluting with mixtures of ethyl acetate and hexane in a gradient manner, from 1:1, 3:2, 7:3 to 4:1 to obtain 144 (9.34 g, 92%) as a yellow solid. The purified solid from chroma-

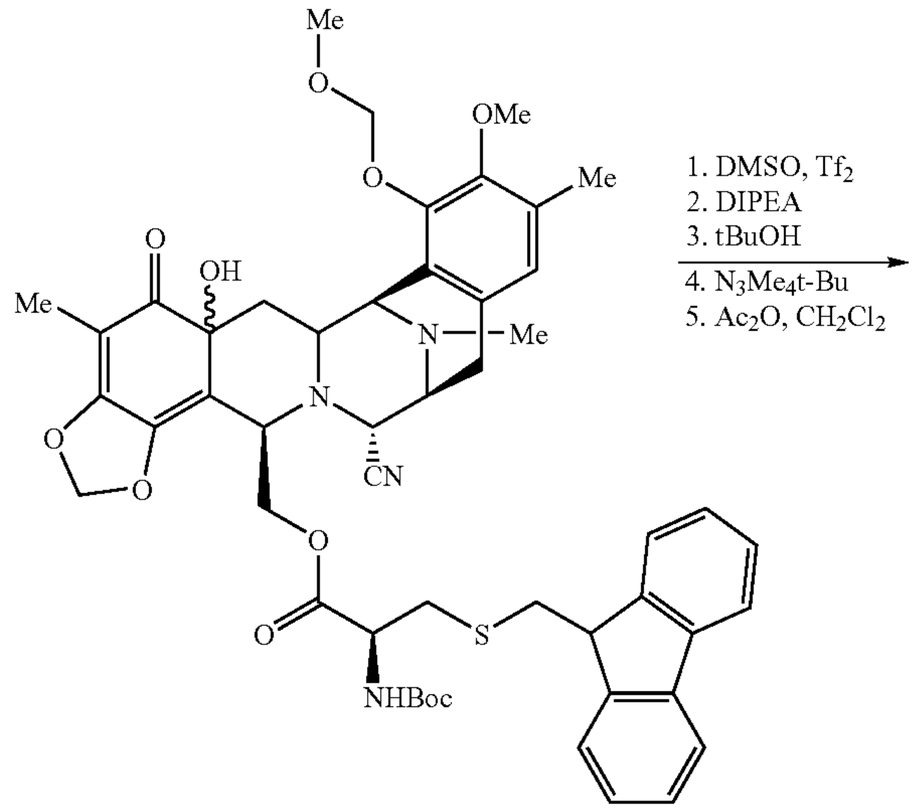
tography was dissolved in dichloromethane (250 mL), charcoal (3.3 g) was added and the suspension was stirred at 23° C. for 1 hour. The mixture was filtered through celite and the celite was washed with dichloromethane (80 mL). The solvent was evaporated at reduced pressure maintaining the temperature at 25-30° C. to yield 144 (8.96 g, 88%) as a yellow solid. Rf=0.30 and 0.25 (mixture of isomers) Hex:EtOAc 1:1.

^{1}H NMR (300 MHz, $CDCl_3$) (mixture of isomers) δ 7.73-7.61 (m, 4H), 7.37-7.30 (m, 4H), 6.62 (s, 1H), 6.59 (s, 1H), 6.53 (s, 1H), 5.72 (s, 1H), 5.70 (s, 1H), 5.61 (s, 1H), 5.55 (bs, 1H), 5.34 (m, 2H), 5.08 (AB sist., J_{AB}=6.7 Hz, 1H), 5.00 (AB sist., J_{AB}=5.9 Hz, 1H), 4.67 (m, 1H), 4.50 (m, 1H), 4.38 (dd, J_1=4.9 Hz, J_2=12.9 Hz, 1H), 4.21 (dd, J_1=6.3 Hz, J_2=12.9 Hz, 1H), 4.11 (t, J=5.9 Hz, 1H), 4.02 (m, 3H), 3.87 (m, 1H), 3.83 (s, 3H), 3.72 (m, 1H), 3.61 (s, 3H), 3.49 (s, 3H), 3.27 (m, 1H), 3.15 (dd, J_1=1.8 Hz, J_2=6.2 Hz, 2H), 3.07 (d, J=6.3 Hz, 1H), 2.94 (m, 4H), 2.86 (m, 2H), 2.42 (m, 2H), 2.25 (s, 3H), 2.20 (s, 3H), 2.15 (s, 3H), 2.08 (dd, J_1=2.4 Hz, J_2=13.9 Hz, 1H), 1.77 (s, 3H), 1.76 (s, 3H), 1.43 (s, 9H).

^{13}C NMR (75 MHz, $CDCl_3$) (mixture of isomers) δ 200.6, 171.2, 160.4, 155.6, 148.9, 148.8, 148.3, 145.9, 145.8, 141.3, 141.2, 138.7, 130.9, 127.9, 127.4, 127.3, 127.3, 125.3, 125.1, 124.2, 120.1, 117.1, 111.9, 108.5, 105.0, 104.7, 101.7, 101.3, 99.5, 99.4, 80.5, 72.5, 70.8, 60.5, 60.1, 58.4, 58.0, 57.9, 56.9, 56.8, 56.3, 55.9, 55.5, 55.4, 53.8, 53.7, 47.1, 42.0, 41.8, 41.5, 37.4, 37.3, 35.6, 35.5, 28.5, 25.8, 25.7, 16.1, 16.0, 7.7, 7.3.

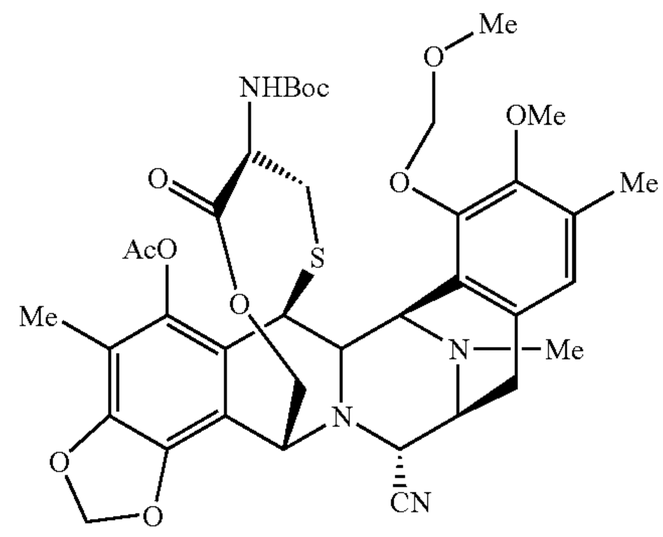
ESI-MS m/z: Calcd. for $C_{50}H_{56}N_4O_{11}S$: 921.3. Found $(M+1)^+$: 922.3.

Example 69



144

-continued



145

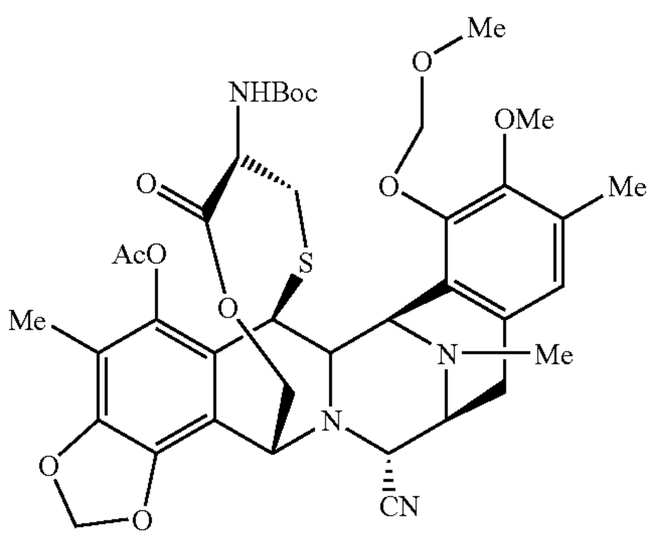
To a solution of DMSO (3.44 mL) in anhydrous dichloromethane (396 mL), triflic anhydride (3.27 mL, 19.45 mmol) was added under argon at −78° C. and the mixture was stirred at that temperature for 20 minutes. Then, a solution of 144 (8.92 g, 9.6 mmol) in anhydrous dichloromethane (124 mL) at −78° C. was added and the mixture was stirred under argon at 40° C. for 35 minutes. Diisopropylethylamine (13.5 mL, 73.43 mmol) was added and the mixture was stirred under argon for 45 minutes at 0° C. Tert-butanol (3.65 mL, 38.6 mmol) and tert-butyl tetramethyl guanidine (11.6 mL, 67.46 mmol) were added and the mixture was stirred under argon for 40 minutes at 23° C. Acetic anhydride (9.15 mL, 96.78 mmol) was then added and the reaction stirred for a further 1 hour at 23° C. The reaction was diluted with dichloromethane (250 mL) and a saturated aqueous ammonium chloride solution (500 mL) was added. The organic layer was separated and washed sequentially with a saturated aqueous sodium bicarbonate solution (500 mL) and a saturated aqueous sodium chloride solution (500 mL). The organic layer was separated, dried over sodium sulphate, filtered and concentrated to dryness at reduced pressure, maintaining the temperature at 25-30° C. The crude solid was then purified by flash column chromatography, eluting with mixtures of ethyl acetate and hexane in a gradient manner, from 1:4 to 2:3 to give 145 (4.99 g, 68%) as a yellow solid. Rf=0.44 Hex:EtOAc 3:2.

^{1}H NMR (300 MHz, $CDCl_3$) (mixture of isomers) δ 6.79 (s, 1H), 6.09 (s, 1H), 6.00 (s, 1H), 5.20 (d, J=5.4 Hz, 1H), 5.14 (d, J=5.6 Hz, 1H), 5.02 (d, J=11.7 Hz, 1H), 4.63 (d, J=9.0 Hz, 1H), 4.50 (m, 1H), 4.33 (d, J=5.4 Hz, 1H), 4.30 (m, 1H), 4.25 (bs, 1H), 4.18 (d, J=2.4 Hz, 1H), 4.17 (dd, J_1=1.3 Hz, J_2=11.7 Hz, 1H), 3.78 (s, 3H), 3.57 (s, 3H), 3.42 (m, 2H), 2.93 (m, 2H), 2.35 (m, 1H), 2.31 (s, 3H), 2.29 (s, 3H), 2.22 (s, 3H), 2.09 (m, 1H), 2.05 (s, 3H), 1.45 (s, 9H).).

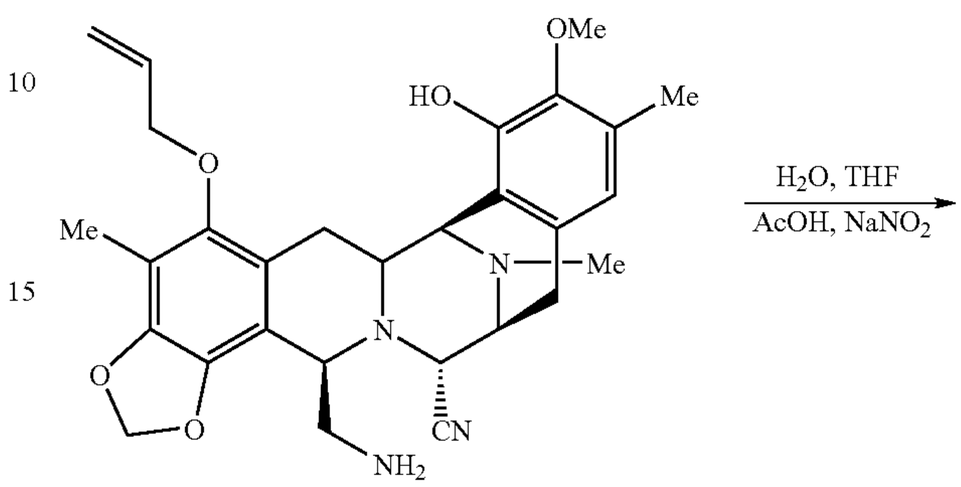
^{13}C NMR (75 MHz, $CDCl_3$) δ 207.3, 170.9, 168.8, 155.4, 149.8, 148.6, 146.0, 141.1, 140.7, 131.7, 130.6, 125.1, 120.6, 118.3, 113.7, 102.2, 99.4, 80.0, 61.6, 60.4, 59.8, 59.4, 59.2, 57.7, 55.0, 54.7, 54.0, 41.9, 41.6, 33.1, 31.8, 28.7, 23.9, 20.6, 16.1, 14.3, 9.8.

ESI-MS m/z: Calcd. for $C_{38}H_{46}N_4O_{11}S$: 766.86. Found $(M+1)^+$: 767.3.

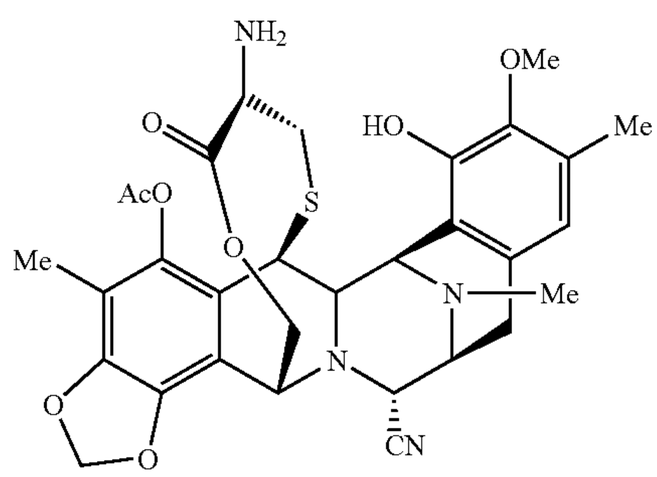
Example 70



145



21

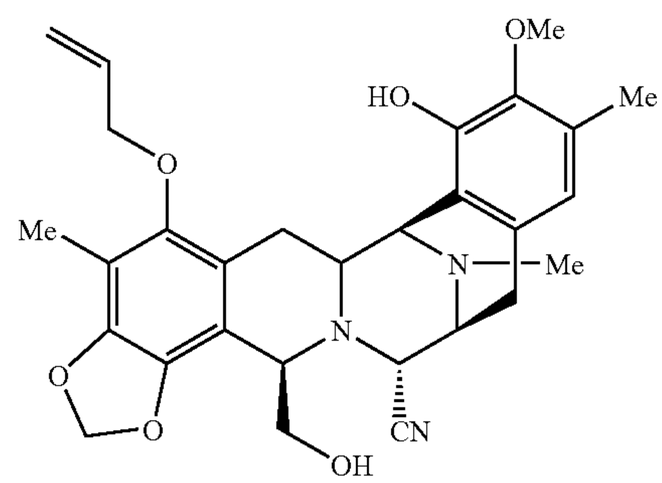


35

To a solution of 145 (1.0 g, 1.3 mmol) in acetonitrile (50 mL) and dichloromethane (25 mL), sodium iodide (1.52 g, 10.01 mmol) was added at 23° C. The mixture was then cooled to 0° C. and aluminium trichloride (1.33 g, 10.01 mmol) was added portionwise maintaining the temperature at 0° C. The mixture was then stirred for 2.5 hours at 0° C. The reaction was diluted with dichloromethane (25 mL) and a saturated aqueous solution of sodium potassium tartrate (100 mL) was added. The aqueous phase is separated and extracted with dichloromethane (2×75 mL). A saturated aqueous sodium bicarbonate solution (50 mL) was then added to the aqueous phase which was further extracted with dichloromethane (2×50 mL). The combined organic extracts were dried over sodium sulphate, filtered and evaporated to dryness under reduced pressure, maintaining the temperature below 25° C. The crude solid was then purified by column chromatography on amino-silicagel and eluting with mixtures of ethyl acetate and hexane in a gradient manner. 35 (487 mg, 60%) was obtained as a yellow solid. Experimental data of 35 were previously described in PCT/GB00/01852.

36, ET-770 and ET-743 were prepared following the same procedures than those previously described in PCT/GB00/01852.

Example 71



146

A solution of 21 (9.84 g, 18.97 mmol) in THP (569 mL) and H_2O (285 mL) was cooled at 0° C. with an ice bath. Then, $NaNO_2$ (1.96 g, 28.45 mmol) and 90% aq. AcOH (18.97 mL, 0.33 mol) were added at 0° C. and the mixture was stirred at 23° C. for 18 h. After cooling down the reaction to 0° C., a saturated aqueous sodium bicarbonate solution (300 mL, basic pH) and dichloromethane (500 mL) were added. After extraction, the aqueous phase was further extracted with dichloromethane (2×300 mL). The combined organic extracts were dried over sodium sulphate and evaporated to dryness under reduced pressure. The crude solid was then disolved in MeOH (379 mL), and 1M NaOH (38 mL) was added at 0° C. The mixture was stirred at 23° C. for 4 h. After dilution with EtOAc (600 mL) at 0° C., the organic layer was washed with a mixture of water (400 mL) and, a saturated aqueous sodium bicarbonate solution (100 mL, basic pH). After extraction, the aqueous phase was further extracted with EtOAc (3×300 mL). The combined organic extracts were dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, Hex:EtOAc gradient from 3:1 to 2:1) to afford 146 (4.55 g, 46%) as a white solid. Rf: 0.33 (Hex:EtOAc 1:1).

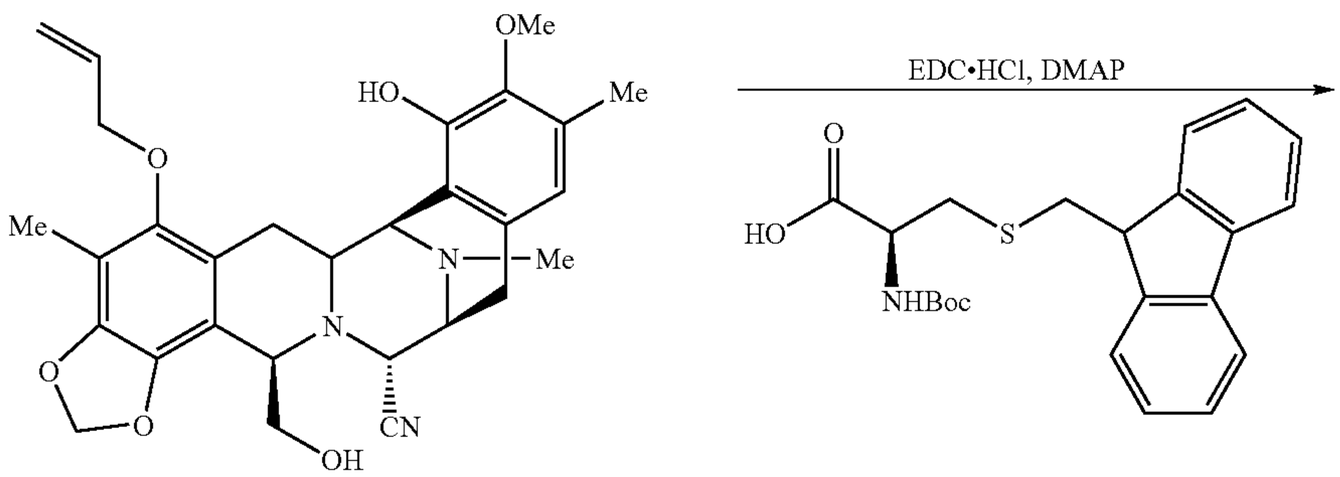
^{1}H NMR (300 MHz, $CDCl_3$) δ 6.48 (s, 1H), 6.15-6.02 (m, 1H), 5.92 (d, J=1.5 Hz, 1H), 5.86 (d, J=1.5 Hz, 1H), 5.77 (s, 1H), 5.39 (dd, J_1=1.5 Hz, J_2=17.1 Hz, 1H), 5.26 (dd, J_1=1.5 Hz, J_2=10.5 Hz, 1H), 4.24-4.15 (m, 3H), 4.04 (d, J=2.4 Hz, 1H), 3.97 (t, J=3.3 Hz, 1H), 3.74 (s, 3H), 3.64 (dt, J_1=3.3 Hz, J_2=11.1 Hz, 1H), 3.43 (dd, J_1=3.3 Hz, J_2=10.5 Hz, 1H), 3.38-3.34 (m, 2H), 3.31 (t, J=2.7 Hz, 1H), 3.22 (dd, J_1=2.4 Hz, J_2=15.6 Hz, 1H), 3.10 (dd, J_1=8.1 Hz, J_2=18.3 Hz, 1H),

2.49 (d, J=18.3 Hz, 1H), 2.34 (s, 3H), 2.24 (s, 3H), 2.11 (s, 3H), 1.88 (dd, J_1=12 Hz, J_2=15.9 Hz, 1H).

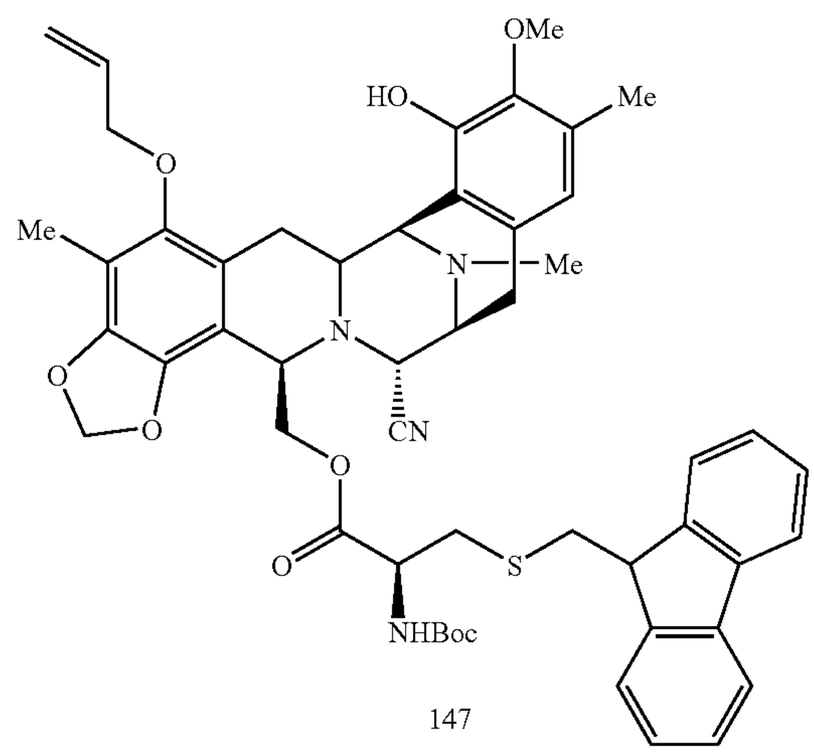
^{13}C NMR (75 MHz, $CDCl_3$) δ 148.6, 146.7, 144.4, 143.0, 138.9, 133.9, 130.2, 129.1, 121.1, 120.9, 117.7, 117.4, 116.8, 113.3, 112.3, 101.1, 74.3, 63.7, 60.6, 60.1, 58.1, 56.9, 56.7, 55.4, 41.7, 26.2, 25.7, 15.7, 9.3.

ESI-MS m/z: Calcd. for $C_{29}H_{33}N_3O_6$: 519.59. Found $(M+1)^+$: 520.5.

Example 72



146



147

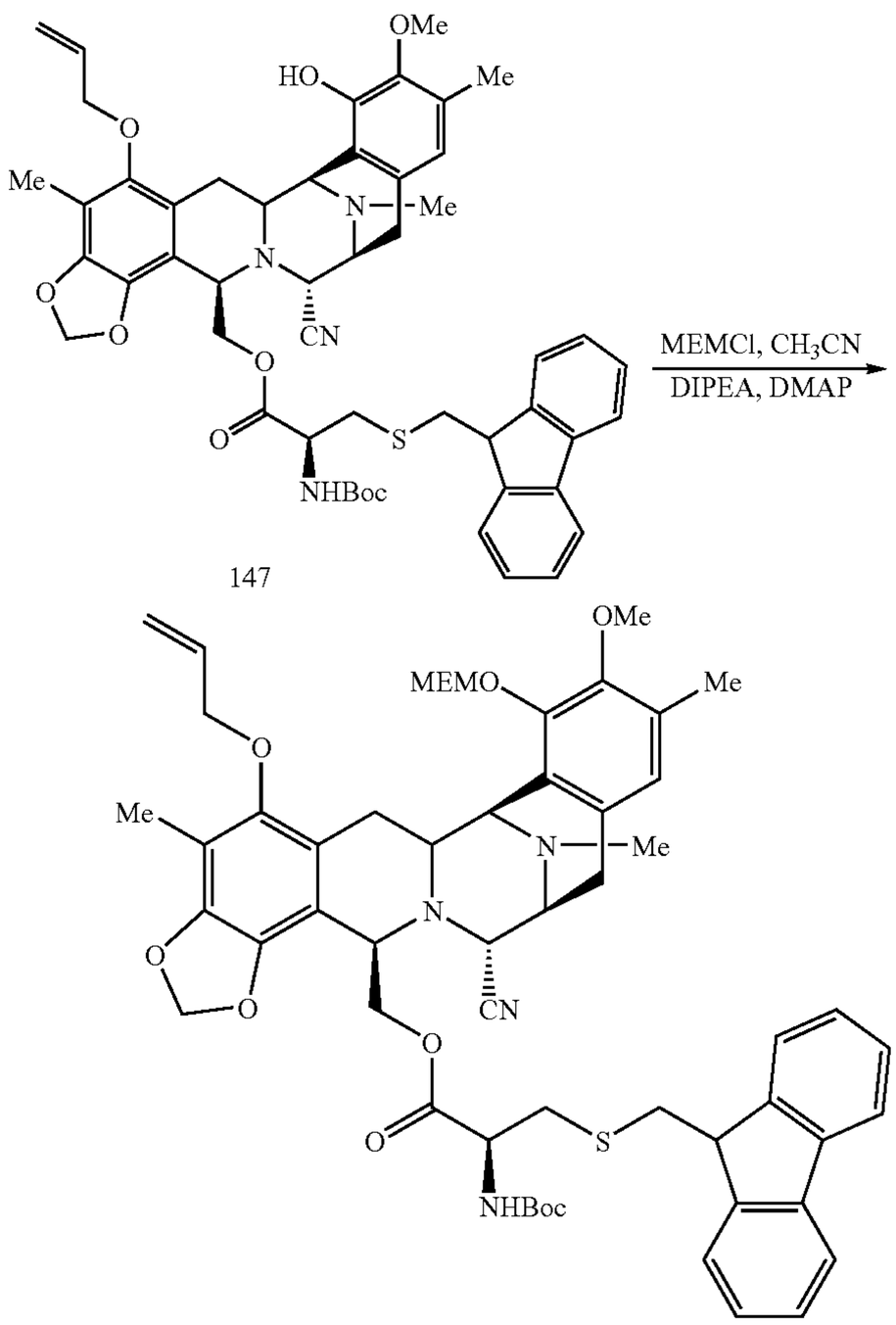
To a stirred solution of 146 (47.35 g, 0.091 mol) and the commercial available Boc-Cys (Fm) derivative (54.6 g, 0.137 mol) in dichloromethane (2.8 L) under argon, dimethylaminopyridine (5.6 g, 0.046 mol) and 1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (43.6 g, 0.227 mol) were added dropwise during 1.5 h at 23° C. The mixture was then stirred at 23° C. for 1 more hour. The reaction was quenched by addition of a saturated aqueous sodium bicarbonate solution (1 L) and the organic phase was separated. The aqueous layer was back-extracted with dichloromethane (2×500 mL). The combined organic extracts were dried over sodium sulphate and evaporated to dryness under reduced pressure. The crude product was purified by flash column chromatography eluting with mixtures of ethyl acetate and hexane in a gradient manner, from 1:4 to 3:1 to yield 147 (74.3 g, 93%) as a white solid. Rf=0.5 Hex: EtOAc 1:1.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.73 (d, J=7.8 Hz, 2H), 7.63-7.55 (m, 2H), 7.39-7.35 (m, 2H), 7.29-7.25 (m, 2H), 6.41 (s, 1H), 6.07-5.97 (m, 1H), 5.92 (d, J=1.2 Hz, 1H), 5.80 (d, J=1.2 Hz, 1H), 5.67 (s, 1H), 5.34 (dd, J_1=1.8 Hz, J_2=17.4 Hz, 1H), 5.23 (dd, J_1=1.8 Hz, J_2=10.5 Hz, 1H), 5.04 (d, J=9.3 Hz, 1H), 4.32-4.29 (m, 1H), 4.13-3.91 (m, 9H), 3.72 (s, 3H), 3.31 (d, J=7.2 Hz, 1H), 3.26-3.17 (m, 2H), 2.96-2.87 (m, 3H), 2.68-2.54 (m, 2H), 2.27 (s, 3H), 2.24 (s, 3H), 2.05 (s, 3H), 1.83 (dd, J_1=12.6 Hz, J_2=15.9 Hz, 1H), 1.45 (s, 9H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 170.9, 155.4, 149.0, 147.1, 146.2, 146.0, 144.7, 143.0, 141.1, 139.4, 134.1, 131.5, 129.1, 127.8, 127.2, 125.0, 121.3, 120.9, 120.1, 118.2, 117.6, 117.2, 112.9, 112.4, 101.4, 80.3, 76.6, 74.4, 65.3, 61.0, 60.4, 57.4, 56.9, 56.7, 55.6, 53.0, 46.9, 41.8, 36.7, 35.3, 31.8, 28.5, 26.6, 25.2, 22.9, 16.0, 14.4, 9.5.

ESI-MS m/z: Calcd. for $C_{51}H_{56}N_4O_9S$: 900.3. Found $(M+1)^+$: 901.3.

Example 73



147 148

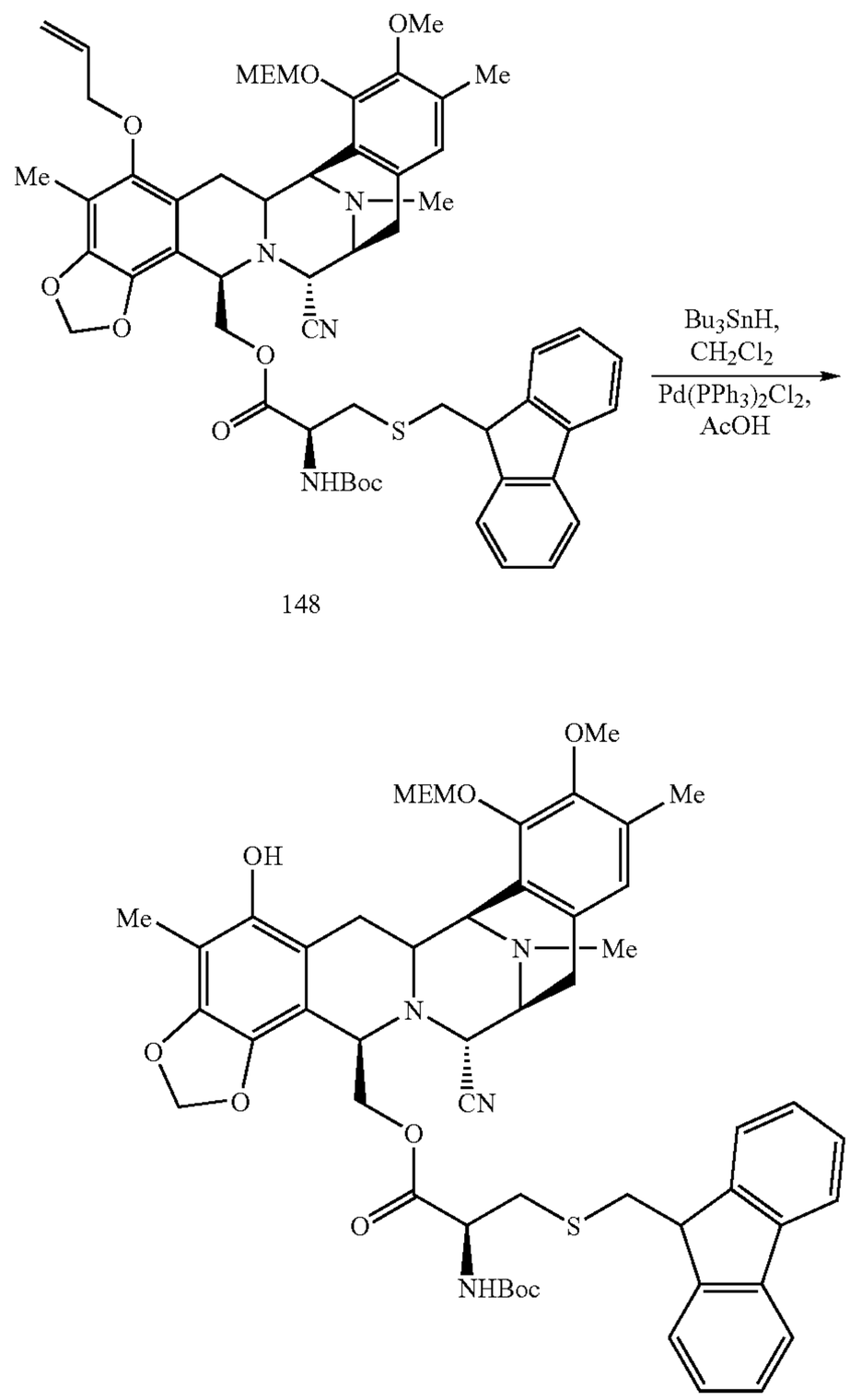
To a solution of 147 (0.562 g, 0.624 mol) in CH_3CN (3.12 mL), MEMCl (1.07 mL, 9.36 mmol), DIPEA (2.17 mL, 12.48 mmol) and DMAP (0.0076 g, 0.06 mmol) were added at 0° C. The mixture was stirred for 5.5 h at 23° C. The reaction was diluted with CH_2Cl_2 (50 mL) and extracted with 0.1N HCl (50 mL). The aqueous phase was extracted again with CH_2Cl_2 (50 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated in vacuo to give a residue which was purified by flash column chromatography (CH_2Cl_2:EtOAc 10:1, 5:1) to give 148 (539 mg, 87%) as a white solid. Rf=0.50 CH_2Cl_2:AcOEt 6:1.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.73-7.71 (m, 2H), 7.57 (dd, J_1=7.2 Hz, J_2=15.3 Hz, 2H), 7.40-7.34 (m, 2H), 7.29-7.26 (m, 2H), 6.62 (s, 1H), 6.08-5.99 (m, 1H), 5.91 (d, J=1.2 Hz, 1H), 5.79 (d, J=1.2 Hz, 1H), 5.35 (dd, J_1=1.2 Hz, J_2=17.1 Hz, 1H), 5.23 (d, J=6.3 Hz, 1H), 5.21 (bs, 1H), 5.13 (d, J=6.3 Hz, 1H), 5.04 (brd, J=9 Hz, 1H), 4.33-4.29 (m, 2H), 4.16-3.90 (m, 8H), 3.85-3.78 (m, 1H), 3.69 (s, 3H), 3.60-3.55 (m, 2H), 3.38 (s, 3H), 3.31 (brd, J=8.1 Hz, 1H), 3.21-3.17 (m, 2H), 2.98-2.88 (m, 3H), 2.64-2.56 (m, 2H), 2.29 (s, 3H), 2.20 (s, 3H), 2.02 (s, 3H), 1.75 (dd, J_1=11.7 Hz, J_2=15.6 Hz, 1H), 1.47 (s, 9H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 170.5, 155.0, 148.6, 148.5, 148.2, 145.77, 145.6, 144.4, 140.8, 140.7, 139.0, 133.6, 130.7, 130.5, 127.4, 126.9, 124.8, 124.6, 123.8, 120.8, 119.7, 117.8, 117.2, 122.5, 111.9, 101.0, 98.1, 80.0, 77.4, 77.0, 76.6, 73.8, 71.6, 69.2, 65.0, 60.2, 60.0, 59.8, 59.0, 56.8, 56.7, 56.6, 55.2, 52.7, 46.6, 41.3, 36.2, 34.9, 29.6, 28.2, 26.3, 24.9, 15.6, 14.1, 9.0.

ESI-MS m/z: Calcd. for $C_{55}H_{64}N_4O_{11}S$: 988.4. Found $(M+1)^+$: 989.3.

Example 74



148 149

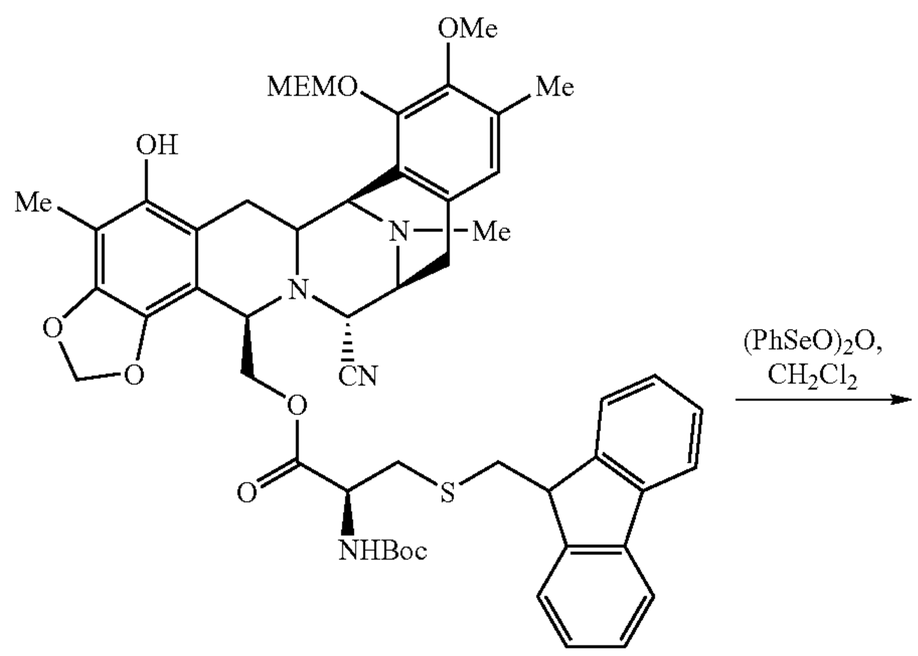
To a stirred solution of 148 (38.32 g. 0.039 mol) in dichloromethane (1 L), dichlorobis(triphenylphosphine) palladium (II) (2.17 g, 0.0031 mol) and acetic acid (11.1 mL, 0.195 mol) were added under argon at 23° C. Then, tributyl tin hydride (36.5 mL, 0.136 mol) was added in a dropwise manner. The mixture was stirred at 23° C. for 15 minutes. The reaction was then filtered through a silica gel column compacted with hexane. 149 (35.07 g, 95%) was obtained as a white solid by subsequent elution with mixtures of ethyl acetate and hexane in a gradient manner, from 0:100, 1:4, 1:3, 2:5, 2:3, 1:1, 2:1, 3:1 to 100:0. Rf=0.25 Hex:EtOAc 2:1.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74 (d, J=7.2 Hz, 2H), 7.63-7.53 (m, 2H), 7.39-7.34 (m, 2H), 7.30-7.27 (m, 2H), 6.62 (s, 1H), 5.87 (m, 1H), 5.75 (s, 1H), 5.69 (bs, 1H), 5.37 (d, J=6 Hz, 1H), 5.23 (d, J=5.7 Hz, 1H), 4.96 (d, J=8.1 Hz, 1H), 4.44 (brd, J=8.7 Hz, 1H), 4.18-3.70 (m, 11H), 3.69 (s, 3H), 3.38 (s, 3H), 3.34-3.18 (m, 3H), 2.99-2.88 (m, 3H), 2.63-2.58 (m, 2H), 2.28 (s, 3H), 2.21 (s, 3H), 2.05 (s, 3H), 1.78 (dd, J_1=12.9 Hz, J_2=15.6.3 Hz, 1H), 1.41 (s, 9H).

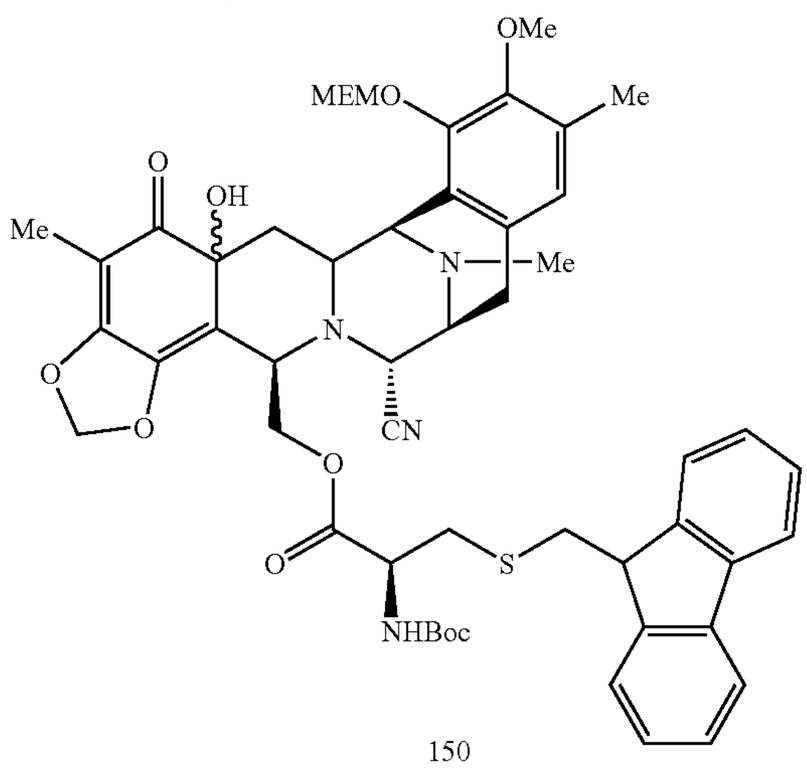
^{13}C NMR (75 MHz, $CDCl_3$) δ 170.8, 155.2, 149.0, 148.0, 146.2, 146.0, 144.8, 141.1, 136.4, 131.3, 131.2, 127.8, 127.2, 125.1, 125.0, 123.2, 120.0, 118.1, 112.6, 111.6, 107.2, 101.0, 98.9, 98.8, 80.3, 71.8, 69.8, 64.9, 60.6, 60.2, 59.2, 57.1, 56.9, 55.5, 53.0, 47.0, 46.9, 41.8, 37.0, 35.3, 28.5, 26.2, 25.2, 21.9, 21.3, 16.1, 14.4, 9.0.

ESI-MS m/z: Calcd. for $C_{52}H_{60}N_4O_{11}S$: 948.4. Found $(M+1)^+$: 949.3.

Example 75



149



150

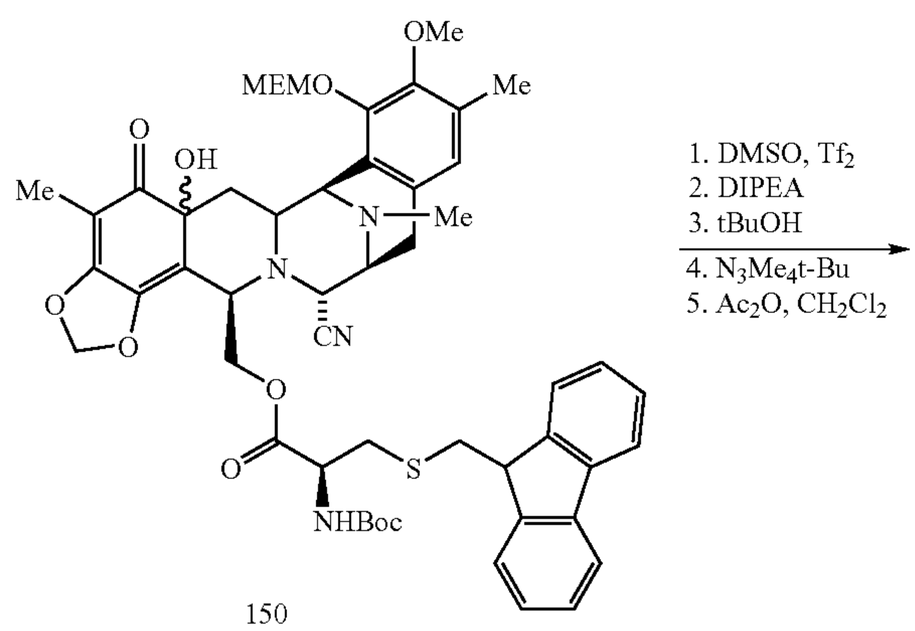
To a solution of 149 (15 g, 0.0158 mol) in anhydrous dichloromethane (265 mL) at −10° C. (bath temperature −15° C.), a solution of benzeneseleninic anhydride (7.4 g, 0.0143 mol) in anhydrous dichloromethane (265 mL) was added dropwise during 30 minutes, discarding any white solid present in the solution. The mixture was stirred for a further 10 minutes at the same temperature. The reaction was diluted with dichloromethane (200 mL) and a saturated aqueous sodium bicarbonate solution (500 mL) was added at −10° C. The organic phase was separated, dried over sodium sulphate, filtered and concentrated to dryness at reduced pressure. The residue was purified by flash column chromatography eluting with mixtures of ethyl acetate and hexane in a gradient manner, from 1:2 to 100:0 to obtain 150 (14.20 g, 89%) as a yellow solid. The purified solid from chromatography is dissolved in dichloromethane (250 mL) and charcoal (4.95 g) was added. The suspension was then stirred at 23° C. for 1 hour. The mixture was filtered through a pad of celite and the celite was washed with dichloromethane (80 mL). The solvent was evaporated at reduced pressure to yield 150 (13.72 g, 86%) as a white solid. Rf=0.37 Hex:EtOAc 1:2.

^{1}H NMR (300 MHz, $CDCl_3$) (mixture of isomers) δ 7.73 (t, J=6.7 Hz, 4H), 7.63 (m, 2H), 7.54 (d, J=7.6 Hz, 2H), 7.40-7.34 (m, 4H), 7.31-7.27 (m, 4H), 6.62 (s, 2H), 5.86 (s, 1H), 5.81 (s, 1H), 5.75 (s, 1H), 5.72 (s, 1H), 5.70 (s, 1H), 5.35 (d, J=5.9 Hz, 1H), 5.30 (d, J=8.4 Hz, 1H), 5.23 (d, J=5.9 Hz, 1H), 5.22 (d, J=5.9 Hz, 1H), 5.13 (d, J=5.9 Hz, 1H), 4.97 (d, J=8.8 Hz, 1H), 4.43 (m, 2H), 4.20-4.01 (m, 8H), 3.97-3.86 (m, 4H), 3.82 (s, 3H), 3.80-3.74 (m, 1H), 3.69 (s, 3H), 3.66-3.64 (m, 4H), 3.54 (m, 2H), 3.38 (s, 3H), 3.35 (s, 3H), 3.34-2.90 (m, 8H), 2.60-2.31 (m, 4H), 2.27 (s, 3H), 2.25 (s, 3H), 2.21 (s, 3H), 1.97 (s, 3H), 1.94-1.81 (m, 2H), 1.77 (s, 3H), 1.43 (s, 9H), 1.41 (s, 9H).

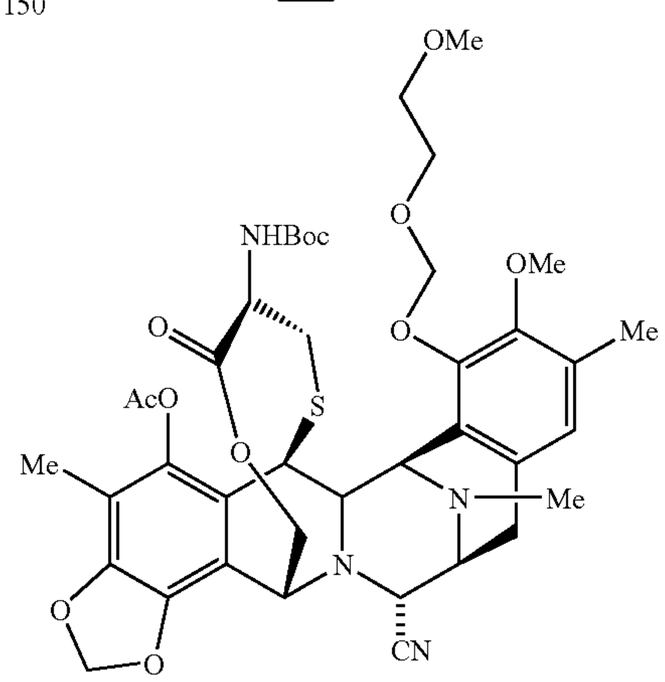
^{13}C NMR (75 MHz, $CDCl_3$) (mixture of isomers) δ 200.2, 198.3, 170.7, 170.5, 160.0, 155.2, 154.9, 148.5, 148.4, 145.5, 142.1, 140.9, 138.3, 130.9, 130.5, 130.0, 129.8, 127.5, 126.9, 125.0, 124.9, 124.7, 123.8, 122.5, 119.8, 117.2, 116.7, 111.5, 108.1, 104.6, 104.3, 101.3, 100.9, 98.0, 80.1, 72.1, 71.5, 70.5, 69.2, 69.0, 66.4, 63.5, 60.7, 60.1, 59.6, 58.9, 58.8, 58.0, 56.7, 56.4, 56.2, 55.9, 55.5, 55.0, 53.5, 46.7, 41.7, 41.3, 41.1, 36.9, 35.2, 35.1, 31.4, 28.1, 25.4, 25.3, 22.5, 15.7, 15.6, 14.0, 7.2.

ESI-MS m/z: Calcd. for $C_{52}H_{60}N_4O_{12}S$: 964.4. Found: 965.3 $(M+1)^+$, 987.3 $(M+23)^+$.

Example 76



150



151

The reaction flask was flamed twice, purged vacuum/Argon several times and kept under Argon atmosphere for the reac-

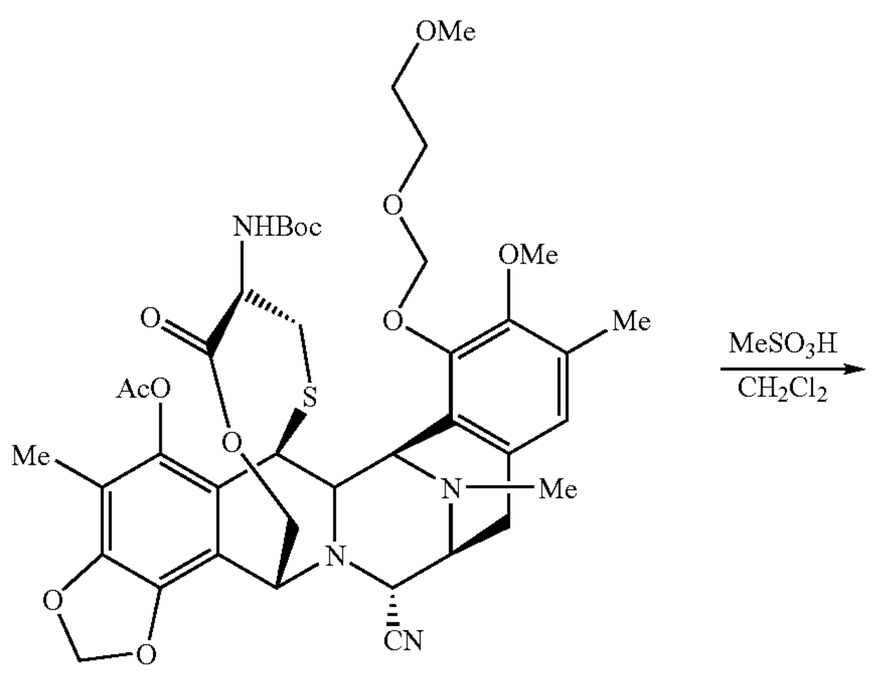
tion. To a solution of DMSO (385.0 μL) in anhydrous CH_2Cl_2 (42 mL) was dropwise added triflic anhydride (366.5 μL, 2.16 mmol) at −78° C. The reaction mixture was stirred at −78° C. for 20 minutes. Then, a solution of 150 (1 g, 1.03 mmol) in anhydrous CH_2Cl_2 (10 mL, for the main addition and 5 mL for washing) was added via canula (addition time: 5 min) at −78° C. During the addition the temperature was kept at −78° C. in both flasks and the color changed from yellow to brown. The reaction mixture was stirred at −40° C. for 35 minutes. During this period of time the solution was turned from yellow to dark green. After this time, $^iPr_2NEt$ (1.51 mL, 9.55 mmol) was dropwise added and the reaction mixture was kept at 0° C. for 45 minutes, the color of the solution turned brown during this time. Then, ^tBuOH (409.5 □L, 4.33 mmol) and tert-butyl tetramethyl guanidine (1.31 mL, 7.61 mmol) were dropwise added and the reaction mixture was stirred at 23° C. for 40 minutes. After this time, acetic anhydride (1.03 mL, 10.89 mmol) was dropwise added and the reaction mixture was kept at 23° C. for 1 hour more. Then, the reaction mixture was diluted with CH_2Cl_2 (25 mL) and washed with aqueous saturated solution of NH_4Cl (50 mL), $NaHCO_3$ (50 mL), and NaCl (50 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash column chromatography (inner diameter: 2.0 cm, height of silica: 9 cm; eluent: ethyl acetate/hexane in a gradient manner, from 20:80, 30:70 to 40:60) to afford 151 (832.6 mg, 99%) as a white solid. Rf=0.48 Hex:EtOAc 3:2.

^1H NMR (300 MHz, $CDCl_3$) δ 6.78 (s, 1H), 6.09 (d, J=1.2 Hz, 1H), 5.99 (d, J=1.2 Hz, 1H), 5.32 (d, J=5.8 Hz, 1H), 5.19 (d, J=5.6 Hz, 1H), 5.01 (d, J=11.7 Hz, 1H), 4.62 (d, J=9.8 Hz, 1H), 4.50 (bs, 1H), 4.34 (d, J=5.1 Hz, 1H), 4.28 (dd, J_1=2.4 Hz, J_2=6.8 Hz, 1H), 4.24 (s, 1H), 4.17 (m, 2H), 3.90 (m, 2H), 3.76 (s, 3H), 3.58 (t, J=4.8 Hz, 2H), 3.42-3.37 (m, 2H), 3.37 (s, 3H), 2.91 (m, 2H), 2.36-2.08 (m, 2H), 2.30 (s, 3H), 2.28 (s, 3H), 2.21 (s, 3H), 2.04 (s, 3H), 1.44 (s, 9H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 170.9, 168.9, 168.0, 155.4, 149.8, 148.6, 146.0, 141.1, 140.6, 131.6, 131.1, 130.6, 129.0, 125.1, 120.6, 118.3, 102.2, 98.4, 79.9, 71.9, 69.4, 61.6, 60.4, 59.8, 59.4, 59.2, 54.9, 54.7, 54.0, 41.6, 30.6, 29.1, 28.7, 23.9, 23.2, 20.6, 16.1, 14.2, 11.2, 9.8.

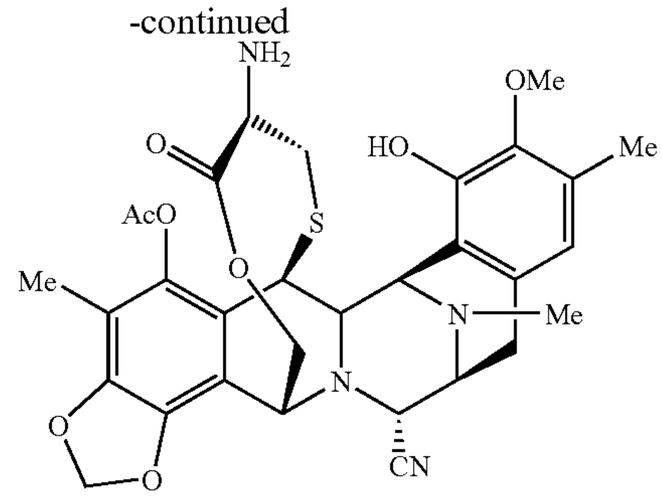
ESI-MS m/z: Calcd. for $C_{40}H_{50}N_4O_{12}S$: 810.91. Found $(M+1)^+$: 811.3.

Example 77



151

-continued



35

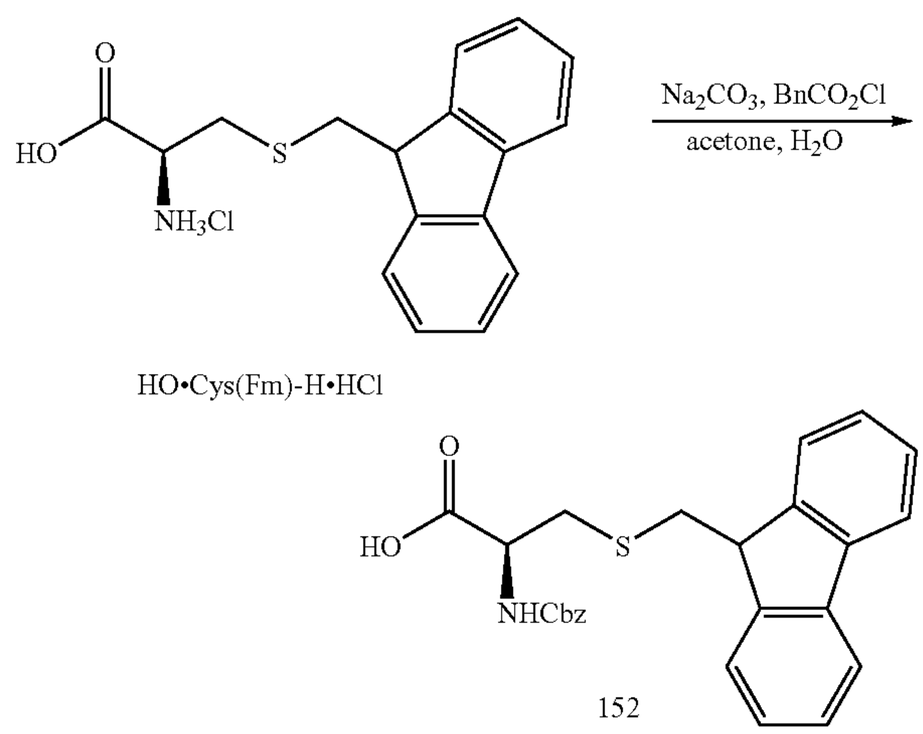
To a solution of 151 (2.9 g, 3.57 mmol) in CH_2Cl_2 (120 mL), $MeSO_3H$ (1.4 mL, 21.46 mmol) was added at 23° C. After stirring the reaction for 30 minutes at 23° C., a saturated aqueous sodium bicarbonate solution (200 mL) was added at 0° C. The organic phase was separated, dried over sodium sulphate, filtered and concentrated to dryness at reduced pressure. The residue was purified by flash column chromatography, eluting with mixtures of ethyl acetate and hexane in a gradient manner, from 0:1 to 1:0 to obtain 35 (1.43 g, 64%) as a pale yellow solid. Experimental data of 35 was previously decribed in PCT/GB00/01852.

36, ET-770 and ET-743 were prepared following the same procedures than those previously described in PCT/GB00/01852.

Route 3

The first step of this Route (transformation of 21 into 146) was described above in Example 71.

Example 78



152

To a solution of the commercial available HO.Cys(Fm)-H.HCl (Bachem) (40 g, 0.119 mol) in acetone (500 mL) and water (500 mL), 1M Na_2CO_3 solution (238 mL) and $BnCO_2Cl$ (18.7 mL, 0.131 mol) were added at 0° C. After stirring the reaction at 60° C. for 30 minutes, the mixture was quenched with 1N HCl (pH 01) and extracted with eter (3×400 mL). The organic phase was separated, dried over magnesium sulphate filtered and concentrated to dryness at

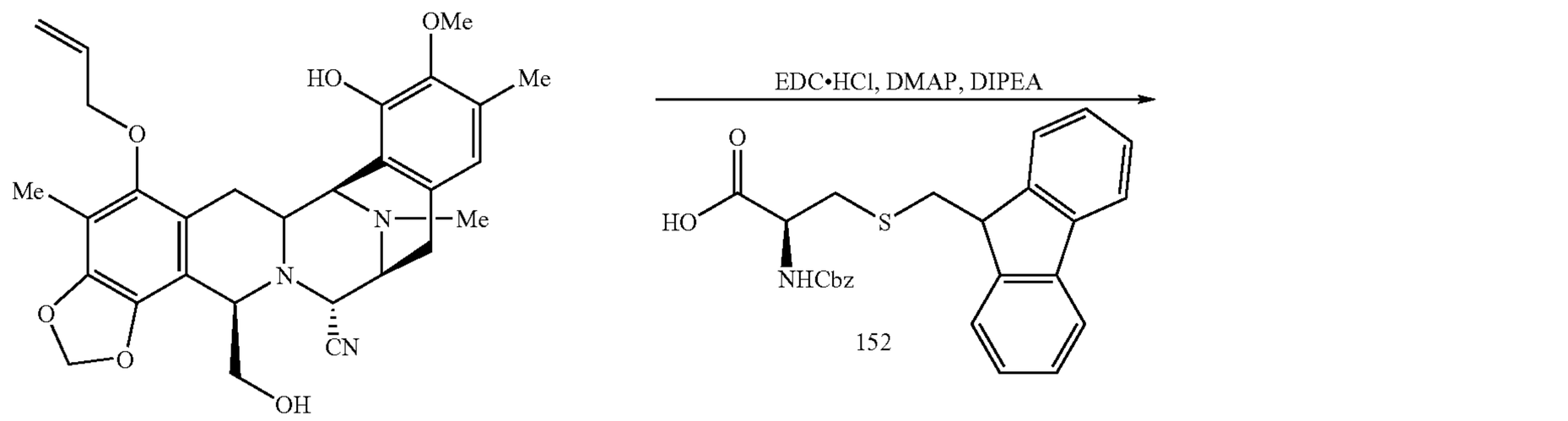
reduced pressure. The crude solid was disolved in a mixture of EtOAc/CH_2Cl_2 1:1, precipitated with hexane and kept at 4° C. overnight. Then, the suspension was filtered off, the solid washed with hexane (200 mL) and the filtrate was dried in vacuo to afford 152 (50.16 g, 97%) as a white solid.

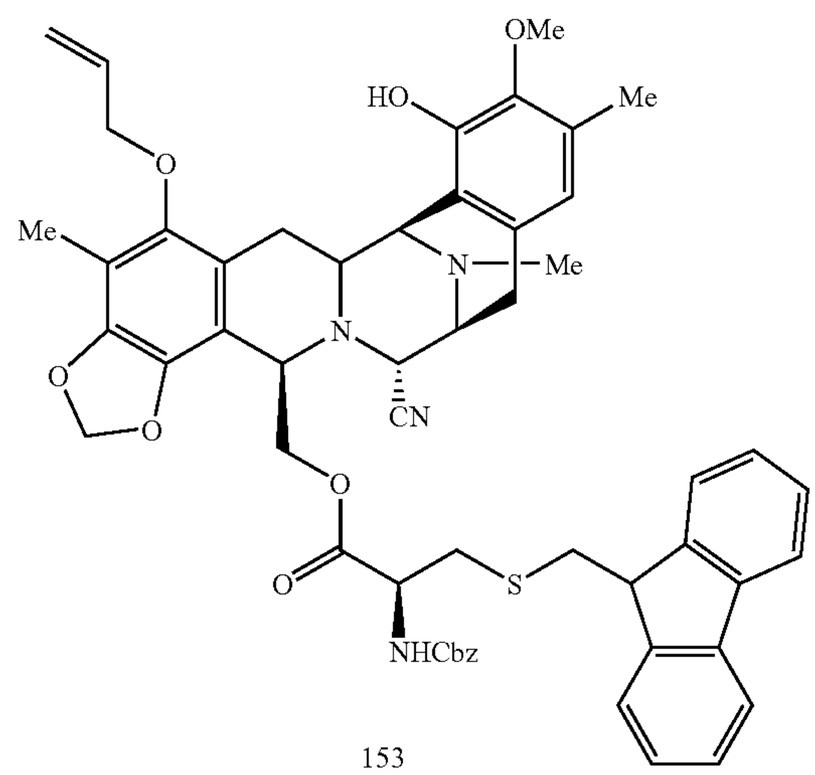
^{1}H NMR (300 MHz, $CDCl_3$) δ 10.66 (bs, 1H), 7.74 (d, J=7.5 Hz, 2H), 7.69-7.64 (m, 2H), 7.62-7.29 (m, 9H), 5.67 (d, J=7.5 Hz, 1H), 5.14 (bs, 2H), 4.70-4.64 (m, 1H), 4.09-4.05 (m, 1H), 3.12-3.09 (m, 2H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 175.2, 155.9, 145.5, 141.0, 135.8, 128.5, 128.2, 128.1, 127.5, 127.0, 124.7, 119.8, 84.8, 67.3, 46.8, 37.0.

ESI-MS m/z: Calcd. for $C_{25}H_{23}NO_4S$: 433.52. Found $(M+1)^+$: 434.4.

Example 79





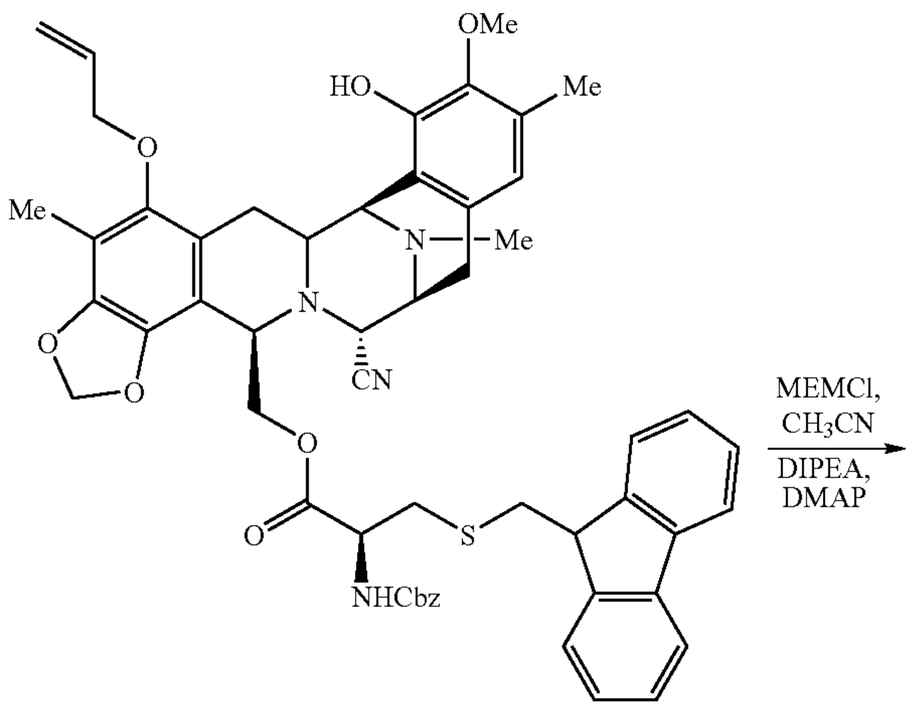
To a stirred solution of 146 (10 g, 19.2 mmol) and 152 (12.5 g, 28.8 mmol) in dichloromethane (800 mL) under argon, dimethylaminopyridine (705 mg, 5.77 mmol), 1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (9.2 g, 48.1 mmol) and diisopropylethy amine (7.4 mL, 42.3 mmol) were added dropwise during 1 h at 0° C. The mixture was then stirred at 23° C. for 1.5 more hour. The reaction was quenched by addition of a saturated aqueous sodium bicarbonate solution (600 mL). The organic phase was separated and washed again with a saturated aqueous amonium chloride solution (500 mL) and a saturated sodium chloride solution (500 mL). The organic extract were dried over sodium sulphate, filtered and evaporated to dryness under reduced pressure. The crude product was purified by flash column chromatography (RP18, CH_3CN:H_2O 4:1) to yield 153 (13.89 g, 77%) as a pale yellow solid.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74-7.72 (m, 2H), 7.61-7.53 (m, 2H), 7.37-7.24 (m, 9H), 6.39 (s, 1H), 6.09-5.96 (m, 1H), 5.90 (s, 1H), 5.84 (s, 1H), 5.78 (s, 1H), 5.34 (dd, J_1=1.5 Hz, J_2=17.4 Hz, 1H), 5.32 (bs, 1H), 5.24 (dd, J_1=1.5 Hz, J_2=10.2 Hz, 1H), 5.17-5.07 (m, 2H), 4.40 (dd, J_1=3.6 Hz, J_2=10.8 Hz, 1H), 4.30 (m, 1H), 4.18-4.01 (m, 6H), 3.92 (brt, J=6.3 Hz, 1H), 3.71 (s, 3H), 3.30-3.19 (m, 3H), 2.99-2.85 (m, 3H), 2.65 (dd, J_1=4.5 Hz, J_2=14.4 Hz, 1H), 2.55 (d, J=18.3 Hz, 1H), 2.26 (s, 3H), 2.21 (s, 3H), 2.06 (s, 3H), 1.86 (dd, J_1=11.7 Hz, J_2=15.9 Hz, 1H).

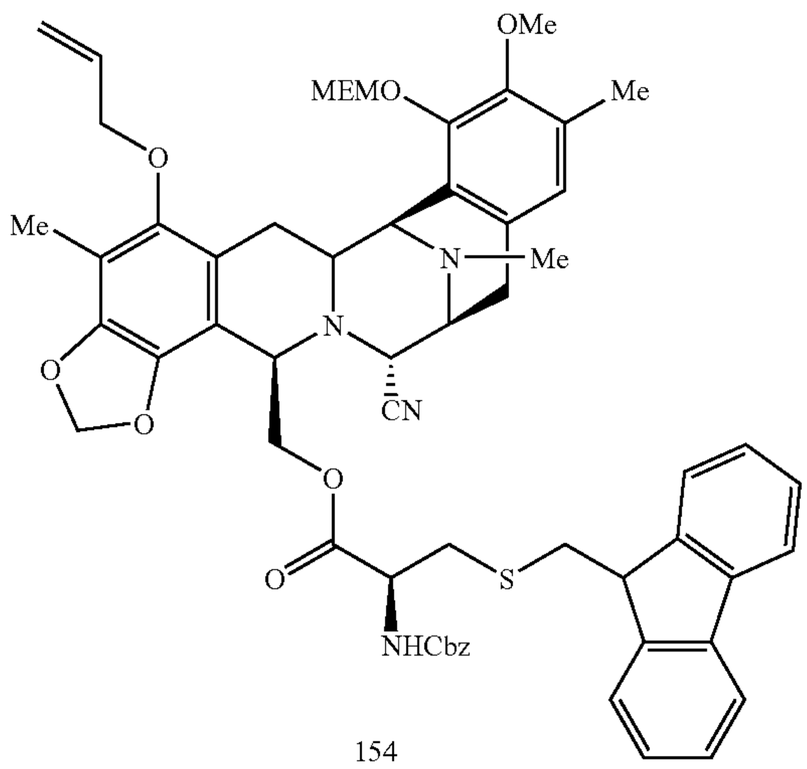
^{13}C NMR (75 MHz, $CDCl_3$) δ 170.2, 155.6, 148.6, 146.8, 145.7, 145.6, 144.3, 142.6, 140.7, 139.0, 133.7, 131.1, 128.8, 128.4, 128.1, 128.0, 127.4, 126.9, 124.7, 124.6, 121.0, 120.5, 119.7, 117.8, 117.3, 116.8, 112.5, 112.0, 101.0, 74.1, 67.0, 64.7, 60.7, 59.9, 57.0, 56.6, 56.3, 55.2, 53.1, 46.5, 41.4, 36.4, 34.8, 26.2, 24.8, 15.6, 9.2.

ESI-MS m/z: Calcd. for $C_{54}H_{54}N_4O_9S$: 934.36. Found $(M+1)^+$: 935.4.

Example 80



153



154

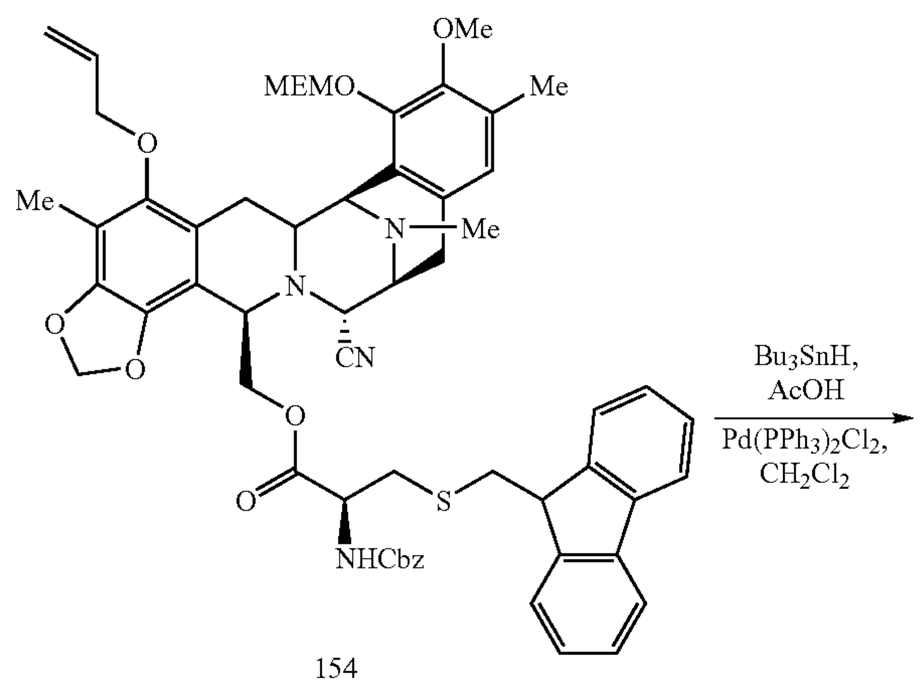
To a solution of 153 (13.89 g, 14.85 mmol) in CH_3CN (74.3 mL), MEMCl (25.4 mL, 223 mmol), DIPEA (52 mL, 297 mmol) and DMAP (0.181 g, 0.15 mmol) were added at 0° C. The mixture was stirred for 5 h at 23° C. The reaction was diluted with CH_2Cl_2 (400 mL) and extracted with 0.1N HCl (300 mL) and 3N HCl (pH=3). The aqueous phase was extracted again with CH_2Cl_2 (2×50 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated in vacuo to give a residue which was purified by flash column chromatography (SiO_2, CH_2Cl_2:EtOAc 10:1, 5:1) to give 154 (13.47 g, 88%) as a white solid. Rf=0.27 CH_2Cl_2:AcOEt 6:1.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.73-7.70 (m, 2H), 7.58-7.50 (m, 2H), 7.38-7.22 (m, 9H), 6.59 (s, 1H), 6.08-5.98 (m, 1H), 5.89 (s, 1H), 5.77 (s, 1H), 5.35 (d, J=17.1 Hz, 1H), 5.31-5.28 (m, 1H), 5.23 (d, J=6.9 Hz, 1H), 5.13 (d, J=6.9 Hz, 1H), 5.12-5.05 (m, 2H), 4.37-4.29 (m, 2H), 4.15-3.77 (m, 9H), 3.68 (s, 3H), 3.58-3.55 (m, 2H), 3.37 (s, 3H), 3.30-3.27 (m, 1H), 3.21-3.16 (m, 2H), 2.96-2.84 (m, 4H), 2.64-2.58 (m, 1H), 2.55 (d, J=18 Hz, 1H), 2.27 (s, 3H), 2.16 (s, 3H), 2.02 (s, 3H), 1.75 (dd, J_1=12.3 Hz, J_2=16.2 Hz, 1H).

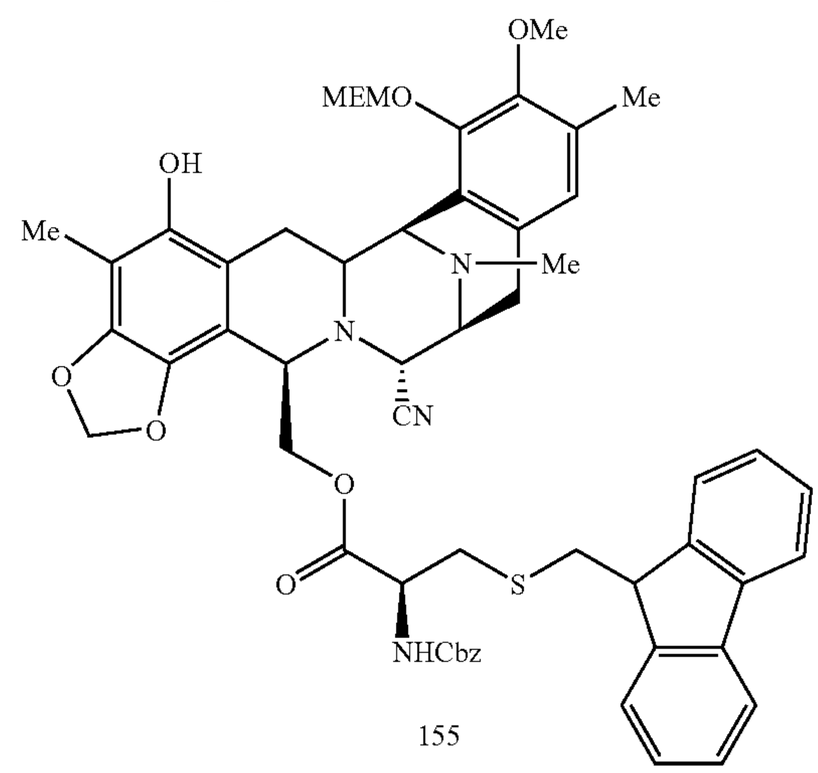
^{13}C NMR (75 MHz, $CDCl_3$) δ 171.9, 170.2, 155.5, 148.7, 148.6, 148.3, 145.8, 145.7, 144.5, 142.1, 140.9, 139.1, 136.1, 133.8, 130.8, 130.5, 128.5, 128.3, 128.1, 127.6, 127.0, 124.9, 124.7, 123.9, 122.2, 120.9, 119.8, 117.8, 117.3, 112.6, 112.0, 101.1, 98.2, 74.0, 71.7, 69.3, 67.1, 65.1, 60.1, 59.8, 59.0, 56.9, 56.8, 56.7, 55.3, 53.3, 46.7, 41.4, 36.5, 35.0, 31.6, 29.7, 26.4, 25.0, 22.6, 15.7, 14.1, 9.2.

ESI-MS m/z: Calcd. for $C_{58}H_{62}N_4O_{11}S$: 1023.2. Found $(M+23)^+$: 1046.3.

Example 81



154



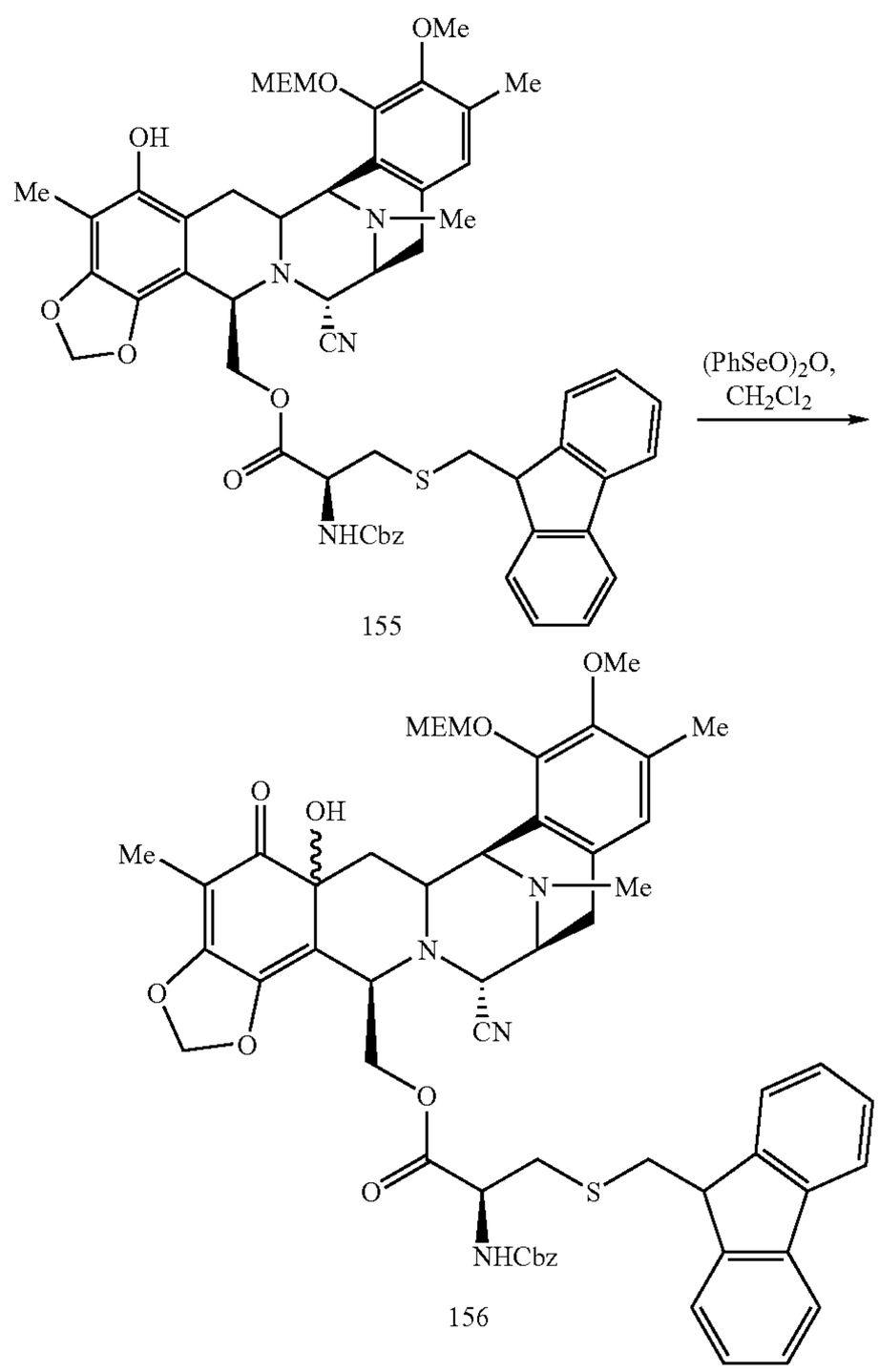
155

To a stirred solution of 154 (20.84 g. 0.02 mol) in dichloromethane (530 mL), dichlorobis(triphenylphosphine) palladium (II) (1.14 g, 1.63 mmol) and acetic acid (11.64 mL, 0.2 mol) were added under argon at 23° C. Then, tributyltin hydride (27.44 mL, 0.1 mol) was added in a dropwise manner. The mixture was stirred at 23° C. for 15 minutes. The reaction was then filtered through a silica gel column compacted with hexane. 155 (18.78 g, 94%) was obtained as a pale yellow solid by subsequent elution with mixtures of ethyl acetate and hexane in a gradient manner, from 1:4, 1:1, 3:2 to 7:3.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71 (d, J=7.2 Hz, 2H), 7.59 (d, J=7.5 Hz, 1H), 7.53 (d, J=7.5 Hz, 1H), 7.41-7.23 (m, 9H), 6.60 (s, 1H), 5.87 (bs, 2H), 5.74 (s, 1H), 5.40 (d, J=6.3 Hz, 1H), 5.33 (d, J=5.8 Hz, 1H), 5.18 (d, J=9 Hz, 1H), 5.09 (d, J=12 Hz, 1H), 4.97 (d, J=12 Hz, 1H), 4.56 (dd, J_1=3 Hz, J_2=11.1 Hz, 1H) 4.19 (d, J=2.1 Hz, 1H), 4.16-3.87 (m, 9H), 3.66 (s, 3H), 3.38 (s, 3H), 3.32-3.20 (m, 3H), 2.96-2.87 (m, 3H), 2.62-2.54 (m, 2H), 2.28 (s, 3H), 2.19 (s, 3H), 1.97 (s, 3H), 1.82 (dd, J_1=13.2 Hz, J_2=15.6 Hz, 1H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 170.0, 155.4, 149.0, 147.5, 145.7, 145.6, 144.4, 140.8, 135.9, 130.9, 128.4, 128.1, 128.0, 127.4, 126.9, 124.7, 124.6, 122.7, 119.7, 117.7, 112.4, 111.4, 100.6, 98.7, 71.5, 69.4, 67.0, 64.9, 63.9, 59.7, 59.6, 58.8, 57.0, 56.5, 56.4, 55.1, 54.9, 53.1, 52.5, 46.5, 41.4, 36.8, 34.9, 25.8, 24.7, 15.7, 8.7.

Example 82

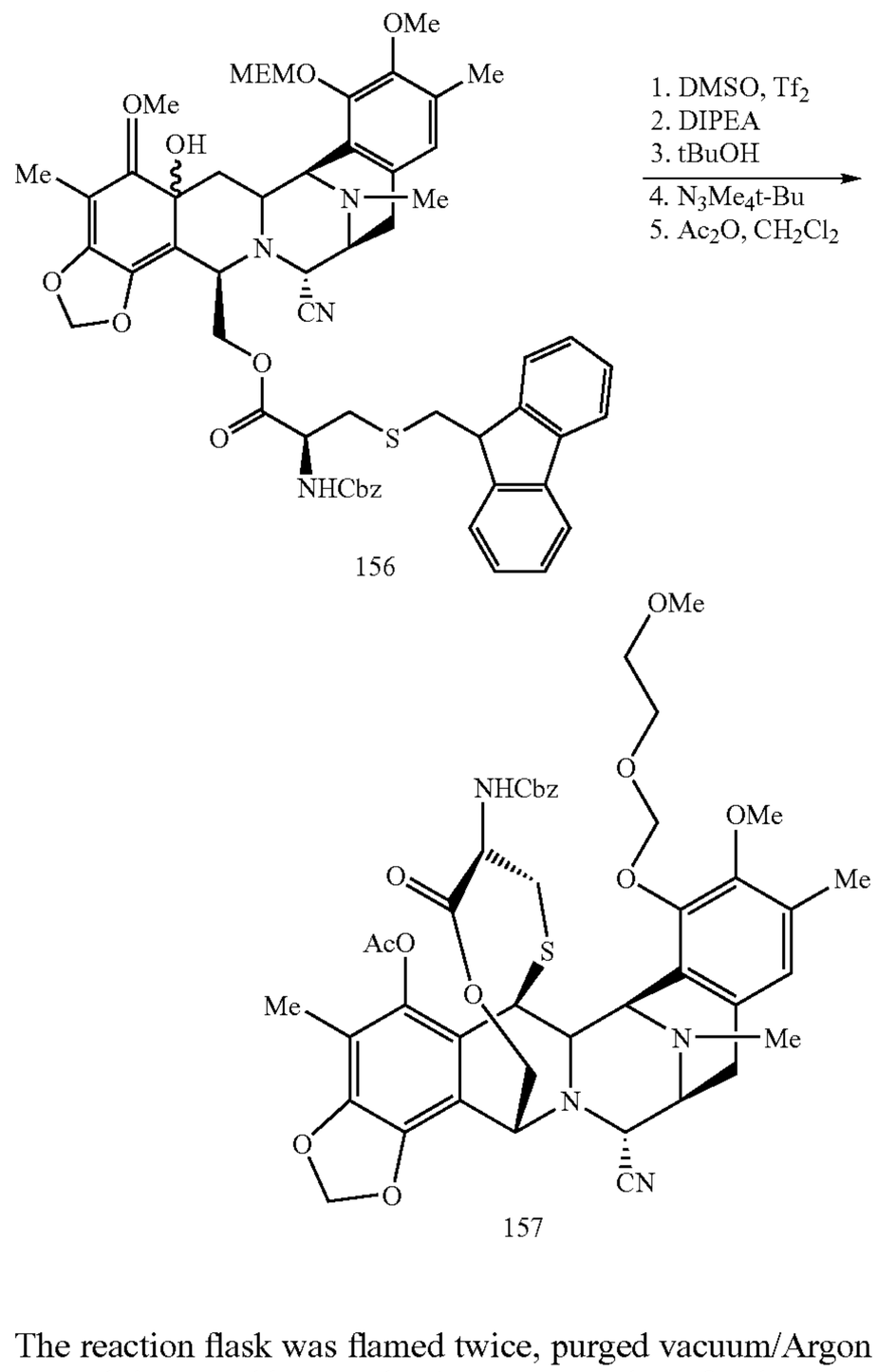


To a solution of 155 (18.5 g, 18.82 mmol) in anhydrous dichloromethane (530 mL) at −10° C. (bath temperature −15° C.), a solution of benzeneseleninic anhydride (9.68 g, 18.82 mmol) in anhydrous dichloromethane (290 mL) was added dropwise, discarding any white solid present in the solution. The mixture was stirred for 10 minutes at the same temperature. The reaction was then quenched with a saturated aqueous sodium bicarbonate solution (600 mL). The organic phase was separated, and the aqueous phase was extracted with CH_2Cl_2 (2×300 mL). The combined organic extracts were dried over sodium sulphate, fitltered, and concentrated to dryness under reduced pressure. The residue was purified by column chromatography, eluting with mixtures of ethyl acetate and hexane in a gradient manner, from 1:1, 3:2, 7:3 to 4:1 to obtain 156 (17.62 g, 88%) as a pale yellow solid.

^{1}H NMR (300 MHz, $CDCl_3$) (mixture of isomers) δ 7.73 (d, J=7.5 Hz, 2H), 7.63 (d, J=7.5 Hz, 2H), 7.40-7.29 (m, 9H), 6.59 (s, 1H), 6.52 (s, 1H), 5.68 (s, 1H), 5.66 (s, 1H), 5.58 (s, 1H), 5.56 (s, 1H), 5.23 (d, J=6 Hz, 1H), 5.15-5.05 (m, 4H), 4.76-4.68 (m, 1H), 4.64-4.55 (m, 1H), 4.40-4.37 (m, 1H), 4.15-3.68 (m, 8H), 3.60 (s, 3H), 3.57 (s, 3H), 3.39 (s, 3H), 3.36 (s, 3H), 3.25-2.78 (m, 7H), 2.38-2.24 (m, 2H), 2.20 (s, 3H), 2.18 (s, 3H), 2.15 (s, 3H), 2.09 (m, 1H), 2.04 (s, 3H), 1.77 (s, 3H), 1.58 (s, 3H).

ESI-MS m/z: Calcd. for $C_{55}H_{58}N_4O_{12}S$: 999.13. Found $(M+1)^+$: 1000.0.

Example 83



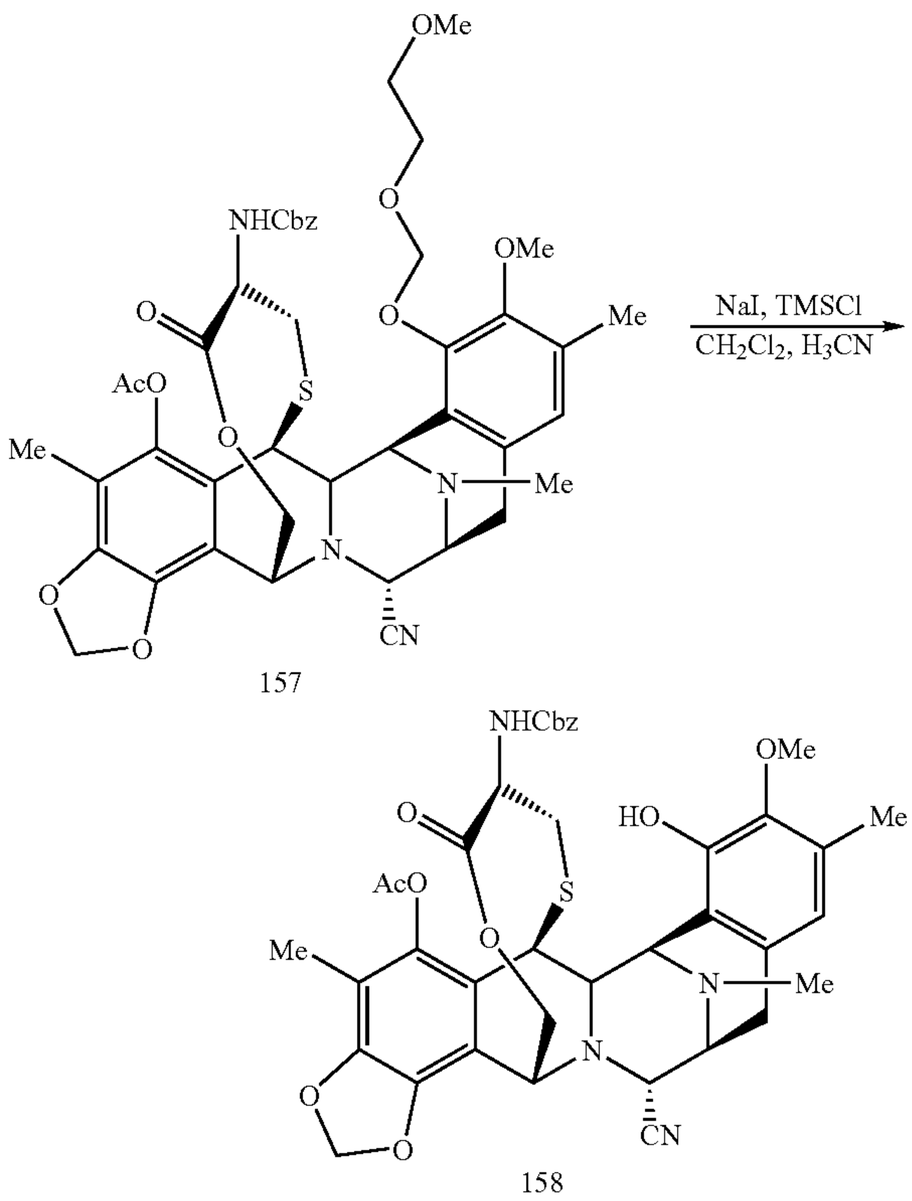
The reaction flask was flamed twice, purged vacuum/Argon several times and kept under Argon atmosphere for the reaction. To a solution of DMSO (178 μL) in anhydrous CH_2Cl_2 (20 mL) was dropwise added triflic anhydride (169 μL, 1 mmol) at −78° C. The reaction mixture was stirred at −78° C. for 20 minutes. Then, a solution of 156 (0.5 g, 0.5 mmol) in anhydrous CH_2Cl_2 (4 mL, for the main addition and 1.5 mL for washing) was added via canula (addition time: 5 min) at −78° C. During the addition the temperature was kept at −78° C. in both flasks and the color changed from yellow to brown. The reaction mixture was stirred at −40° C. for 35 minutes. During this period of time the solution was turned from yellow to dark green. After this time, $^iPr_2NEt$ (0.7 mL, 4.42 mmol) was dropwise added and the reaction mixture was kept at 0° C. for 45 minutes, the color of the solution turned brown during this time. Then ^tBuOH (189 μL, 2 mmol) and tert-butyl tetramethyl guanidine (0.6 mL, 3.49 mmol) were dropwise added and the reaction mixture was stirred at 23° C. for 40 minutes. After this time, acetic anhydride (0.47 mL, 4.97 mmol) was dropwise added and the reaction mixture was kept at 23° C. for 1 hour more. Then, the reaction mixture was diluted with CH_2Cl_2 (15 mL) and washed with aqueous saturated solution of NH_4Cl (25 mL), $NaHCO_3$ (25 mL), and

NaCl (25 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash column chromatography (inner diameter: 2.0 cm, height of silica: 9 cm; eluent: ethyl acetate/hexane in a gradient manner, from 1:4, 1:3, 1:2 to 1:1) to afford 157 (128 mg, 30%) as a light yellow solid. Rf=0.37 Hex:EtOAc 3:2.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 (bs, 5H), 6.66 (s, 1H), 6.09 (s, 1H), 5.99 (s, 1H), 5.30 (d, J=5.4 Hz, 1H), 5.17 (d, J=6 Hz, 1H), 5.06 (d, J=7.8 Hz, 1H), 5.00 (s, 1H), 4.83 (d, J=9.3 Hz, 1H), 4.50 (s, 1H), 4.34-4.17 (m, 7H), 3.90-3.87 (m, 2H), 3.66 (s, 3H), 3.65-3.56 (m, 2H), 3.37 (s, 3H), 2.89-2.90 (m, 2H), 2.28 (s, 3H), 2.18 (s, 3H), 2.15-2.04 (m, 2H), 2.03 (s, 3H), 1.99 (s, 3H).

ESI-MS m/z: Calcd. for $C_{43}H_{48}N_4O_{12}S$: 844.93. Found $(M+1)^+$: 845.8.

Example 84



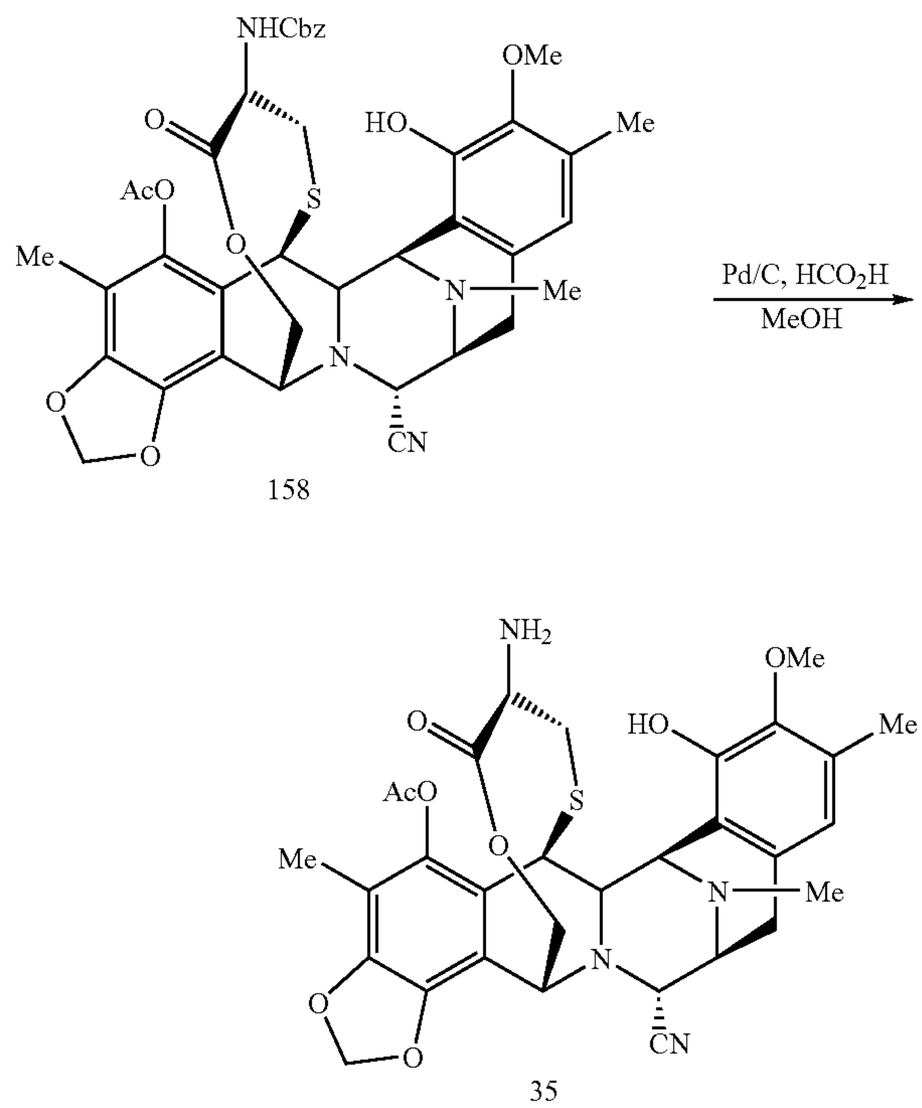
To a solution of 157 (100 mg, 0.118 mmol) in CH_2Cl_2 (2 mL) and CH_3CN (2 mL), NaI (71 mg, 0.472 mmol) and TMSCl (60 □L, 0.472 mmol) were added at 0° C. After stirring the reaction at 23° C. for 50 minutes, the mixture was quenched with water (30 mL) and extracted with CH_2Cl_2 (2×20 mL). The combined organic phases were washed successively with a saturated solution of NaCl (20 mL) and a saturated solution of sodium ditionite (20 mL), dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by flash column chromatography (eluent: ethyl acetate/hexane gradient from 1:4, 1:2 to 1:1) to afford 158 (62 mg, 70%) as white solid. Rf=0.21 Hex:EtOAc 1:1.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36 (bs, 5H), 6.44 (s, 1H), 6.07 (d, J=1.2 Hz, 1H), 5.97 (d, J=1.2 Hz, 1H), 5.81 (bs, 1H), 5.10-5.00 (m, 3H), 4.82 (d, J=9.3 Hz, 1H), 4.49 (bs, 1H), 4.35-4.30 (m, 1H), 4.21-4.17 (m, 2H), 4.16-4.14 (m, 2H), 3.65 (s, 3H), 3.41-3.36 (m, 2H), 2.88-2.85 (m, 2H), 2.28 (s, 3H), 2.24-2.03 (m, 2H), 2.17 (s, 3H), 2.02 (s, 3H), 2.00 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 170.5, 168.8, 155.9, 148.3, 146.0, 143.1, 141.2, 140.6, 136.6, 130.6, 130.0, 128.8, 128.7, 128.5, 121.0, 120.3, 118.3, 118.2, 113.7, 113.6, 102.2, 67.2, 61.5, 60.8, 60.3, 59.6, 59.5, 54.8, 54.7, 54.1, 41.9, 41.6, 32.9, 23.9, 20.8, 15.5, 9.8.

ESI-MS m/z: Calcd. for $C_{39}H_{40}N_4O_{10}S$: 756.82. Found $(M+1)^+$: 757.3.

Example 85



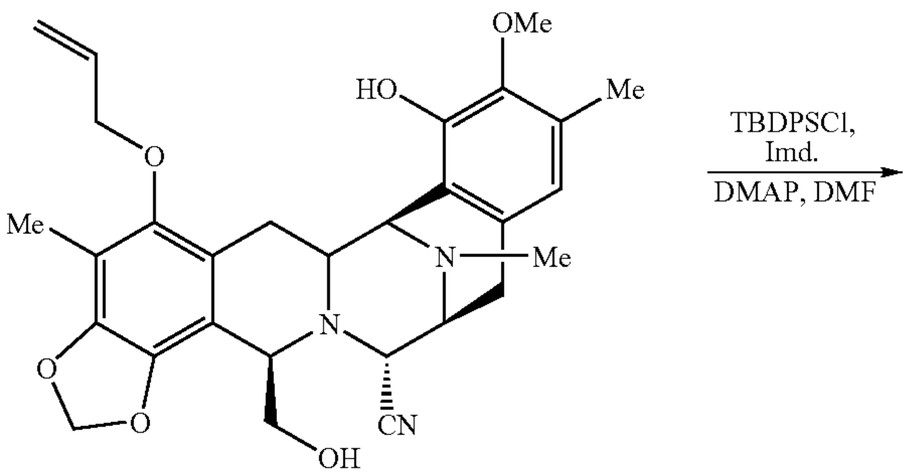
To a solution of 158 (100 mg, 0.132 mmol) in MeOH (6.8 mL), HCO_2H (360 μL) and 10% Pd/C (140 mg, 0.132 mmol) were added at 23° C. and the mixture was stirred for 15 minutes. Then, toluene (7 mL) was added to the reaction and the solvent was evaporated under reduced pressure. The azeotropic destination with toluene was repeated 3 times. The residue was then diluted with dichloromethane (15 mL) and a saturated aqueous solution of sodium bicarbonate (15 mL) was added. The aqueous phase was separated and extracted with dichloromethane (2×10 mL). The combined organic extracts were dried over sodium sulphate, filtered and evaporated to dryness under reduced pressure. The residue was then purified by flash column chromatography on amino-silicagel and eluting with mixtures of ethyl acetate and hexane in a gradient manner, from 1:2, 1:1 to 2:1 to give 35 (57 mg, 70%) as a yellow solid. Experimental data of 35 were previously described in PCT/GB00/01852.

36, ET-770 and ET-743 were prepared following the same procedures than those previously described in PCT/GB00/01852.

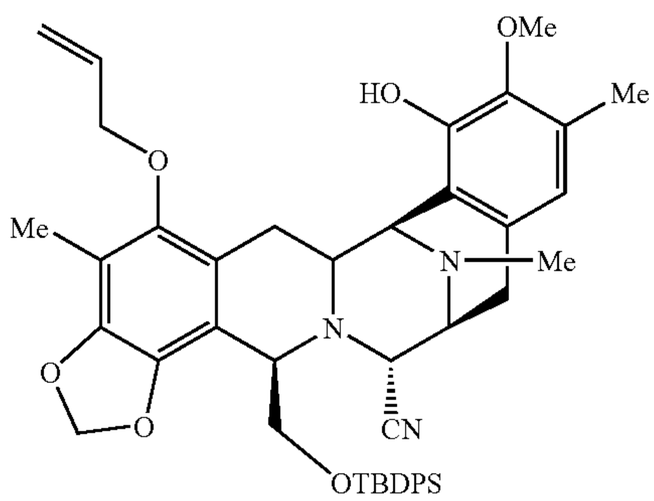
Route 4

The first step of this Route (transformation of 21 into 146) was described above in Example 71.

Example 86



146



159

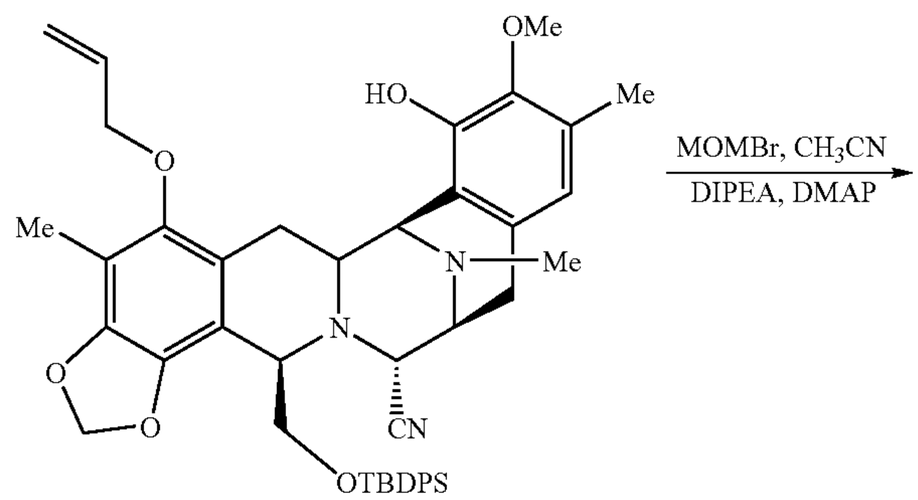
To a solution of 146 (18 mg, 0.032 mmol), cat. DMAP and imidazole (5 mg, 0.08 mmol) in DMF (0.05 mL) at 0° C., tert-buthyldiphenylsilyl chloride (12.5 μL, 0.048 mmol) was added and the reaction was stirred for 4 hours at 23° C. Then, water (30 mL) was added at 0° C. and the mixture was extracted with Hex:EtOAc 1:10 (2×40 mL). The combined organic phases were dried over sodium sulphate, filtered, and the solvent was removed under reduced pressure. The residue was puified by flash column chromatography (SiO_2, Hex: EtOAc 3:1) to afford 159 (27 mg, 88%) as a white solid. Rf=0.29 Hex:EtOAc 3:1.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.72-7.41 (m, 2H), 7.40-7.20 (m, 8H), 6.46 (s, 1H), 6.16-6.00 (m, 1H), 5.77 (d, J=1.5 Hz, 1H), 5.71 (s, 1H), 5.63 (d, J=1.5 Hz, 1H), 5.24 (dd, J_1=1.2 Hz, J_2=17.1 Hz, 1H), 5.23 (dd, J_1=1.2 Hz, J_2=10.2 Hz, 1H), 4.18 (d, J=2.4 Hz, 1H), 4.13-4.00 (m, 4H), 3.77 (s, 3H), 3.63 (dd, J_1=2.4 Hz, J_2=7.5 Hz, 1H), 3.39-3.19 (m, 4H), 2.99 (dd, J_1=8.1 Hz, J_2=18.0 Hz, 1H), 2.68 (d, J=17.7 Hz, 1H), 2.30 (s, 3H), 2.28 (s, 3H), 2.08 (s, 3H), 1.99 (dd, J_1=12.6 Hz, J_2=16.3 Hz, 1H), 0.89 (s, 9H).

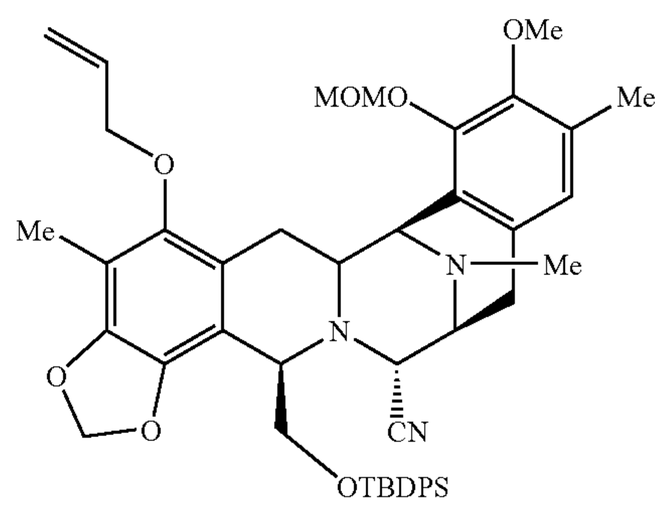
^{13}C NMR (75 MHz, $CDCl_3$) δ 148.3, 146.6, 144.0, 142.5, 139.0, 135.7, 135.4, 133.9, 133.6, 132.2, 131.2, 129.5, 129.4, 128.3, 127.5, 127.4, 121.8, 120.9, 118.7, 117.3, 117.2, 112.9, 111.7, 100.8, 74.2, 68.0, 61.6, 60.6, 60.3, 59.0, 57.4, 56.7, 55.4, 41.7, 29.6, 26.6, 26.5, 25.5, 18.9, 15.8, 9.3.

ESI-MS m/z: Calcd. for $C_{45}H_{51}N_3O_6Si$: 757.9. Found $(M+1)^+$: 758.4.

Example 87



159

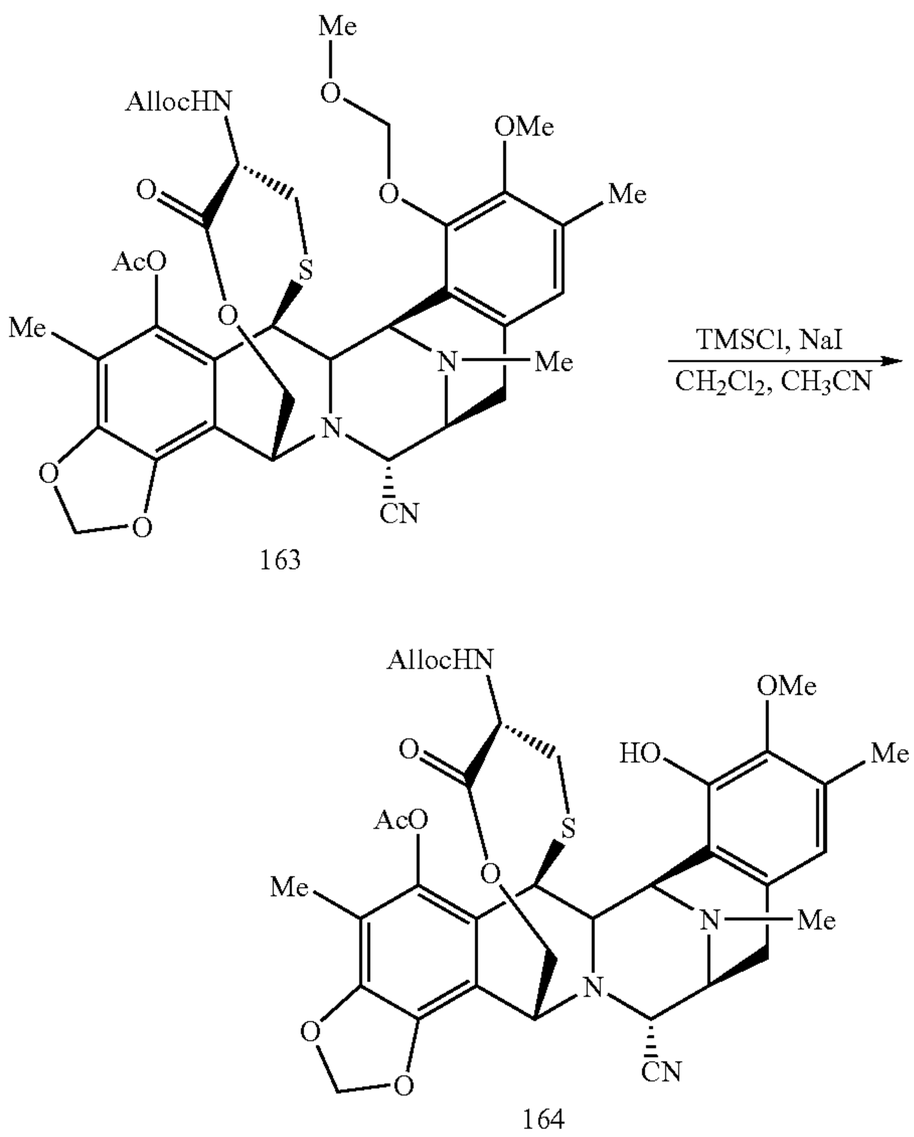


26

To a solution of 159 (2.4 g, 3.17 mmol) in CH_3CN (16 mL), MOMBr (2.6 mL, 31.75 mmol), DIPEA (8.3 mL, 47.6 mmol) and DMAP (16 mg, 0.127 mmol) were added at 0° C. The mixture was stirred for 6 h at 23° C. The reaction was diluted with CH_2Cl_2 (50 mL) and extracted with 0.1 N HCl (50 mL). The aqueous phase was extracted again with CH_2Cl_2 (50 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated in vacuo to give a residue which was purified by flash column chromatography (SiO_2, CH_2Cl_2: EtOAc 15:1, 5:1) to give 26 (1.78 g, 70%) as a white solid. Experimental data of 26 were described previously in PCT/GB00/01852.

Experimental procedures for Int. 11, 160, 161, 162, and 163 were previously described in U.S. Pat. No. 5,721,362.

Example 88



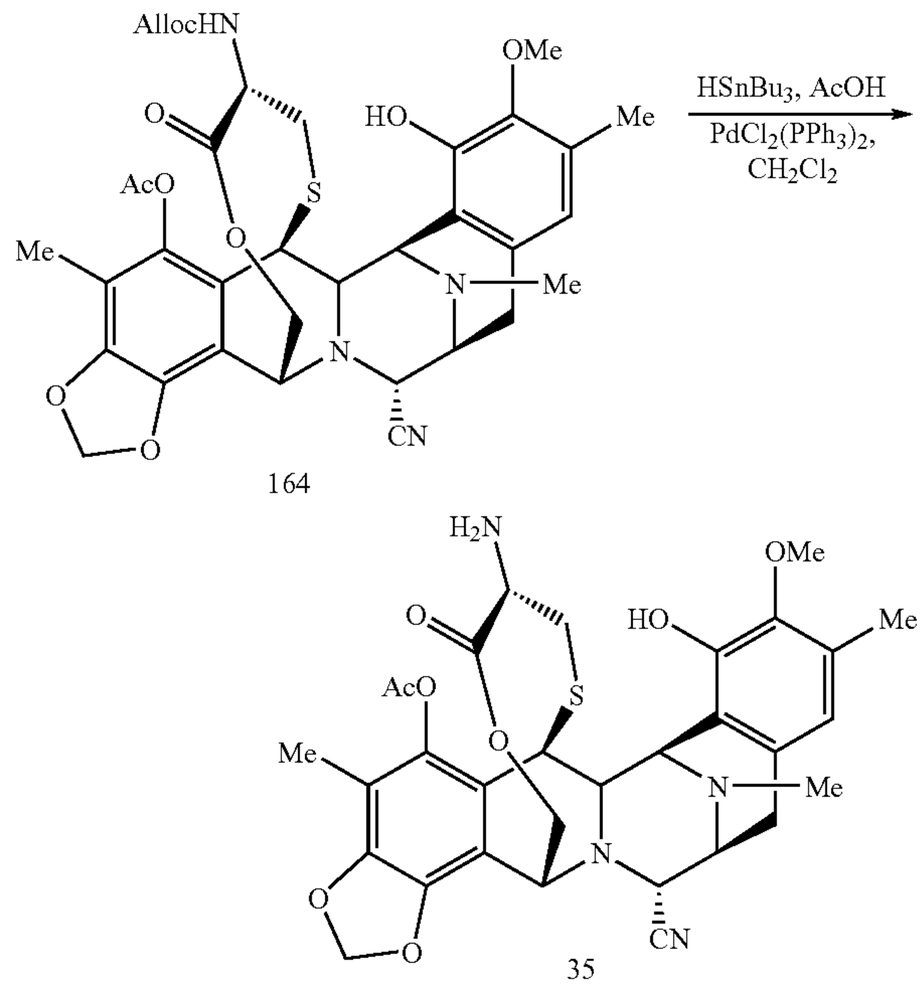
To a solution of 163 (15.8 g, 0.02 mol) in anhydrous CH_2Cl_2 (250 mL) and acetonitrile (300 mL), NaI (31.5 g, 0.21 mol) and ClTMS (freshly distilled over CaH_2, 26.7 mL, 0.21 mol) were added under argon atmosphere at 23° C. The reaction mixture was stirred for 40 minutes. Then the reaction was partitioned between CH_2Cl_2 (200 mL) and water (300 mL). The organic layer was washed with a saturated aqueous solution of NaCl (2×300 mL). The organic phase was dried over Na_2SO_4, filtered and the solvent was eliminated under reduced pressure. The crude was purified by flash column chromatography using ethyl acetate/hexane 2:3 as eluent to afford 164 (10.74 g, 76%) as a pale yellow solid. Rf=0.25 Hex:EtOAc 3:2.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.57 (s, 1H), 6.08 (d, J=1.5 Hz, 1H), 5.98 (d, J=1.5 Hz, 1H), 5.96-5.85 (m, 1H), 5.76 (bs, 1H), 5.30 (dd, J_1=1.5, J_2=17.3 Hz, 1H), 5.23 (dd, J_1=1.5, J_2=10.2 Hz, 1H), 5.00 (d, J=12.1 Hz, 1H), 4.81 (d, J=9.8 Hz, 1H), 4.58-4.45 (m, 3H), 4.34-4.28 (m, 1H), 4.23 (m, 2H), 4.17-4.00 (m, 2H), 3.76 (s, 3H), 3.40-3.38 (m, 2H), 2.91-2.85 (m, 2H), 2.30 (s, 3H), 2.29 (s, 3H), 2.24-2.23 (m, 2H), 2.19 (s, 3H), 2.02 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) 170.1, 168.4, 155.2, 148.0, 145.5, 142.8, 140.7, 140.1, 132.7, 130.2, 129.6, 120.7, 119.9, 117.8, 113.3, 101.9, 65.6, 61.0, 60.4, 59.9, 59.2, 59.0, 54.3, 53.6, 41.5, 41.2, 32.6, 29.5, 23.5, 20.4, 15.6, 9.4.

ESI-MS m/z: Calcd. for $C_{35}H_{38}N_4O_{10}S$: 706.76. Found $(M+1)^+$: 707.2.

Example 89



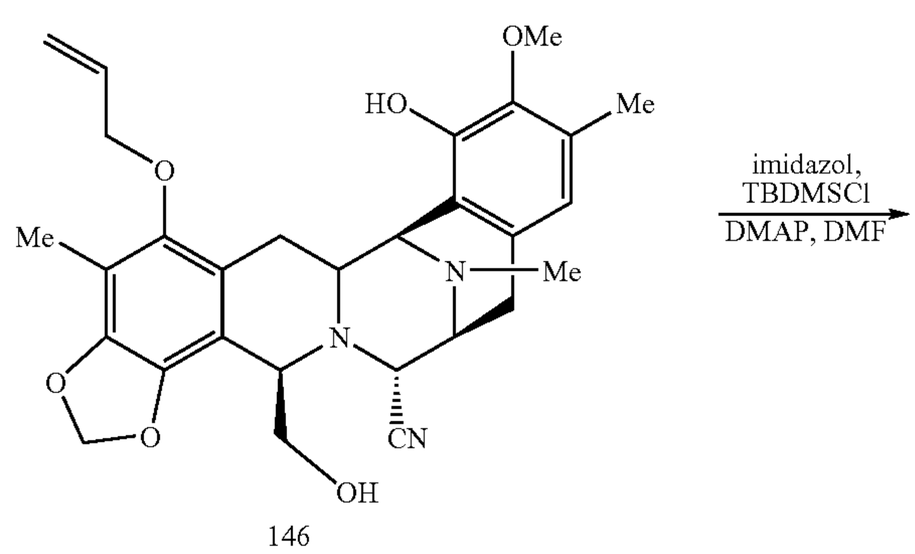
To a stirred solution of 164 (2 g. 2.85 mmol) in dichloromethane (142 mL), dichlorobis(triphenylphosphine) palladium (II) (0.2 g, 0.28 mmol) and acetic acid (0.65 mL, 11.4 mmol) were added under argon at 23° C. Then, tributyltin hydride (4.51 mL, 17.02 mmol) was added in a dropwise manner during 25 minutes. After addition of $HsnBu_3$, the mixture was stirred at 23° C. for 20 minutes more. The reaction was filtered through a silical gel column compacted with hexane. 35 (1.38 g, 78%) was obtained by subsequent elution with mixtures of ethyl acetate and hexane in a gradient manner, from 1:2 to 15:1. Experimental data of 35 were previously described in PCT/GB00/01852.

36, ET-770 and ET-743 were prepared following the same procedures than those previously described in PCT/GB00/01852.

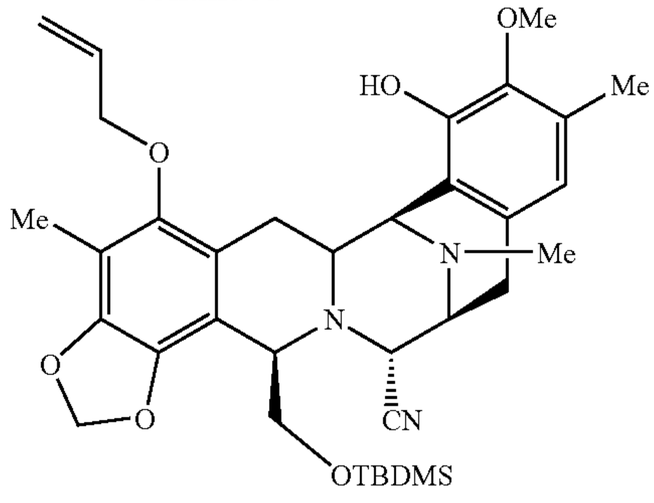
Route 5

The first step of this Route (transformation of 21 into 146) was described above in Example 71.

Example 90



-continued



165

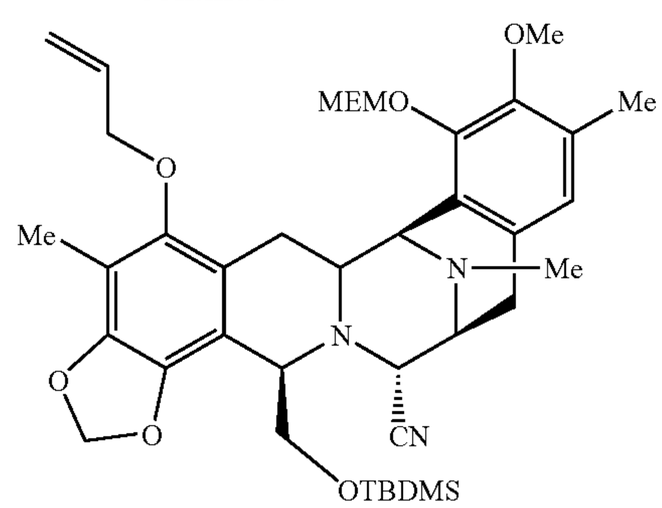
To a solution of 146 (8.72 g, 16.78 mmol) in DMF (20.1 mL), imidazol (3.43 g, 50.34 mmol), tert-butyl dimethyl chlorosilane (7.58 mL, 50.34 mmol) and DMAP (0.2 g, 1.7 mmol) were added at 0° C. After being stirred at 23° C. for 3.5 h, the reaction mixture was quenched with water (100 mL) and extracted with EtOAc/Hex 1:3 (2×75 mL). The combined organic phases were washed with 0.1 M HCl (50 mL) and the aqueous phase was extracted again with EtOAc/Hex 1:3 (40 mL). The combined organic phases were dried over sodium sulphate, filtered and concentrated in vacuo. The residue was purified by flash column chromatography (Hex:EtOAc 10:1, 3:1) to obtain 165 (9.85 g, 93%) as a white solid. Rf=0.39 in Hex:AcOEt 2:1.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.43 (s, 1H), 6:15-6.03 (m, 1H), 5.92 (d, J=1.2 Hz, 1H), 5.84 (d, J=1.2 Hz, 1H), 5.67 (s, 1H), 5.41 (dd, J_1=1.5, J_2=17.1 Hz, 1H), 5.26 (dd, J_1=1.5, J_2=10.5 Hz, 1H), 4.44 (d, J=2.7 Hz, 1H), 4.20-4.08 (m, 3H), 3.97 (dd, J_1=2.7, J_2=8.1 Hz, 1H), 3.75 (s, 3H), 3.61 (dd, J_1=2.71, J_2=9.9 Hz, 1H), 3.18 (brd, J=8.7 Hz, 1H), 3.22-3.16 (m, 2H), 2.99 (dd, J_1=8.1, J_2=17.4 Hz, 1H), 2.65 (d, J=17.4 Hz, 1H), 2.28 (s, 3H), 2.25 (s, 3H), 2.11 (s, 3H), 1.89 (dd, J_1=12, J_2=15.6 Hz, 1H), 0.8 (s, 9H), −0.05 (s, 3H), −0.09 (s. 3H).

^{13}C NMR (75 MHz, $CDCl_3$) □ 148.2, 146.5, 143.8, 142.4, 138.9, 133.8, 131.0, 128.0, 121.5, 120.4, 118.4, 117.1, 112.8, 111.6, 100.7, 74.0, 68.2, 61.5, 60.2, 58.6, 57.1, 56.5, 55.2, 41.3, 26.2, 25.4, 25.2, 20.6, 17.8, 15.3, 13.8, 9.0, −3.9, −6.0.

ESI-MS m/z: Calcd. for $C_{35}H_{47}N_3O_6Si$: 633.85. Found $(M+1)^+$: 634.2.

-continued



166

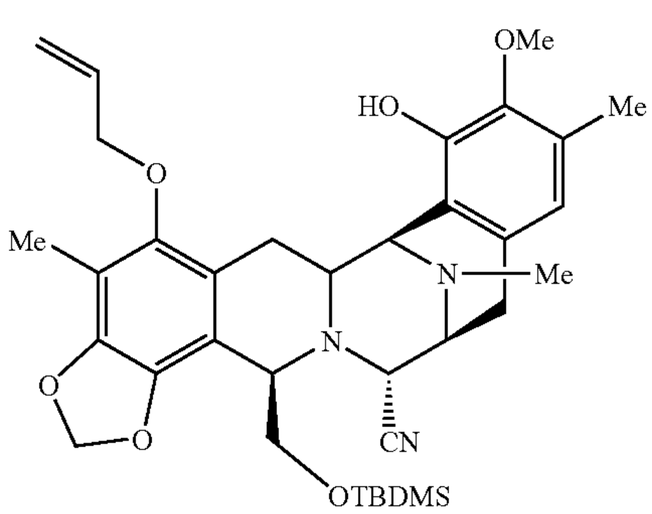
To a solution of 165 (7.62 g, 12.02 mmol) in THF (87.64 mL) and water (0.24 mL), MEMCl (2.33 mL, 20.43 mmol) was added at −6° C. After addition of 60% NaH (0.72 g, 18.03 mmol) in portions over 45 min, the mixture was stirred for 1.5 h at that temperature. The reaction was quenched with water (150 mL) and extracted with CH_2Cl_2 (2×100 mL). The combined organic phases were dried over Na_2SO_4, filtered and concentrated in vacuo to give 166 (8.69 g, 100%) as a white solid which was used in following steps with no further purification. Rf=0.24 Hex:AcOEt 2:1.

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.64 (s, 1H), 6.16-6.05 (m, 1H), 5.92 (d, J=1.2 Hz, 1H), 5.85 (d, J=1.2 Hz, 1H), 5.41 (dd, J_1=1.51, J_2=17.1 Hz, 1H), 5.29-5.24 (m, 2H), 5.14 (d, J=6 Hz, 1H), 4.42 (d, J=2.7 Hz, 1H), 4.21-4.06 (m, 3H), 4.01-3.95 (m, 2H), 3.88-3.82 (m, 1H), 3.72 (s, 3H), 3.64-3.57 (m, 3H), 3.39 (s, 3H), 3.29 (brd J=7.5 Hz, 1H), 3.25-3.15 (m, 2H), 3.00 (dd, J_1=8.1, J_2=17.4 Hz, 1H), 2.65 (d, J=18 Hz, 1H), 2.30 (s, 3H), 2.21 (s, 3H), 2.11 (s, 3H), 1.82 (dd J_1=12, J_2=15.6 Hz, 1H), 0.79 (s, 9H), −0.06 (s, 3H), −0.11 (s, 3H).

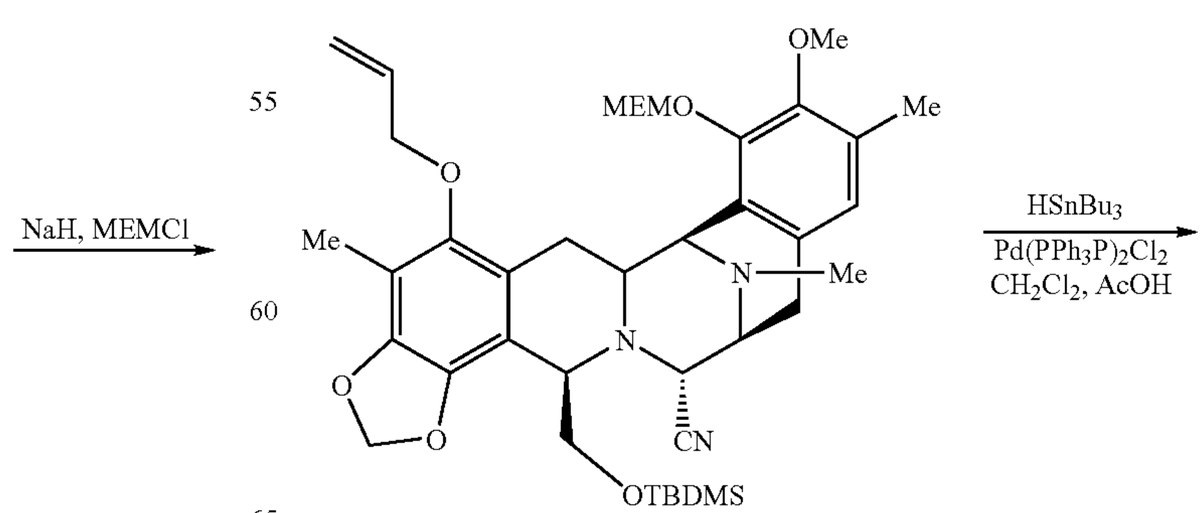
^{13}C NMR (75 MHz, $CDCl_3$) δ 148.4, 148.1, 144.1, 139.2, 133.9, 130.9, 130.8, 130.2, 128.8, 125.1, 124.2, 121.5, 118.8, 117.45, 113.0, 111.9, 101.0, 98.2, 74.1, 71.7, 69.3, 68.3, 61.7, 59.6, 59.0, 58.9, 57.3, 57.1, 55.5, 41.6, 29.7, 26.4, 25.8, 25.5, 25.4, 15.7, 9.2, −5.6, −5.6.

ESI-MS m/z: Calcd. for $C_{39}H_{55}N_3O_8Si$: 721.3. Found $(M+1)^+$: 722.3.

Example 91



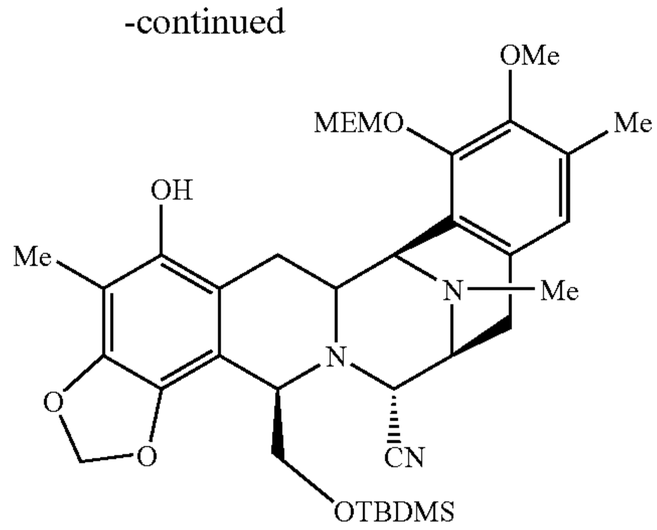
165



166

Example 92

-continued



167

To a solution of 166 (10.76 g, 14.90 mmol) in anhydrous CH_2Cl_2 (275 mL), $Pd(PPh_3P)_2Cl_2$ (837 mg, 1.19 mmol), acetic acid (4.26 mL, 74.5 mmol) and tributyltin hydride (11.85 mL, 44.7 mmol) were added under Argon atmosphere at 23° C. The reaction mixture was stirred at 23° C. for 15 minutes. (TLC AcOEt/Hexane 1:1 showed no starting material). Hexane (100 mL) was added and the mixture was poured into a flash column chromatography, (SiO_2, EtOAc:Hexane in a gradient manner, from 0:100, 1:4, 2:3 to 1:1) to afford 167 (9.95 g, 98%) as a yellow solid. Rf=0.42 Hex:EtOAc 3:7.

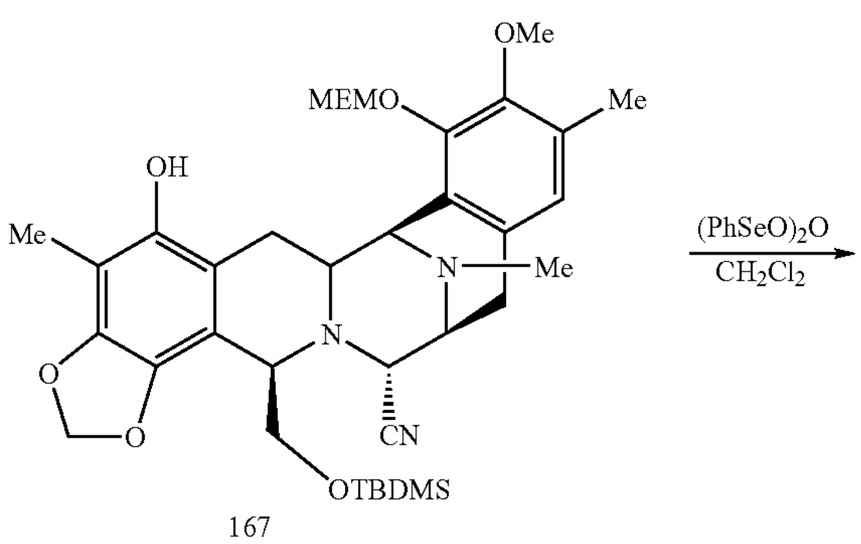
^{1}H-RMN (300 MHz, $CDCl_3$): δ 6.63 (s, 1H), 5.89 (d, J=1.4 Hz, 1H), 5.79 (d, J=1.4 Hz, 1H), 5.76 (m, 1H), 5.38 (d, J=5.6 Hz, 1H), 5.23 (d, J=5.9 Hz, 1H), 4.53 (d, J=2.7 Hz, 1H), 4.17 (dd, J_1=1.95 Hz, J_2=6.05 Hz, 1H), 4.11 (dd, J_1=7.0 Hz, J_2=12.5 Hz, 1H), 4.01-3.92 (m, 2H), 3.70 (s, 3H), 3.67 (m, 3H), 3.40 (s, 3H), 3.29 (m, 1H), 3.24-3.13 (m, 3H), 2.99 (dd, J_1=8.0 Hz J_2=17.5 Hz, 1H), 2.67 (d, J=17.5 Hz, 1H), 2.28 (s, 3H), 2.09 (s, 3H), 2.05 (s, 3H), 1.80 (dd, J_1=11.2 Hz, J_2=14.9 Hz, 1H), 0.82 (s, 9H), −0.03 (s, 3H), −0.07 (s, 3H).

^{13}C-RMN (75 MHz, $CDCl_3$): δ 148.4, 147.3, 145.5, 144.1, 136.2, 134.9, 134.8, 130.9, 130.2, 124.8, 123.1, 118.6, 112.8, 112.1, 106.2, 100.4, 98.4, 71.5, 69.2, 68.9, 61.7, 59.6, 58.7, 58.6, 56.9, 56.6, 55.3, 41.5, 29.5, 25.7, 25.3, 17.9, 15.5, 8.7, −5.7, −5.8.

ESI-MS m/z: Calcd. for $C_{36}H_{51}N_3O_8Si$: 681.89. Found $(M+1)^+$: 682.3.

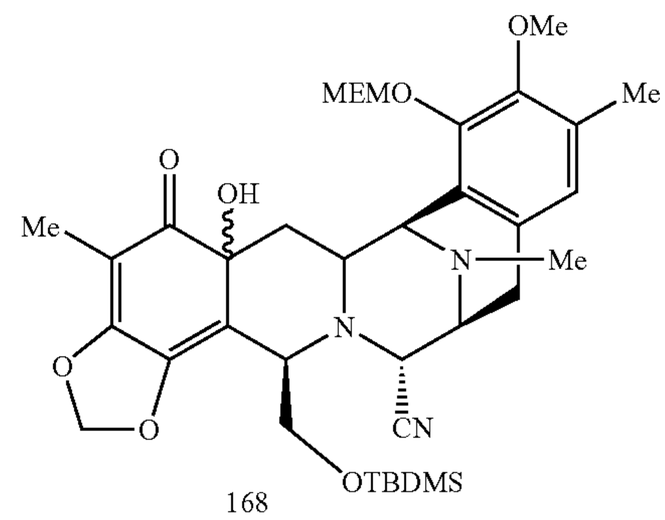
HPLC: Conditions: Column: Symmetry C18; mobile phase: AcN—buffer phosphate 25 mM, pH=5, isocratic of AcN (65%) in 5 minutes and gradient in AcN from 65-92% in 31 minutes, Ø: 0.6 mL/min, t^a: 40° C. Retention time: 27.89 minutes. HPLC purity in area: 89.62%.

Example 93



167

-continued



168

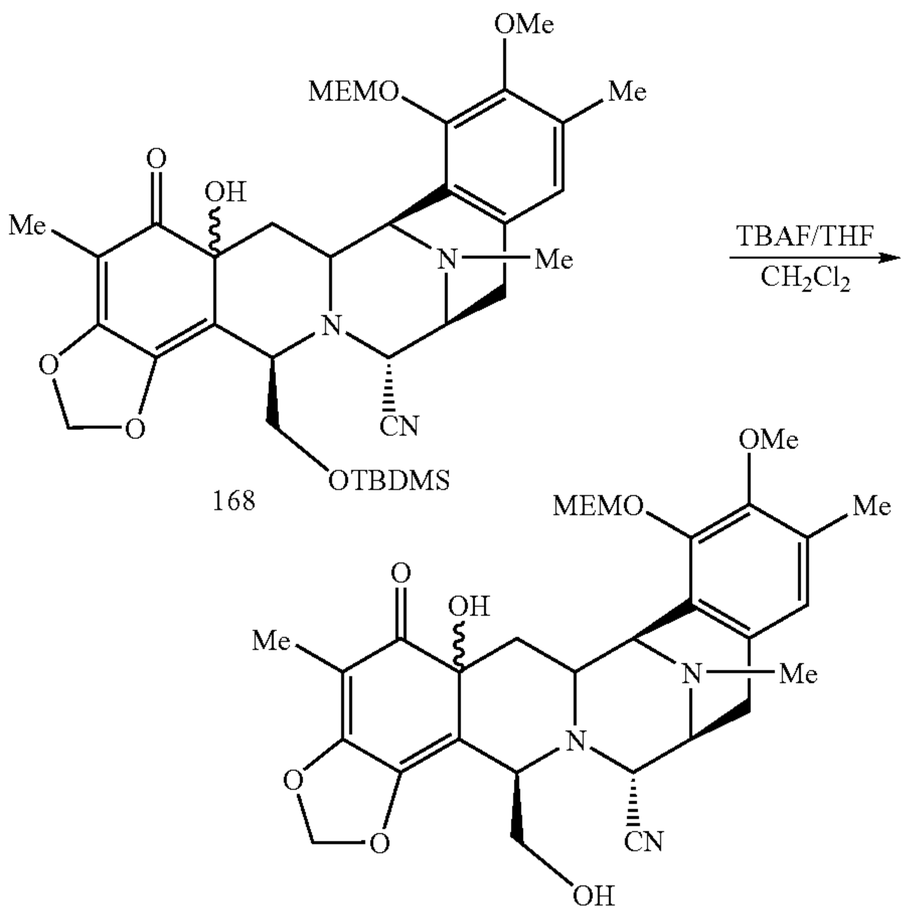
To a solution of 167 (9.95 g, 14.6 mmol) in anhydrous CH_2Cl_2 (300 mL), a solution of benceneseleninic anhydride (7.51 g, 14.6 mmol, reagent purity 70%) in anhydrous CH_2Cl_2 (120 mL) was dropwise added, under Argon atmosphere at −15° C. (the remaning white solid was discarded). The solution was then stirred at −15° C. for 15 minutes (TLC EtOAc/Hexane 2:3, showed no starting material). A saturated aqueous solution of sodium bicarbonate (500 mL) was added to the reaction mixture at this temperature. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (500 mL). The combined organic extracts were dried over sodium sulphate, filtered and the solvent was eliminated under reduced pressure. The crude of the reaction was purified by flash column chromatography, (SiO_2, EtOAc:Hexane in a gradient manner, from 2:3 to 3:1) to afford 168 (9.86 g, 97%) as a yellow solid. Rf=0.33 Hex:EtOAc 3:7).

^{1}H-RMN (300 MHz, $CDCl_3$) (Isomers ratio: ≈3:2): δ 6.59 (s, 1H), 6.57 (s, 1H), 5.77 (s, 1H), 5.76 (s, 1H), 5.68 (s, 1H), 5.63 (s, 1H), 5.19 (d, J=6.0 Hz, 1H), 5.09 (d, J=6.0 Hz, 1H), 5.07 (d, J=6.1 Hz, 1H), 5.00 (d, J=6.1 Hz, 1H), 4.40 (d, J=2.7 Hz, 1H), 4.27 (d, J=2.44 Hz, 1H), 4.22 (d, J=10.5 Hz, 1H), 3.95 (d, J=1.7 Hz, 1H), 3.86-3.75 (m, 2H), 3.81 (s, 3H), 3.72-3.68 (m, 2H), 3.65 (m, 2H), 3.54 (s, 3H), 3.50 (m, 3H), 3.31 (s, 3H), 3.29 (s, 3H); 3.24 (m, 1H), 3.09 (dt, J=3.2 Hz, J=7.6 Hz, 1H), 3.02 (d, J=11.2 Hz, 1H), 2.92 (m, 2H), 2.48 (d, J=9.5 Hz, 1H), 2.43 (d, J=9.3 Hz, 1H), 2.21 (s, 3H), 2.14 (s, 3H), 2.13 (s, 3H), 2.03 (m, 2H), 1.73 (s, 3H), 1.71 (s, 3H), 0.86 (s, 9H), 0.77 (s, 9H), 0.04 (s, 3H), 0.02 (s, 3H).

^{13}C-RMN (75 MHz, $CDCl_3$): 200.5, 197.2, 159.8, 157.7, 148.4, 148.2, 147.7, 140.0, 137.6, 130.5, 130.2, 129.9, 129.4, 124.9, 124.7, 124.0, 122.7, 117.1, 116.9, 113.4, 110.8, 103.9, 103.8, 101.0, 100.4, 97.8, 72.8, 71.3, 69.7, 68.9, 68.8, 65.4, 64.1, 60.2, 59.9, 59.3, 59.1, 59.0, 58.6, 58.5, 56.8, 56.5, 56.2, 55.5, 54.9, 54.8, 42.5, 41.1, 40.9, 35.8, 25.6, 25.5, 25.4, 25.3, 20.6, 17.9, 17.8, 15.5, 15.3, 13.8, 7.0, 6.7, −5.7, −6.0, −6.1.

ESI-MS m/z: Calcd. for $C_{36}H_{51}N_3O_9Si$: 697.89. Found $(M+1)^+$: 698.8 HPLC: Conditions: Column: Symmetry C18; mobile phase: AcN, buffer phosphate 25 mM, pH=5, gradient in AcN from 30-100% in 50 minutes. Ø: 1.2 mL/min, t^a: 40° C. Retention time: 30.70 minutes and 30.95 minutes (the two isomers). HPLC purity in area: 60.77% and 31.99%.

Example 94



168

169

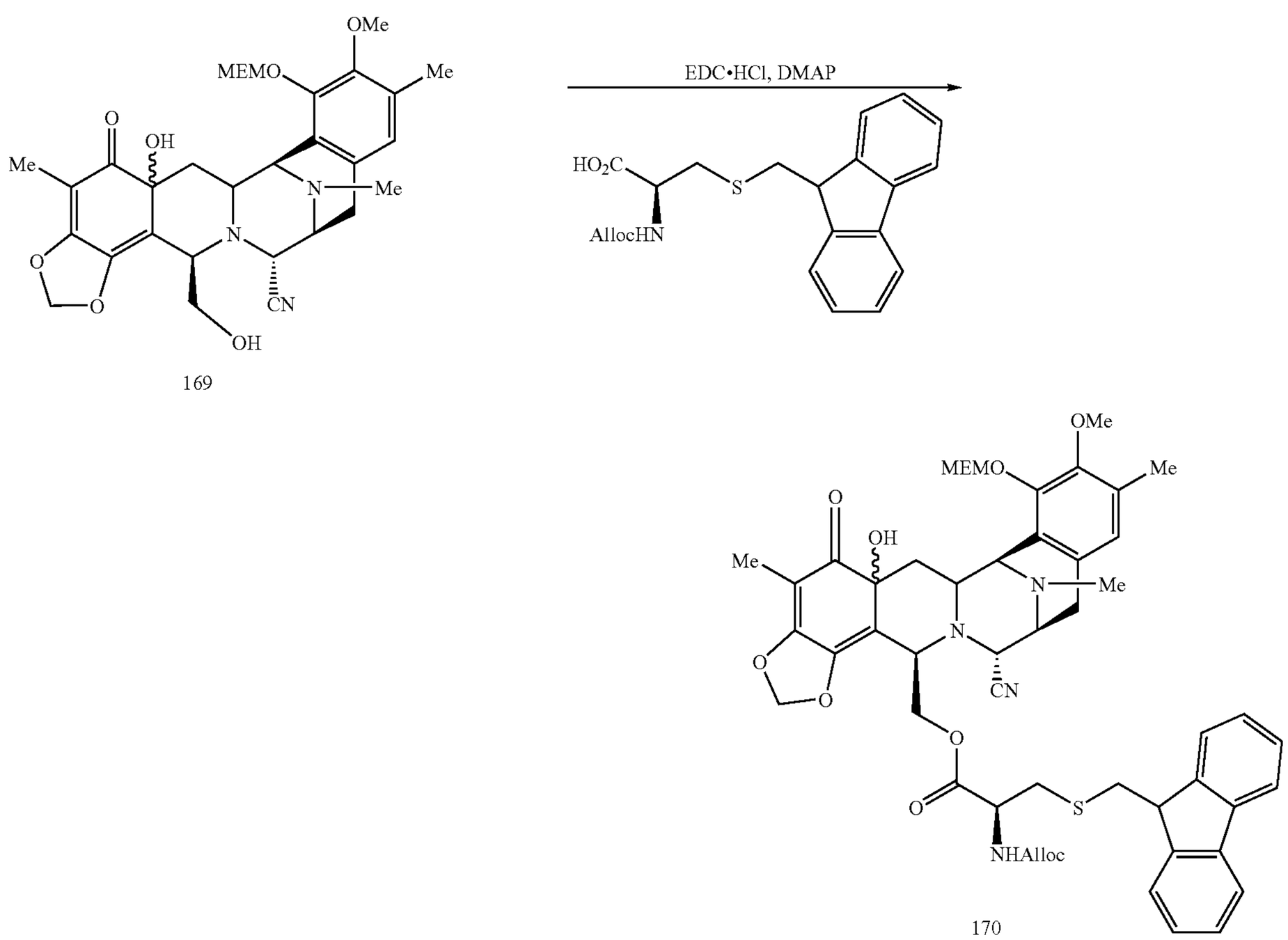
To a solution of 168 (16.38 g, 23.47 mmol) in anhydrous THF (727 mL, 0.03 M), a solution of TBAF in 1M THF (59 mL, 59 mmol) was dropwise added at 23° C. The reaction mixture was stirred at 23° C. for 45 minutes. Then, the mixture was partitioned between a saturated aqueous NaCl solution (850 mL) and CH_2Cl_2 (950 mL). Both layers were separated and the organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, EtOAc:Hexane in a gradient manner, from 40:60, 50:50, 70:30, 90:10 to 100:0) to afford 169 (12.17 g, 89%) as a light yellow solid. Rf=0.1 Hex:EtOAc 3:7.

^{1}H-RMN (300 MHz, $CDCl_3$) (Isomers ratio: 3:2): δ 6.63 (s, 1H), 6.57 (s, 1H), 5.79 (s, 1H), 5.77 (s, 1H), 5.75 (s, 1H), 5.62 (s, 1H), 5.23 (s, 1H), 5.18 (d, J=6.1 Hz, 1H), 5.08 (d, J=6.1 Hz, 1H), 5.01 (d, J=6.1 Hz, 1H), 4.22 (d, J=2.7 Hz, 1H), 4.09 (d, J=2.4 Hz, 1H), 4.00 (m, 4H), 3.82 (s, 3H), 3.87-3.64 (m, 6H), 3.55 (s, 3H), 3.51-3.44 (m, 2H), 3.30 (s, 3H), 3.29 (s, 3H), 3.26 (m, 1H), 3.18 (dt, J_1=2.9 Hz, J_2=7.3 Hz, 1H), 2.94 (m, 4H) 2.50 (m, 4H), 2.22 (s, 3H), 2.16 (s, 3H), 2.15 (s, 3H), 2.11 (s, 3H), 2.02 (d, J=7.3 Hz, 2H), 1.72 (s, 3H), 1.69 (s, 3H).

^{13}C-RMN (75 MHz, $CDCl_3$): 200.2, 200.1, 159.6, 158.5, 148.5, 148.4, 148.1, 147.9, 140.5, 137.4, 130.9, 130.4, 130.1, 130.0, 125.1, 124.9, 123.8, 122.7, 116.9, 116.6, 113.3, 110.7, 104.5, 103.9, 101.4, 100.7, 98.1, 97.9, 71.9, 71.5, 71.4, 70.1, 69.0, 69.0, 62.0, 60.1, 59.5, 58.7, 58.5, 58.1, 57.4, 56.9, 56.8, 56.4, 55.9, 55.1, 55.0, 41.3, 41.0, 36.1, 31.3, 25.3, 25.2, 22.4, 15.6, 15.5, 13.8, 7.0, 6.8.

ESI-MS m/z: Calcd. for $C_{30}H_{37}N_3O_9$: 583.63. Found $(M+1)^+$: 584.2.

Example 95



170

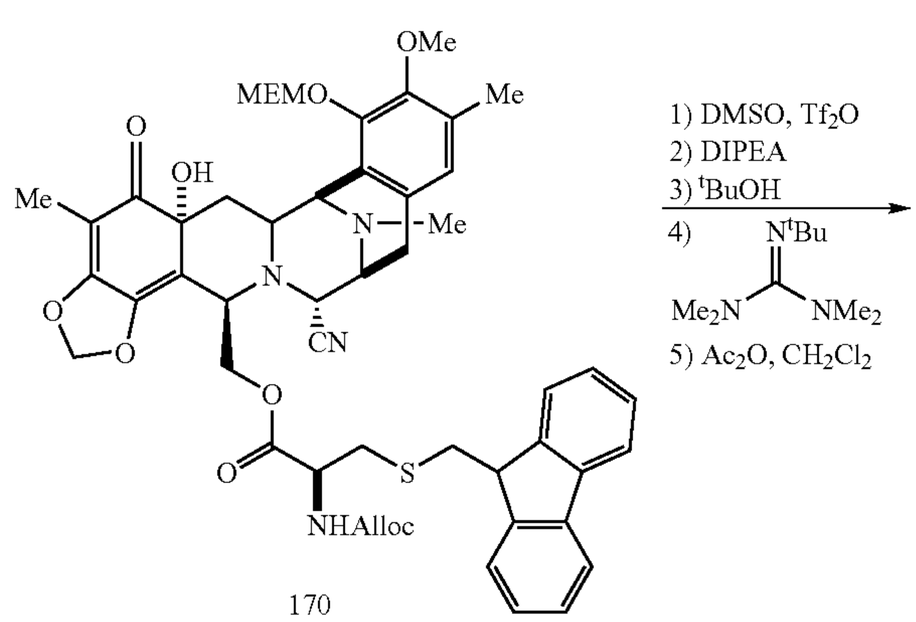
To a solution of 169 (11.49 g, 19.69 mmol) and Alloc-Cys-(Fm) (11.32 g, 29.53 mmol) (for its preparation see Kruse, C. H.; Holden, K. G., *J. Org. Chem.*, 1985, 50, pp. 2792-2794) in anhydrous CH_2Cl_2 (688 mL), DMAP (2.4 g, 19.69 mmol) and EDC-HCl (9.44 g, 49.22 mmol) were added at 23° C. Then, DIPEA (5.14 mL, 29.53 mmol) was added at 0° C. and the reaction was stirred at 23° C. for 3 hour. The mixture was washed successively with a saturated aqueous solution of $NaHCO_3$ (500 mL), NaCl (400 mL) and NH_4Cl (2×300 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, AcOEt:Hex in a gradient manner, from 1:1, 6:4 to 7:3) to afford 170 (14.76 g, 79%) as a pale yellow solid. Rf=0.31 and 0.40 Hex:EtOAc 3:7 (mixture of isomers).

^{1}H-RMN (300 MHz, $CDCl_3$): 7.74 (d, J=7.6 Hz, 4H), 7.63 (dd, J=7.0 Hz, J=15.3 Hz, 4H), 7.38 (t, J=7.3 Hz, 4H), 7.29 (m, 4H), 6.61 (s, 1H), 6.54 (s, 1H), 5.89 (m, 2H); 5.73 (s, 1H), 5.70 (s, 1H), 5.69 (s, 1H), 5.62 (s, 1H), 5.55 (m, 1H), 5.32 (d, J=15.1 Hz, 1H), 5.23 (d, J=6.1 Hz, 1H), 5.22 (d, J=10.6 Hz, 1H), 5.14 (d, J=5.9 Hz, 1H), 5.13 (d, J=6.0 Hz, 1H), 5.07 (d, J=6.3 Hz, 1H), 4.68 (m, 1H), 4.56 (m, 4H), 4.51 (m, 2H), 4.38 (dd, J_1=4.5 Hz, J_2=12.6 Hz, 1H), 4.22 (dd, J_1=6.2 Hz, J_2=11.1 Hz, 1H), 4.14-3.88 (m, 12H), 3.83 (s, 3H), 3.79-3.69 (m, 4H), 3.61 (s, 3H), 3.56 (m, 4H), 3.39 (s, 3H), 3.36 (s, 3H), 3.23 (m, 2H), 3.16 (d, J=6.0 Hz, 2H), 3.07 (d, J=6.1 Hz, 2H), 3.00-2.81 (m, 6H), 2.46-2.34 (m, 4H), 2.25 (s, 3H), 2.20 (s, 3H), 2.16 (s, 3H), 2.07 (m, 1H), 1.83 (dd, J_1=9.5 Hz, J_2=15.1 Hz, 1H), 1.78 (s, 3H), 1.77 (s, 3H).

^{13}C-RMN (75 MHz, $CDCl_3$): δ 200.3, 198.4, 170.3, 160.0, 158.1, 148.7, 148.7, 148.5, 148.2, 145.6, 145.6, 145.5, 142.2, 141.1, 141.0, 141.0, 138.5, 132.4, 132.3, 131.1, 130.6, 130.1, 129.8, 128.8, 127.6, 127.1, 127.1, 125.1, 125.0, 124.8, 124.7, 124.7, 124.0, 122.7, 119.9, 118.1, 118.0, 117.2, 116.8, 111.6, 108.3, 104.8, 104.5, 101.5, 101.0, 98.2, 98.2, 72.3, 71.7, 71.7, 70.6, 69.3, 69.2, 66.4, 66.0, 66.0, 65.5, 63.8, 60.8, 60.2, 59.8, 59.0, 58.9, 58.1, 56.8. 56.6, 56.5, 56.3, 56.1, 55.7, 55.3, 55.2, 53.9, 46.9, 41.9, 41.4, 41.2, 37.2, 36.9, 35.4, 31.5, 29.6, 25.6, 25.4, 22.6, 15.8, 15.7, 14.1, 7.3, 7.0.

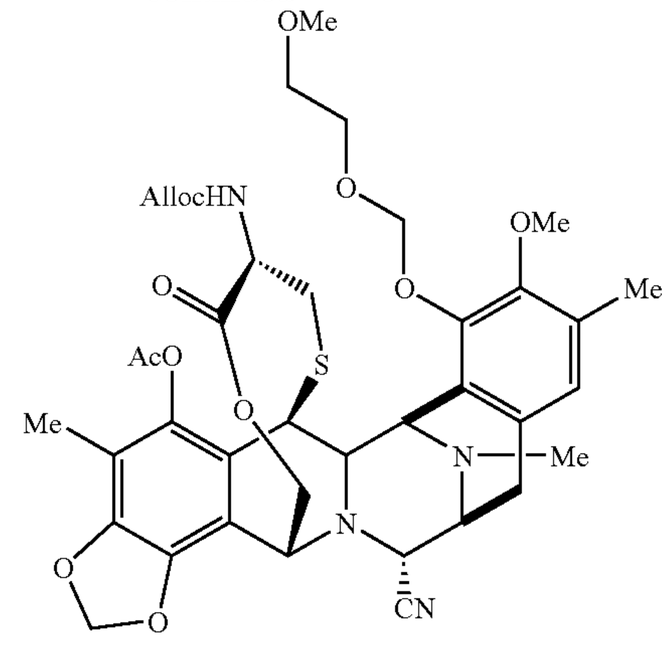
ESI-MS m/z: Calcd. for $C_{51}H_{56}N_4O_{12}S$: 948.36. Found $(M+1)^+$: 949.3.

Example 96



170

-continued



171

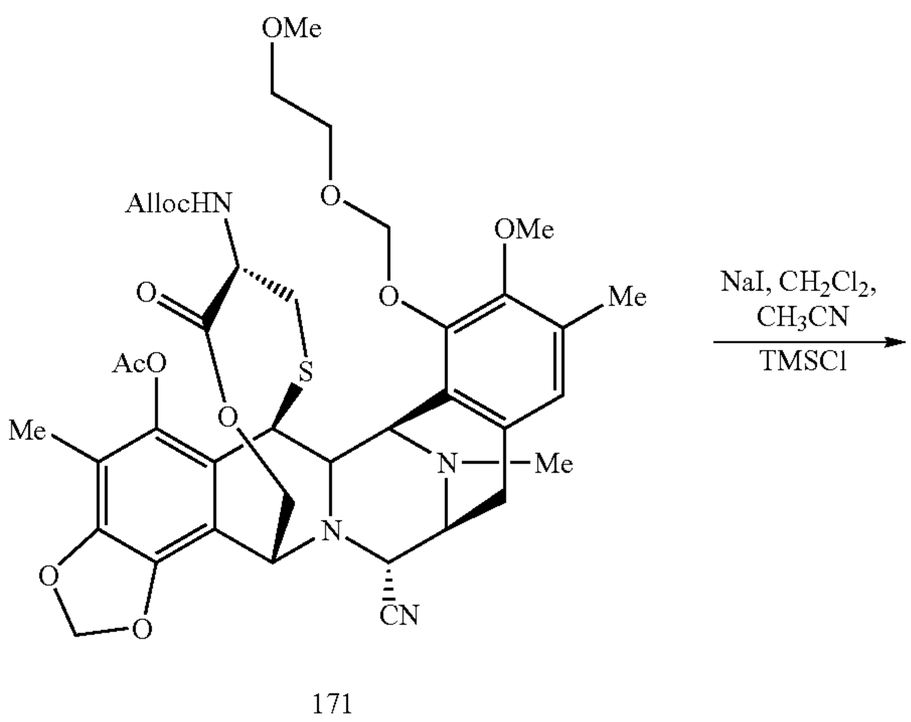
The reaction flask was flamed twice, purged vacuum/Argon several times and kept under Argon atmosphere for the reaction. To a solution of DMSO (5.4 mL) in anhydrous CH_2Cl_2 (554 mL) was dropwise added triflic anhydride (5.11 mL, 30.4 mmol) at −78° C. The reaction mixture was stirred at −78° C. for 20 minutes. Then, a solution of 170 (14.43 g, 15.2 mmol) in anhydrous CH_2Cl_2 (188 mL) at −78° C. was added via canula. During the addition the temperature was kept at −78° C. in both flasks and the color of the reaction was yellow. The reaction mixture was stirred at −40° C. for 35 minutes. During this period of time the solution was turned from yellow to dark green. After this time, $^iPr_2NEt$ (21.2 mL, 121.6 mmol) was dropwise added and the reaction mixture was kept at 0° C. for 45 minutes. The color of the solution turned to pale brown during this time. Then, ^tBuOH (5.8 mL, 60.8 mmol) and tert-butyl tetramethyl guanidine (18.3 mL, 106.4 mmol) were dropwise added and the reaction mixture was stirred at 23° C. for 40 minutes. After this time, acetic anhydride (14.34 mL, 152 mmol) was dropwise added and the reaction mixture was kept at 23° C. for 1 hour more. Then, the reaction mixture was diluted with CH_2Cl_2 (38 mL) and washed with a saturated aqueous solution of NH_4Cl (500 mL), $NaHCO_3$ (500 mL), and NaCl (500 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated. The residue was purified by flash column chromatography (SiO_2, EtOAc:Hex in a gradient manner, from 3:7 to 4:6) to afford 171 (6.24 g, 52%) as a pale yellow solid. Rf=0.38 Hex:EtOAc 1:1.

^{1}H-RMN ($CDCl_3$): 6.78 (s, 1H), 6.07 (d, J=1.2 Hz, 1H), 5.98 (d, J=1.2 Hz, 1H), 5.92 (m, 1H), 5.32 (d, J=5.9 Hz, 1H), 5.31 (dd, J_1=1.5 Hz, J_2=17.1 Hz, 1H), 5.23 (dd, J_1=1.5 Hz, J_2=10.4 Hz, 1H), 5.19 (d, J=5.6 Hz, 1H), 5.01 (d, J=11.5 Hz, 1H), 4.81 (d, J=9.8 Hz, 1H), 4.53-4.51 (m, 3H), 4.35-4.27 (m, 2H), 4.24 (s, 1H), 4.18-4.13 (m, 2H), 3.94-3.84 (m, 2H), 3.73 (s, 3H), 3.58 (t, J=4.7 Hz, 2H), 3.43-3.37 (m, 2H), 3.36 (s, 3H), 2.91 (m, 2H), 2.27 (s, 3H), 2.26 (s, 3H), 2.20 (s, 3H), 2.36-2.06 (m, 2H), 2.02 (s, 3H).

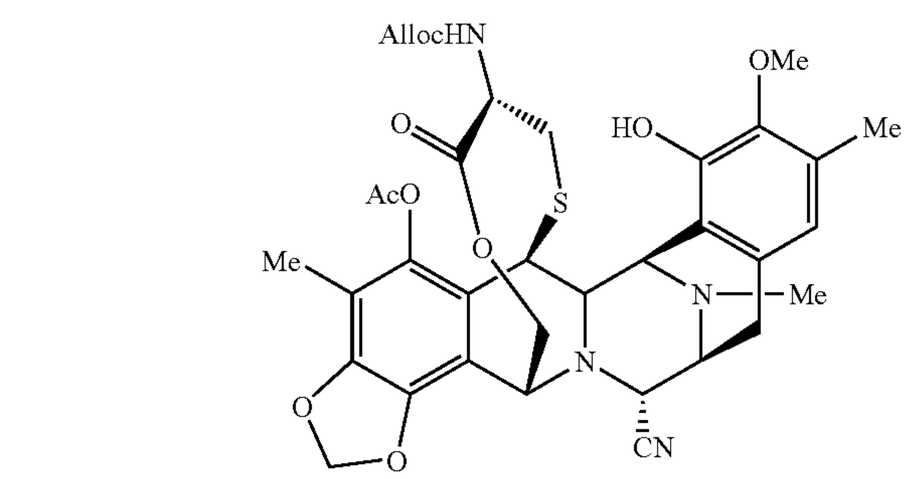
^{13}C-RMN ($CDCl_3$): 170.23, 168.49, 155.26, 149.62, 148.26, 145.63, 140.85, 140.24, 132.74, 131.60, 130.11, 124.89, 124.70, 120.14, 117.89, 117.84, 113.21, 101.89, 98.03, 92.67, 71.60, 69.04, 65.70, 61.20, 60.35, 59.36, 59.01, 58.89, 54.71, 54.42, 53.79, 41.53, 41.19, 32.68, 29.53, 23.57, 20.26, 15.62, 9.45.

ESI-MS m/z: Calcd. for $C_{39}H_{46}N_4O_{12}S$: 794.87. Found: 796 $(M+1)^+$, 817 $(M+23)^+$. HPLC: Conditions: Column: Simmetry C18, Mobile phase: AcN/buffer phosphate (pH: 5) in gradient from 45 to 65% in 15 minutes and 65-90% in 36 minutes. Ø=0.8 ml/min, t^a=40° C. Retention time: 19.734 minutes. HPLC purity in area: 83.17%

Example 97



171



164

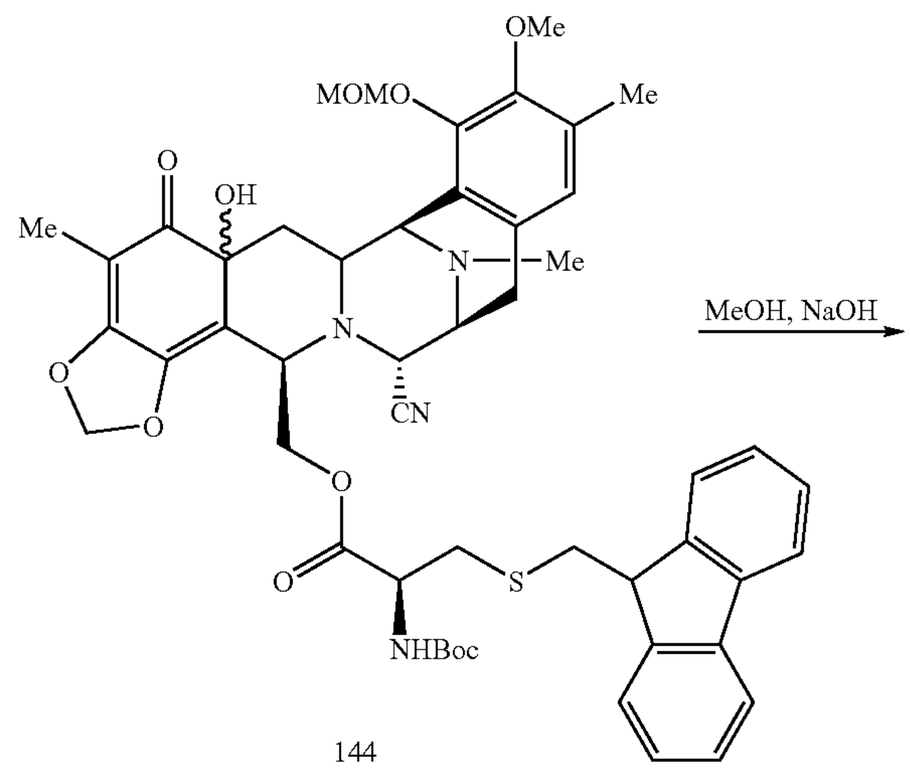
To a solution of 171 (2.26 g, 2.85 mmol) in anhydrous CH_2Cl_2 (74 mL) and acetonitrile (74 mL), NaI (3.42 g, 22.8 mmol) and TMSCl (freshly distilled over CaH_2) (2.6 mL, 22.8 mmol) were added at 0° C. and the reaction was stirred for 35 minutes. A saturated aqueous solution of sodium bicarbonate (150 mL) was added to the reaction mixture at this temperature. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (2×100 mL). The combined organic extracts were dried over sodium sulphate, filtered and the solvent was eliminated under reduced pressure to give 164 (2.4 g, 100%) as a pale yellow solid which was used in subsequent reactions with no further purification. Experimental data of 164 were described above in Example 88.

Transformation of 164 into 35 was previously described above in Example 89.

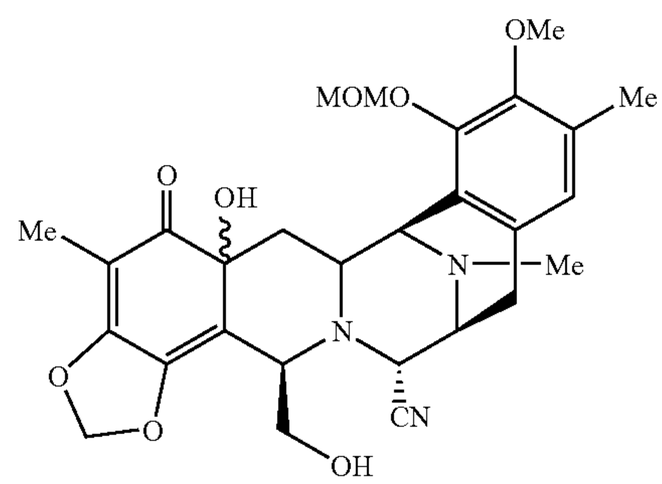
Intermediates 35, 36, ET-770 and ET-743 were prepared following the same procedures than those previously described in PCT/GB00/01852.

Route 6

Example 98



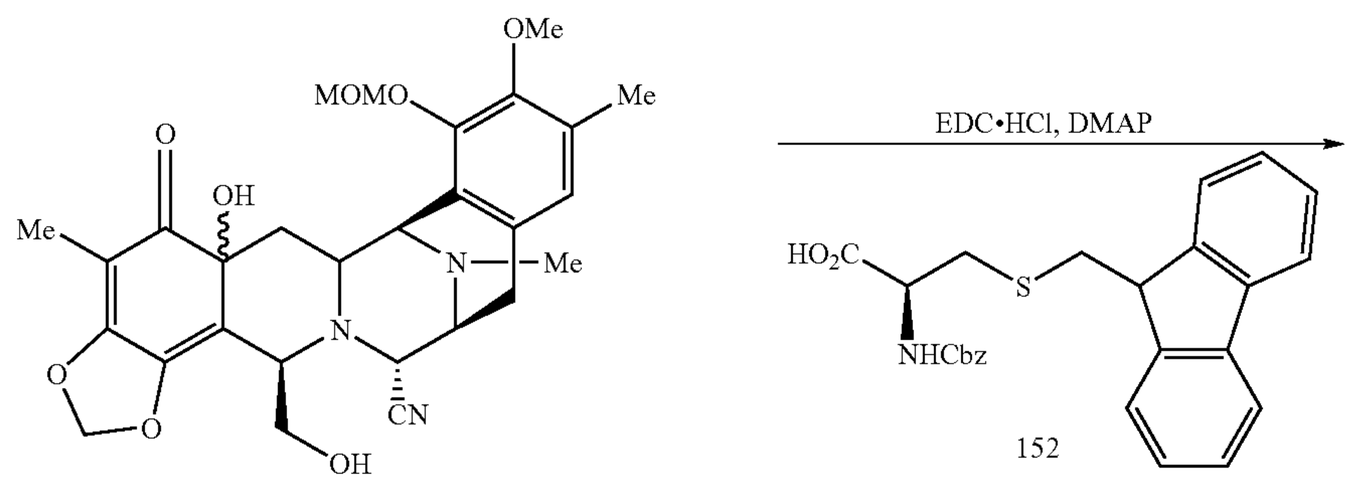
144

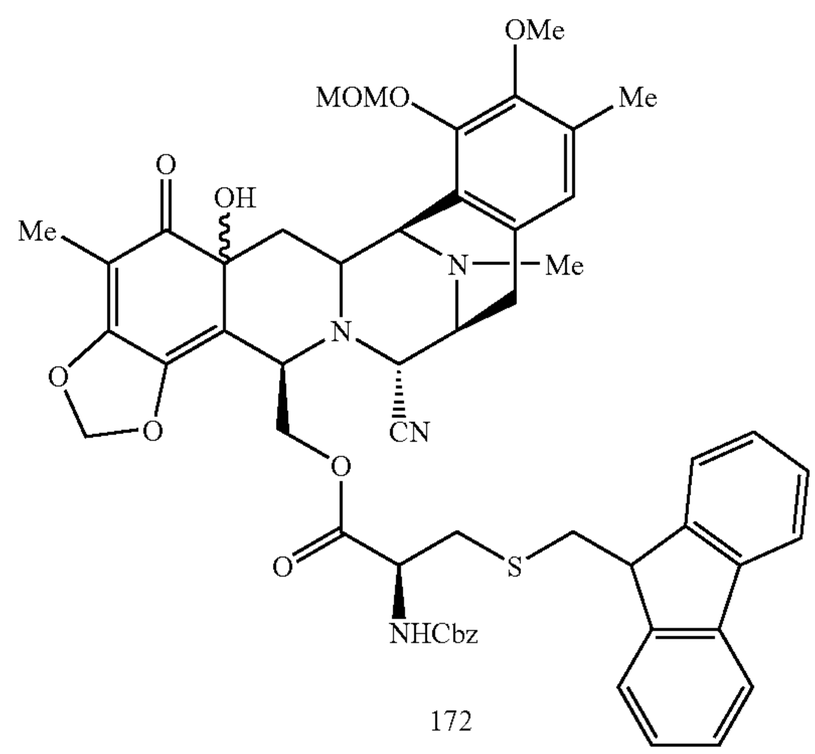


161

To a solution of 144 (7 g, 7.6 mmol) in MeOH (140 mL), 1M NaOH (15.1 mL) was added and the reaction was stirred for 10 minutes at 23° C. A saturated aqueous solution of NH_4Cl (100 mL) was added to the reaction mixture. The organic phase was separated and washed with 5% HCl until the colour turned into yellow. The organic extract was dried over sodium sulphate, filtered and the solvent was eliminated under reduced pressure. The residue was purified by flash column chromatography (SiO_2, EtOAc:Hexane in a gradient manner, from 0:1, 1:3, 1:2, 1:1, 1:1 to 3:1) to afford 161 (3.76 g, 85%). Experimental data of 161 were previously described in U.S. Pat. No. 5,721,362.

Example 99





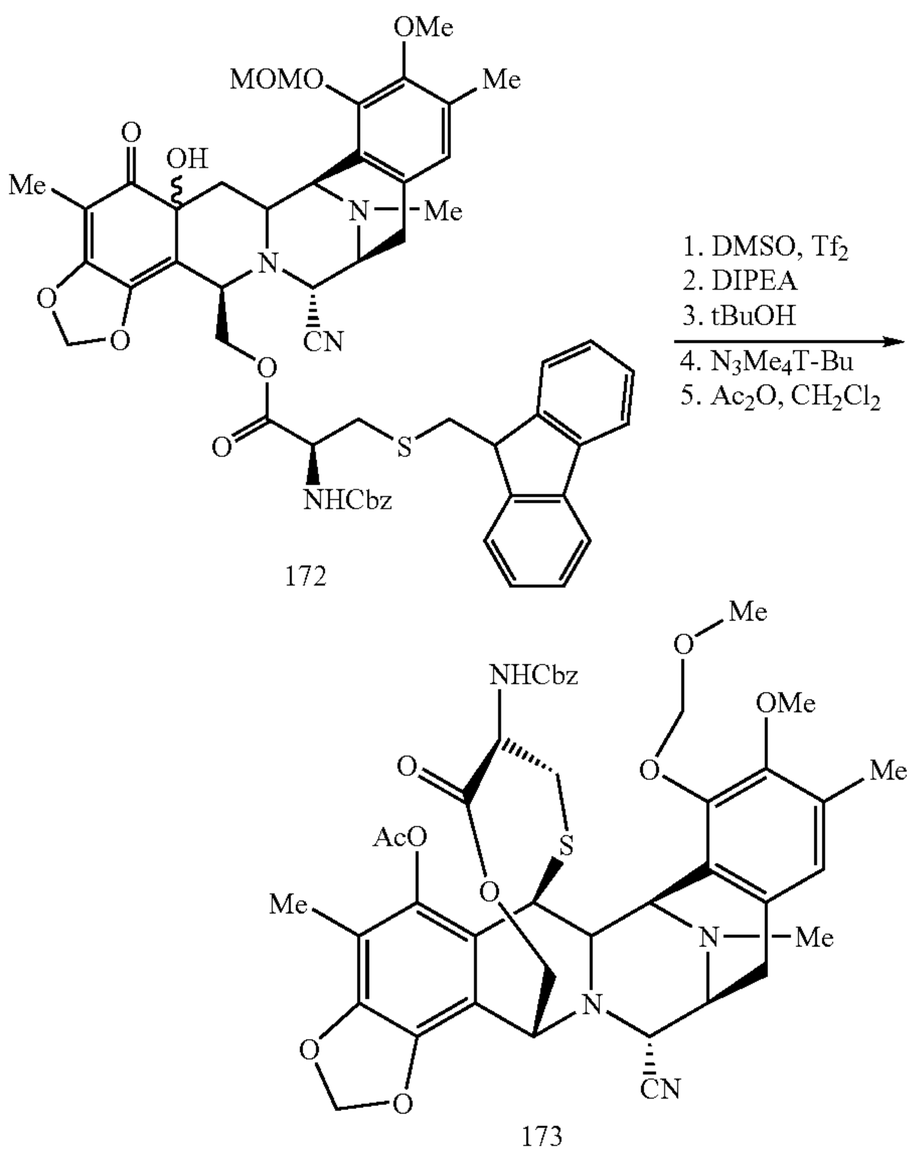
To a solution of 161 (200 mg, 0.37 mmol) and the cysteine 152 (240 mg, 0.55 mmol) in anhydrous CH_2Cl_2 (20 mL), DMAP (110 mg, 0.925 mmol) and EDC-HCl (170 mg, 0.925 mmol) were added at 23° C. and the reaction was stirred at that temperature for 1.5 hours. The mixture was then washed successively with a saturated aqueous solution of $NaHCO_3$ (15 mL), NaCl (15 mL) and NH_4Cl (2×10 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by flash column chromatography with silica gel (SiO_2, AcOEt/Hexane in a gradient manner, from 1:4 to 1:2), to afford 172 (285 mg, 80%) as a white solid. Rf=0.3 Hex:EtOAc 2:1.

^{1}H RMN ($CDCl_3$) δ 7.73 (d, J=7.5 Hz, 2H), 7.59-7.57 (m, 2H), 7.40-7.28 (m, 9H), 6.60 (s, 1H), 5.69 (s, 1H), 5.65 (s, 1H), 5.54 (d, J=7.8 Hz, 1H), 5.11-5.08 (m, 4H), 4.52-4.49 (m, 1H), 4.21-3.90 (m, 6H), 3.83 (s, 3H), 3.49 (s, 3H), 3.21 (d, J=6.6 Hz, 1H), 3.09-2.90 (m, 6H), 2.41 (d, J=18 Hz, 1H), 2.34-2.31 (m, 1H), 2.25 (s, 3H), 2.19 (s, 3H), 1.88-1.83 (m, 1H), 1.77 (s, 3H).

^{13}C-RMN ($CDCl_3$) δ 198.7, 170.5, 158.4, 155.9, 148.9, 148.8, 145.8, 142.5, 141.3, 136.2, 131.4, 130.0, 128.8, 128.6, 128.4, 127.9, 127.3, 125.3, 125.0, 124.9, 123.0, 120.1, 117.5, 108.5, 104.8, 101.7, 99.5, 70.8, 67.4, 60.5, 57.8, 57.0, 56.5, 56.0, 55.5, 47.1, 41.6, 37.4, 37.1, 31.8, 25.8, 22.8, 15.9, 14.3, 7.6.

ESI-MS m/z: Calcd. for $C53H_{54}N_4O_{11}S$: 954.35. Found $(M+23)^+$: 977.8.

Example 100



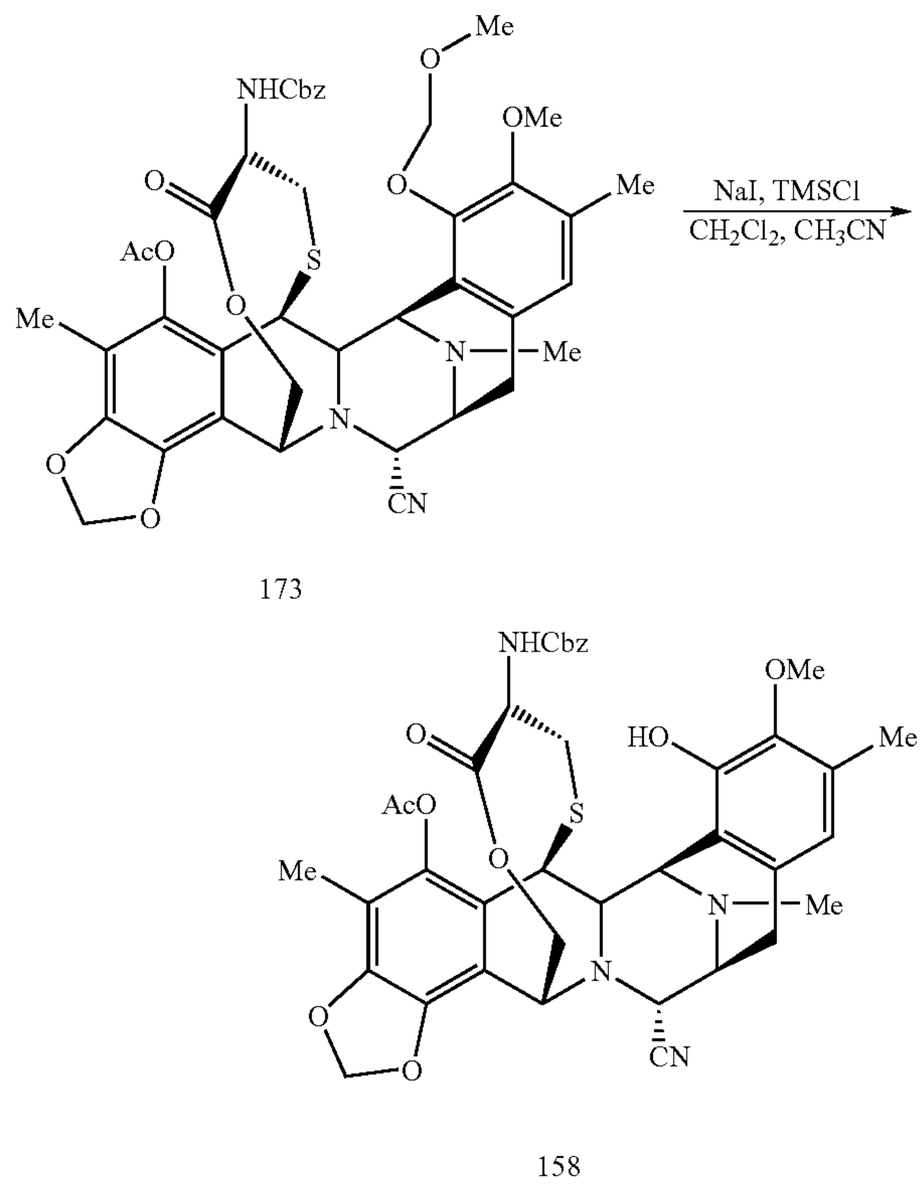
The reaction flask was flamed twice, purged vacuum/Argon several times and kept under Argon atmosphere for the reaction. To a solution of DMSO (977 μL) in anhydrous CH_2Cl_2 (118 mL) was dropwise added triflic anhydride (930 □L, 5.5 mmol) at −78° C. The reaction mixture was stirred at −78° C. for 20 minutes. Then, a solution of 172 (2.63 g, 2.75 mmol) in anhydrous CH_2Cl_2 (26 mL, for the main addition and 13 mL for washing) was added via canula (addition time: 5 min) at −78° C. During the addition the temperature was kept at −78° C. in both flasks and the color changed from yellow to brown. The reaction mixture was stirred at −40° C. for 35 minutes. During this period of time the solution was turned from yellow to dark green. After this time, $^iPr_2NEt$ (3.48 mL, 22 mmol) was dropwise added and the reaction mixture was kept at 0° C. for 45 minutes, the color of the solution turned brown during this time. Then, ^tBuOH (1.04 mL, 11 mmol) and tert-butyl tetramethyl guanidine (3.31 mL, 19.25 mmol) were dropwise added and the reaction mixture was stirred at 23° C. for 40 minutes. After this time, acetic anhydride (2.6 mL, 27.5 mmol) was dropwise added and the reaction mixture was kept at 23° C. for 1 hour more. Then, the reaction mixture was diluted with CH_2Cl_2 (70 mL) and washed successively with a saturated aqueous solution of NH_4Cl (180 mL), $NaHCO_3$ (180 mL), and NaCl (180 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated at reduced pressure. The residue was purified by flash column chromatography (SiO_2, Hex:EtOAc in a gradient manner, from 4:1, 3:1, to 2:1) to afford 173 (1.145 g, 52%) as a white solid. Rf=0.31 Hex:EtOAc 3:2.

^{1}H RMN ($CDCl_3$) δ 7.37 (bs, 5H), 6.67 (s, 1H), 6.08 (d, J=1.2 Hz, 1H), 5.99 (d, J=1.2 Hz, 1H), 5.19-5.00 (m, 4H), 4.82 (d, J=9.3 Hz, 1H), 4.49 (bs, 1H), 4.32-4.15 (m, 5H), 3.67 (s, 3H), 3.55 (s, 3H), 3.44 (d, J=4.8 Hz, 1H), 3.39 (d, J=6 Hz, 1H), 2.90-2.87 (m, 2H), 2.28 (s, 3H), 2.19 (s, 3H), 2.15-2.07 (m, 2H), 2.03 (s, 3H), 2.00 (s, 3H).

^{13}C-RMN ($CDCl_3$) δ 170.6, 168.8, 155.8, 149.9, 148.5, 146.0, 141.2, 140.6, 136.6, 132.0, 130.4, 128.8, 128.7, 128.5, 125.2, 124.9, 120.5, 118.2, 113.7, 113.6, 102.2, 99.4, 67.2, 61.6, 60.7, 59.7, 59.3, 57.6, 55.1, 54.8, 54.2, 41.9, 41.6, 33.0, 29.9, 23.9, 20.6, 15.6, 9.8.

ESI-MS m/z: Calcd. for $C_{41}H_{44}N_4O_{11}S$: 800.87. Found $(M+23)^+$: 823.7.

Example 101



To a solution of 1713 (100 mg, 0.125 mmol) in CH_2Cl_2 (2 mL) and CH_3CN (2 mL), NaI (75 mg, 0.5 mmol) and TMSCl (63 □L, 0.5 mmol) were added at 0° C. After stirring the reaction at 23° C. for 50 minutes, the mixture was quenched with water (30 mL) and extracted with CH_2Cl_2 (2×20 mL). The combined organic phases were washed successively with a saturated aqueous solution of NaCl (20 mL) and sodium ditionite (20 mL), dried over Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by flash column chromatography (SiO_2, EtOAc:Hexane in a gradient manner, from 1:4, 1:2 to 1:1) to afford 158 (66 mg, 70%) as white solid. Rf=0.21 Hex:EtOAc 1:1. Experimental data of 158 was described above in Example 19.

Transformation of 158 into 35 was described above in Example 85.

Intermediates 36, ET-770 and ET-743 were prepared following the same procedures than those previously described in PCT/GB00/01852.

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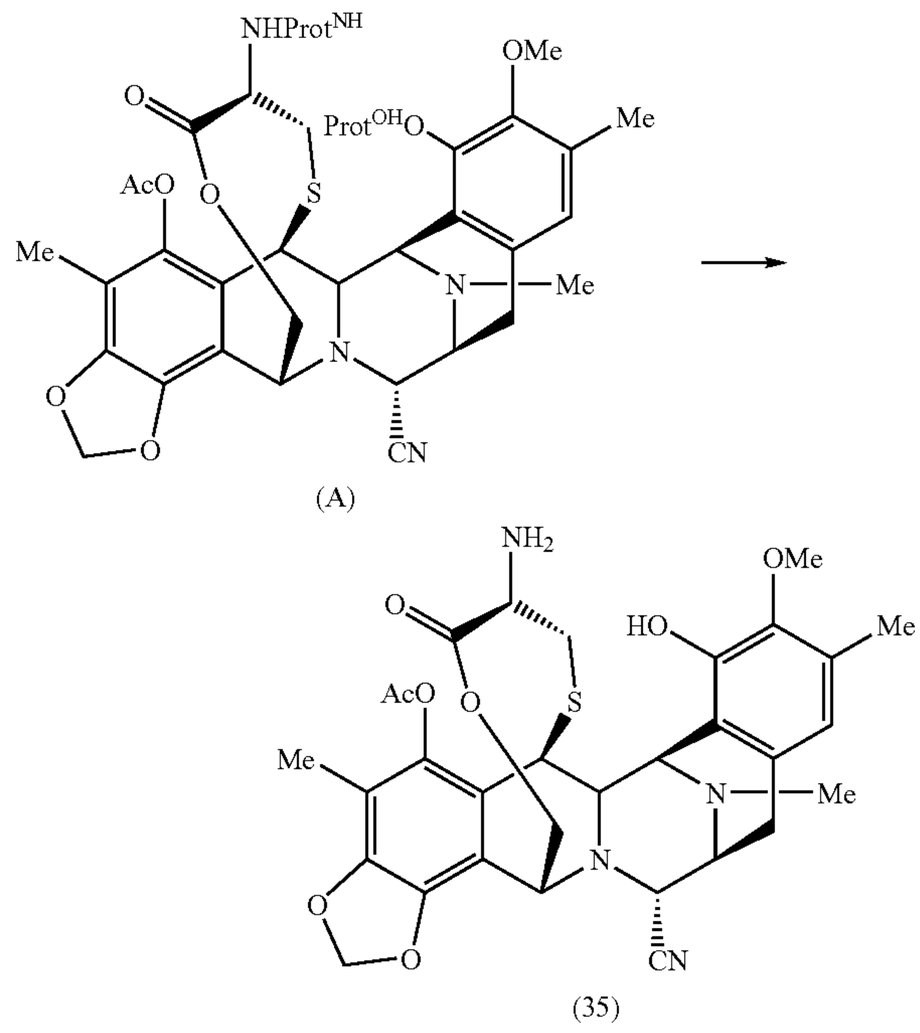
The invention claimed is:

1. A process for the manufacture of an ecteinascidin compound, wherein the process comprises deprotecting a compound of formula (A) to give an α-aminelactone of formula (35), in accordance with the following scheme:

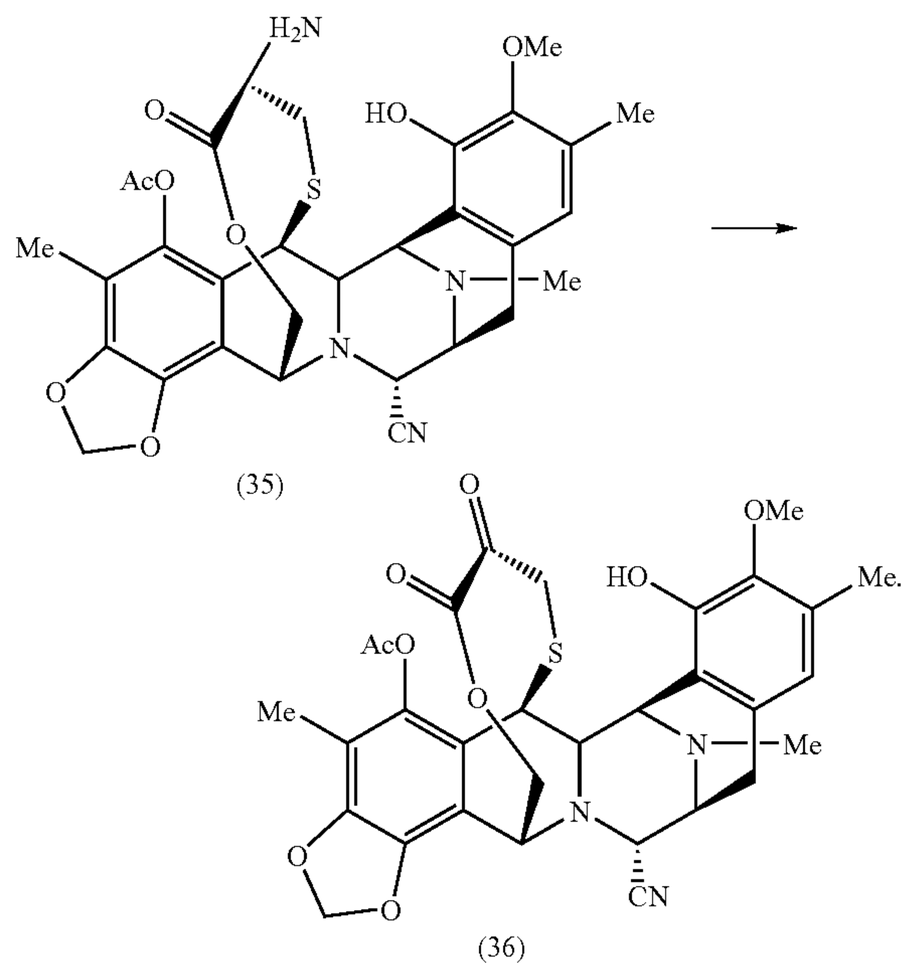


where $Prot^{NH}$ is amino protecting group, and $Prot^{OH}$ is a hydroxy protecting group, and wherein deprotecting the amino group protected with $Prot^{NH}$ and deprotecting the hydroxyl group protected with $Prot^{OH}$ is performed in a single step.

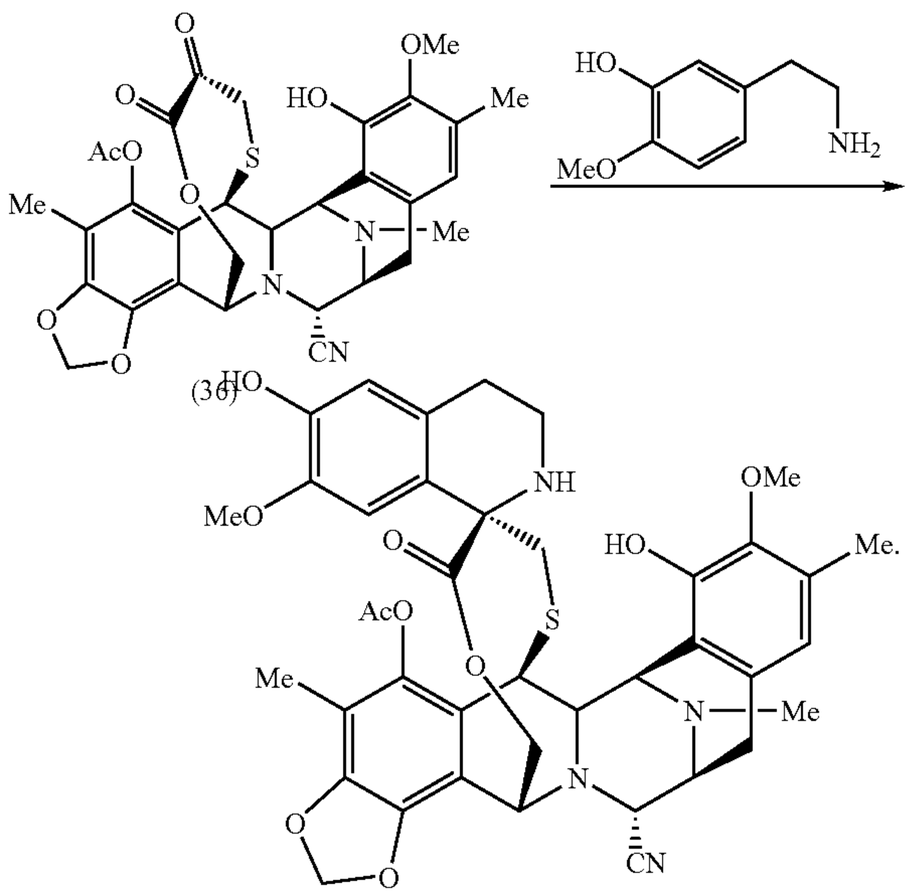
2. The process of claim **1**, wherein $Prot^{NH}$ is t-butyloxycarbonyl and $Prot^{OH}$ is methoxymethyl.

3. The process of claim **1**, wherein $Prot^{NH}$ is t-butyloxycarbonyl and $Prot^{OH}$ is methoxyethoxymethyl.

4. The process of claim **1**, which further comprises the step of oxidising the α-aminelactone of formula (35) to form an α-ketolactone of formula (36) in accordance with the following scheme:

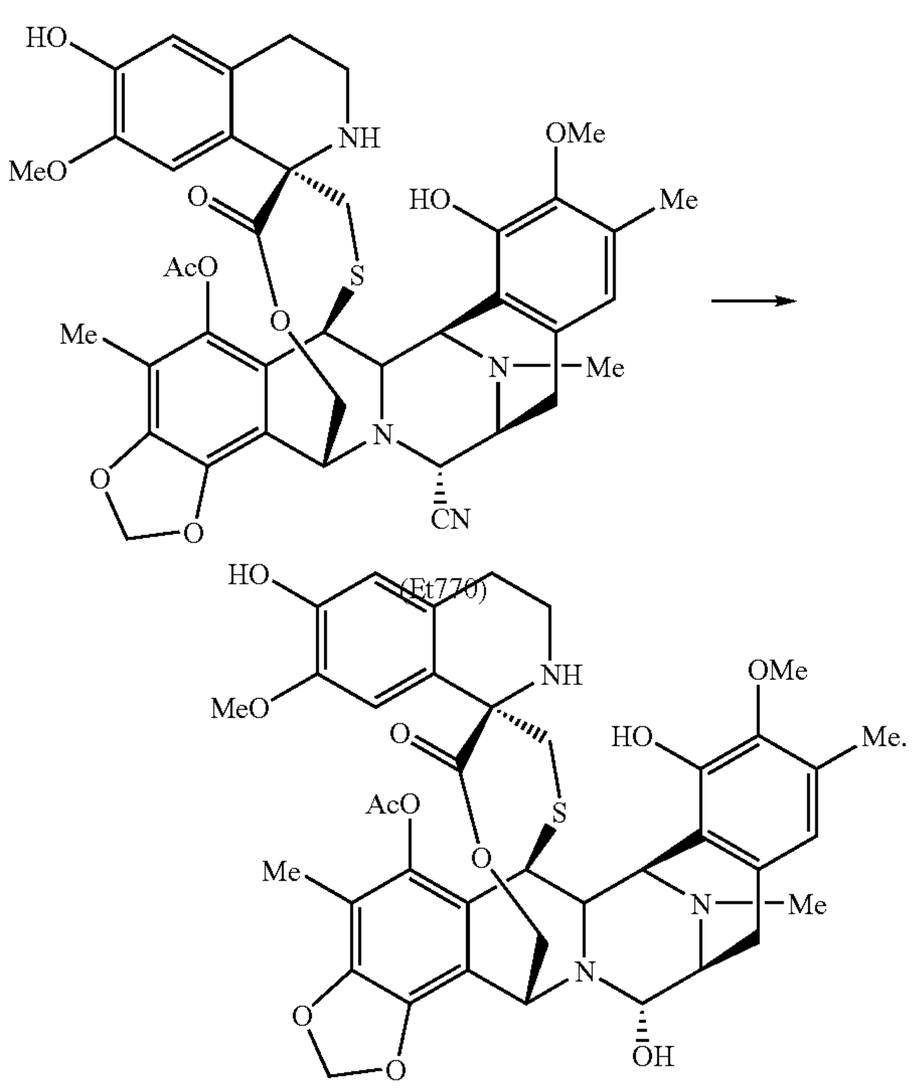


5. The process of claim **4**, which further comprises converting stereospecifically the α-ketolactone of formula (36) to a spirotetrahydroisoquinoline compound Et770, in accordance with the following scheme:

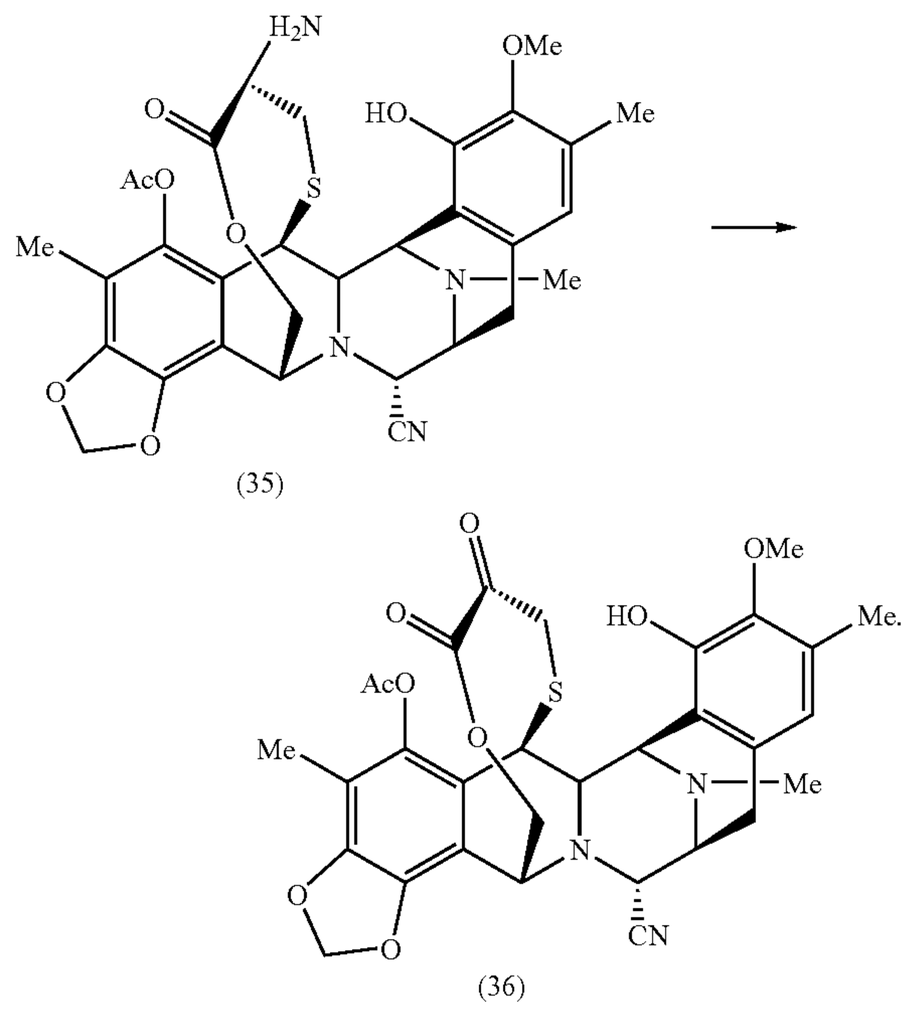


6. The process of claim **5**, wherein converting stereospecifically the α-ketolactone of formula (36) comprises performing a Pictet-Spengler reaction.

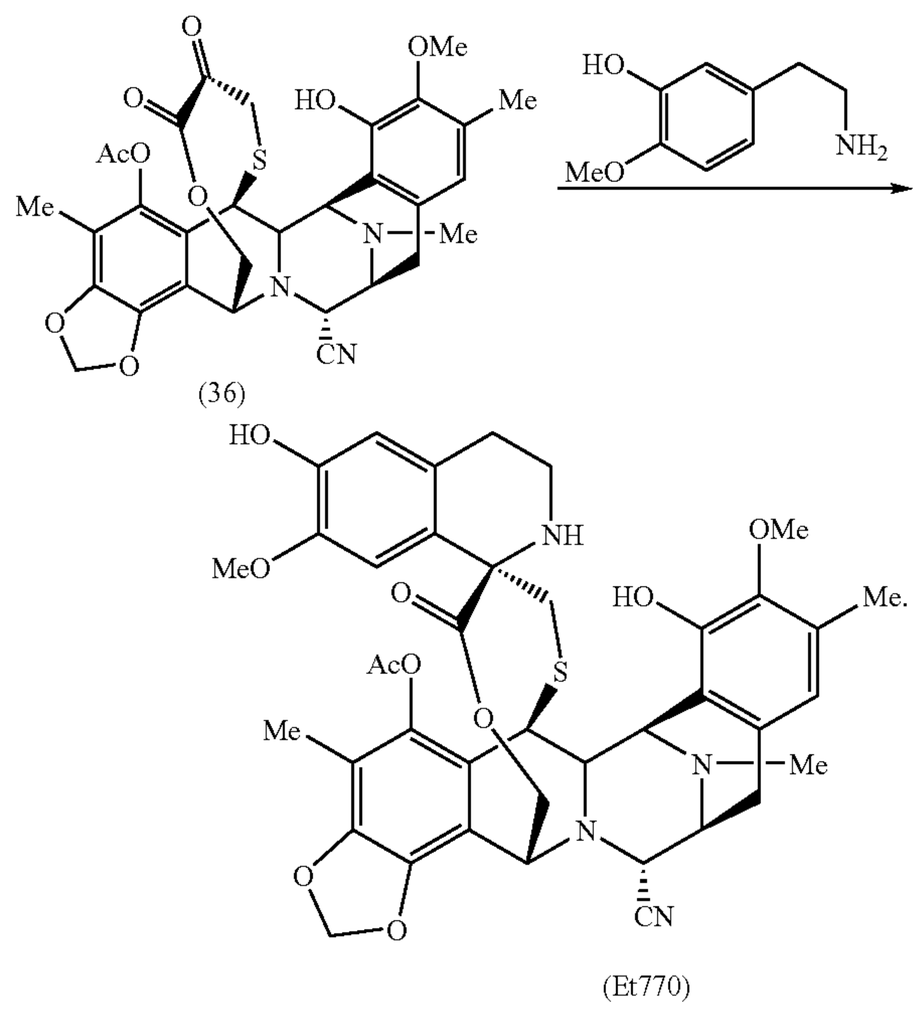
7. The process of claim **5**, which further comprises replacing the nitrile group at C-21 of Et770 with a hydroxy group to form Et743:



8. A process for the manufacture of an ecteinascidin compound, wherein the process comprises oxidizing an α-aminelactone of formula (35) to form an α-ketolactone of formula (36) in accordance with the following scheme:

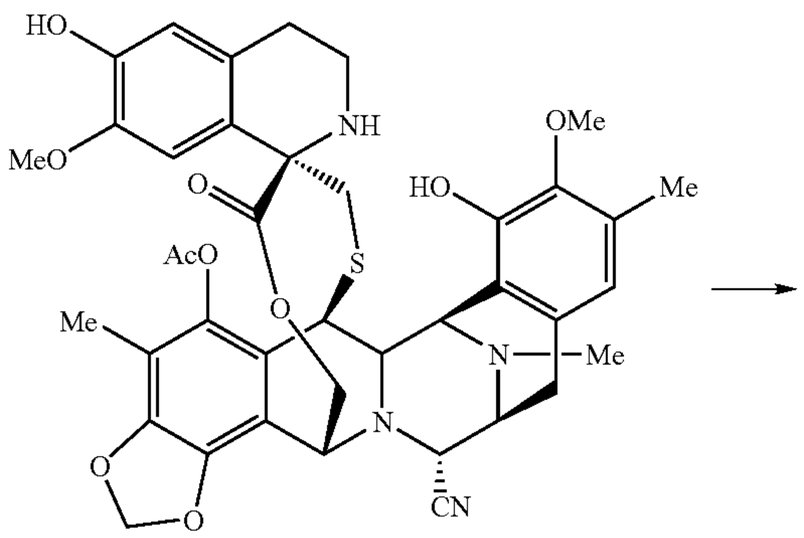


9. The process of claim **8**, which further comprises converting stereospecifically the α-ketolactone of formula (36) to a spirotetrahydroisoquinoline compound Et770, in accordance with the following scheme:

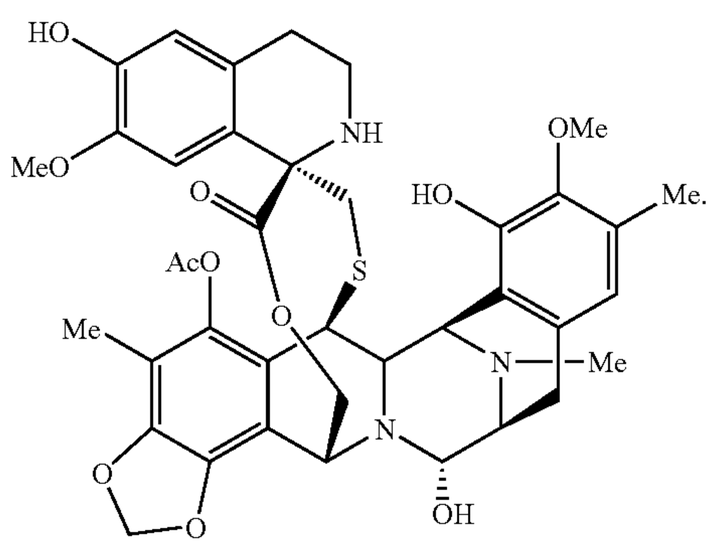


10. The process of claim **9**, wherein converting stereospecifically the α-ketolactone of formula (36) comprises performing a Pictet-Spengler reaction.

11. The process of claim **9**, which further comprises replacing the nitrile group at C-21 of Et770 with a hydroxy group to form Et743:

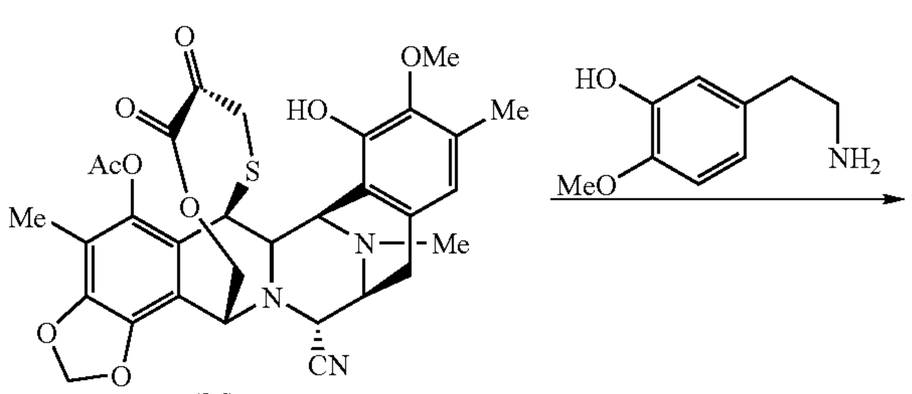


(Et770)



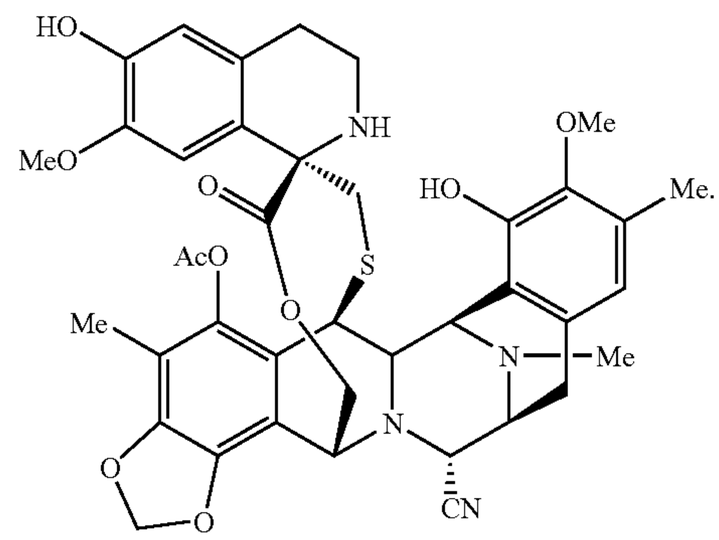
(Et743)

12. A process for the manufacture of an ecteinascidin compound, wherein the process comprises converting stereospecifically an α-ketolactone of formula (36) to a spirotetrahydroisoquinoline compound Et770, in accordance with the following scheme:



(36)

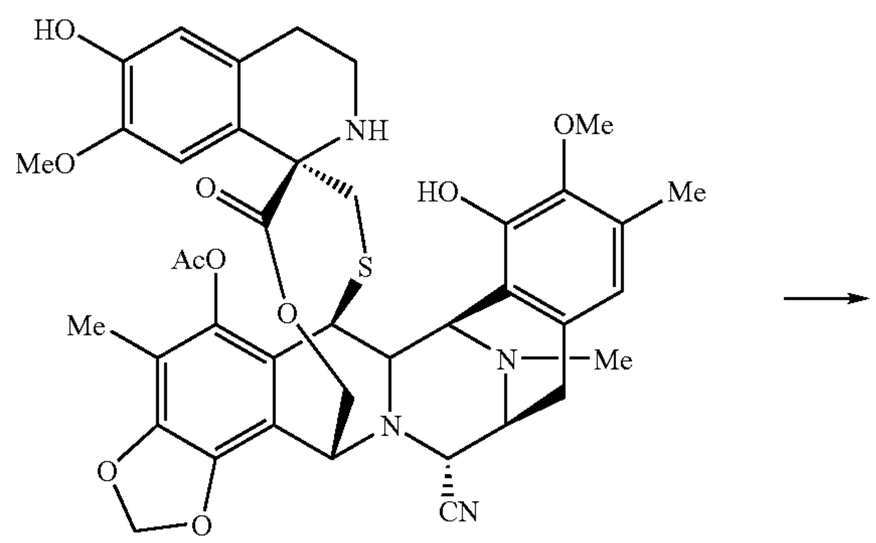
-continued



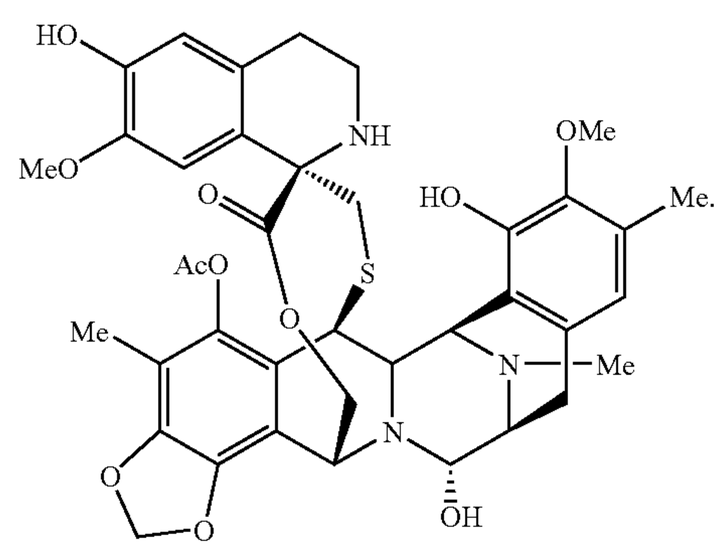
(Et770)

13. The process of claim **12**, wherein converting stereospecifically the α-ketolactone of formula (36) comprises performing a Pictet-Spengler reaction.

14. The process of claim **12**, which further comprises replacing the nitrile group at C-21 of Et770 with a hydroxy group to form Et743:



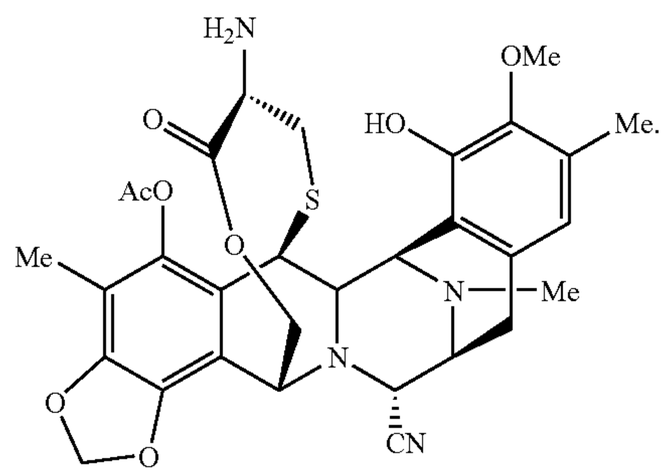
(Et770)



(Et743)

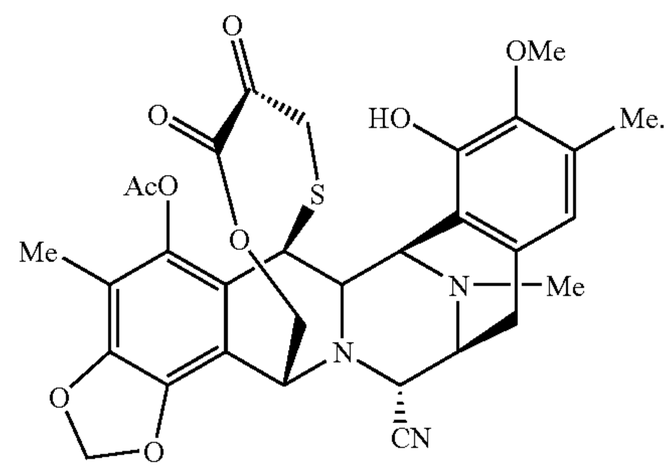
15. An intermediate for the synthesis of an ecteinascidin compound, the intermediate being of the formula (35):

(35)



16. An intermediate for the synthesis of an ecteinascidin compound, the intermediate being of the formula (36):

(36)



* * * * *

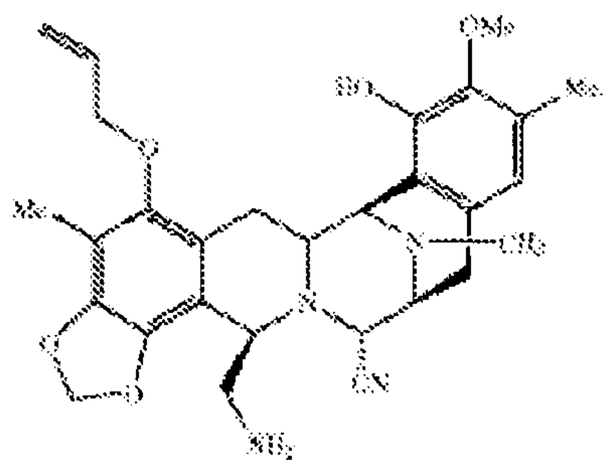
UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 7,420,051 B2
APPLICATION NO. : 11/249172
DATED : October 11, 2005
INVENTOR(S) : Andres Francesch et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 11, line 30: The number "21" should be added below the formula as follows:

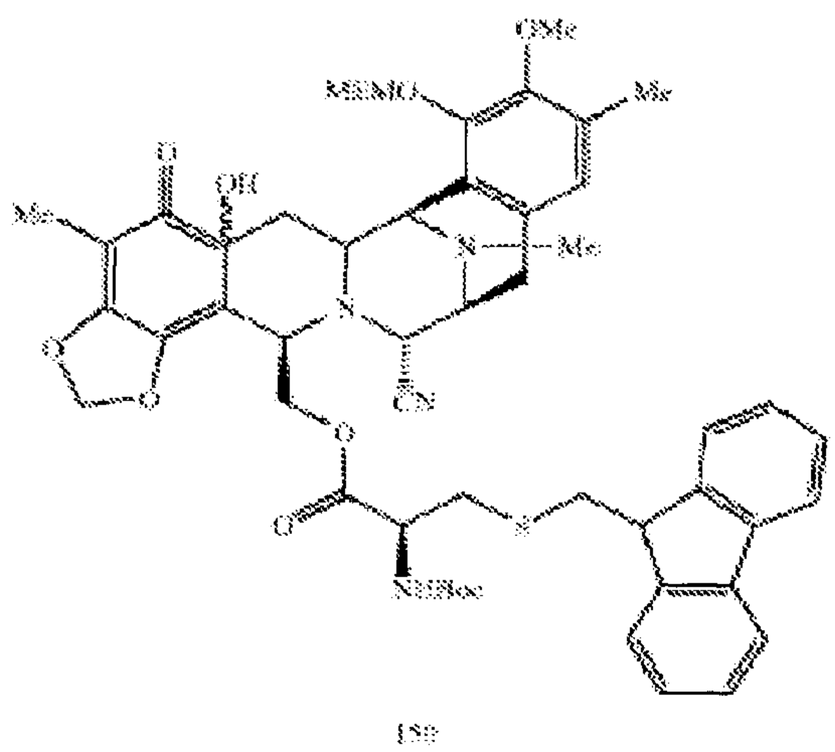


21

Column 17, lines 60-65: The reactive in scheme 2 should be amended from $NaNO_2$" to --$NaNO_3$--.

Column 21, lines 30-35: The formula 150 reading:

"



150

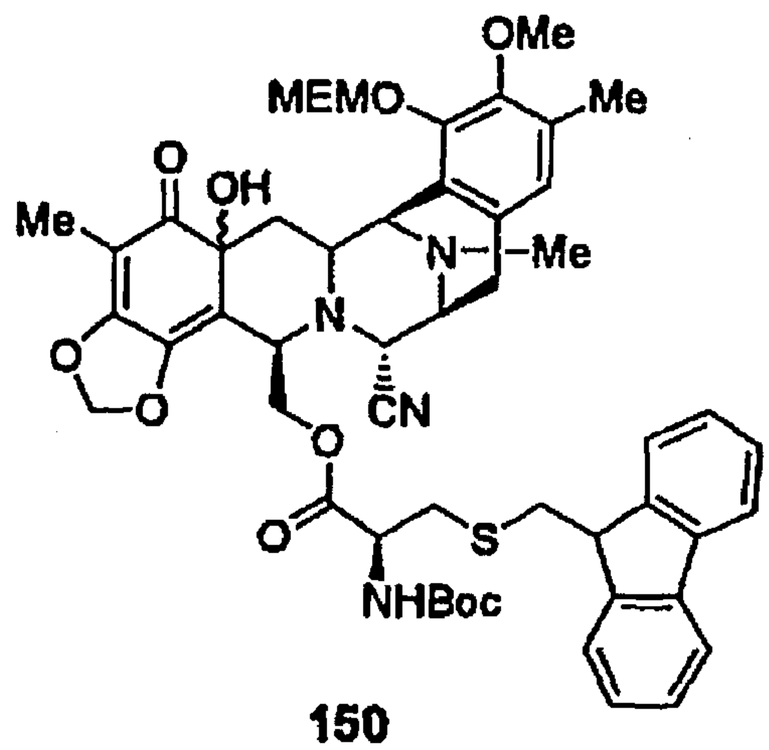
"

Signed and Sealed this
Eighteenth Day of January, 2011

David J. Kappos

David J. Kappos
Director of the United States Patent and Trademark Office

should read:



150

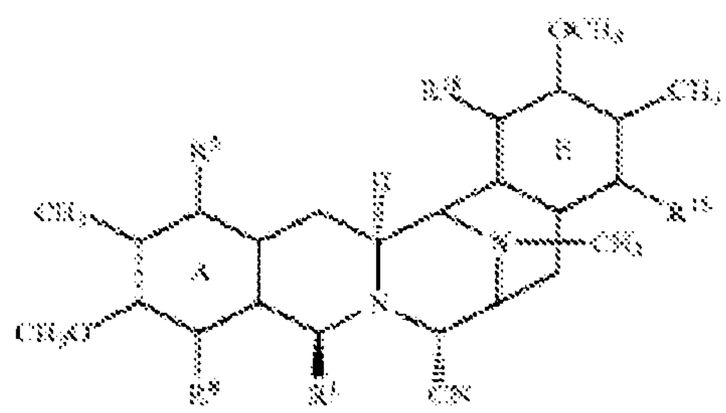
-- --.

Column 30, line 25-30: scheme 4, structure 146, the reactive "tmd" should read --Imd--.

Column 34, line 40-45: scheme 5, structure 146, the reactive "tmd" should read --Imd--.

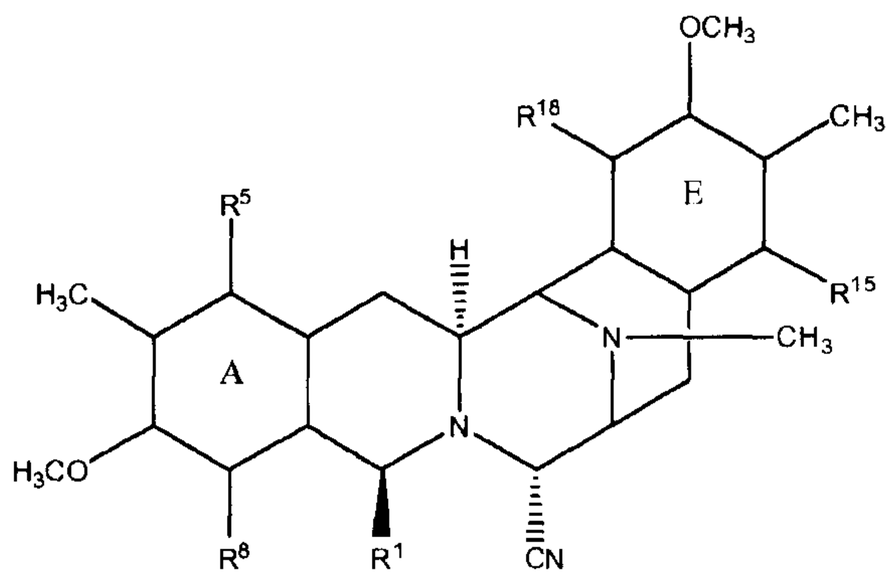
Column 43, line 60: "-CH^2-NH-CO-$CR^{25a}R^{25b}R^{25c}$" should read -- -CH_2-NH-CO-$CR^{25a}R^{25b}R^{25c}$--.

Column 44, line 55-60: The formula (XVI) reading:



" "

should read:



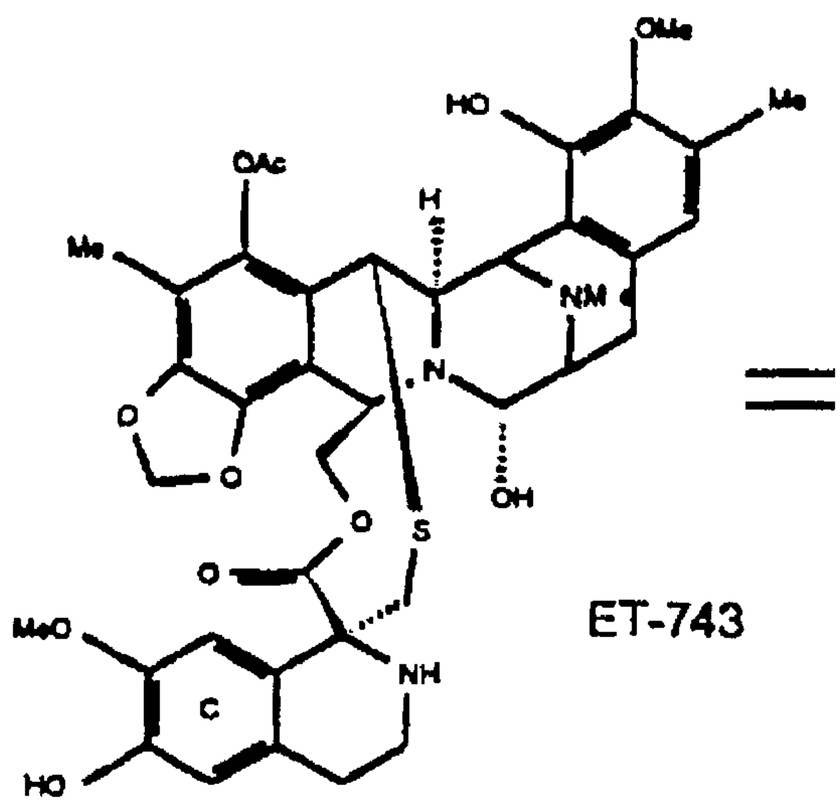
(XVI)

-- --.

Column 45, line 32: "allyl" should read --alkyl--.

Column 52, line 31: "(I" should read --(IV)--.

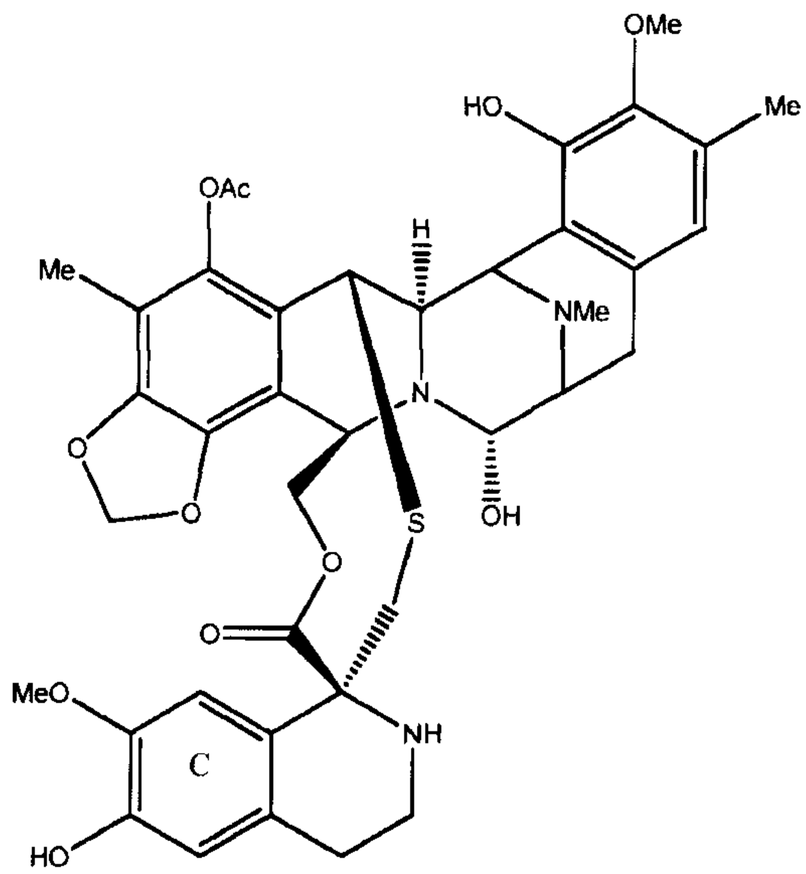
Column 66, line 50-65: The formula of the compound ET-743 reading:



ET-743

" "

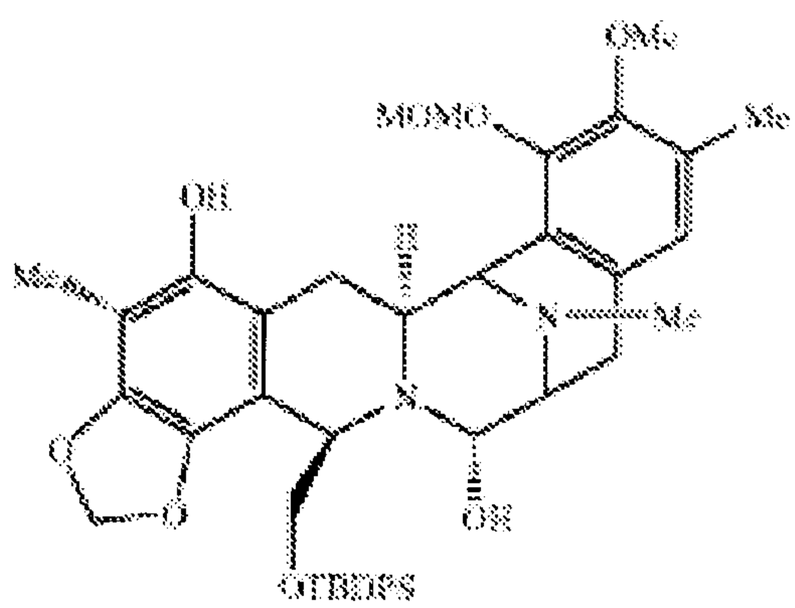
should read:



ET-743

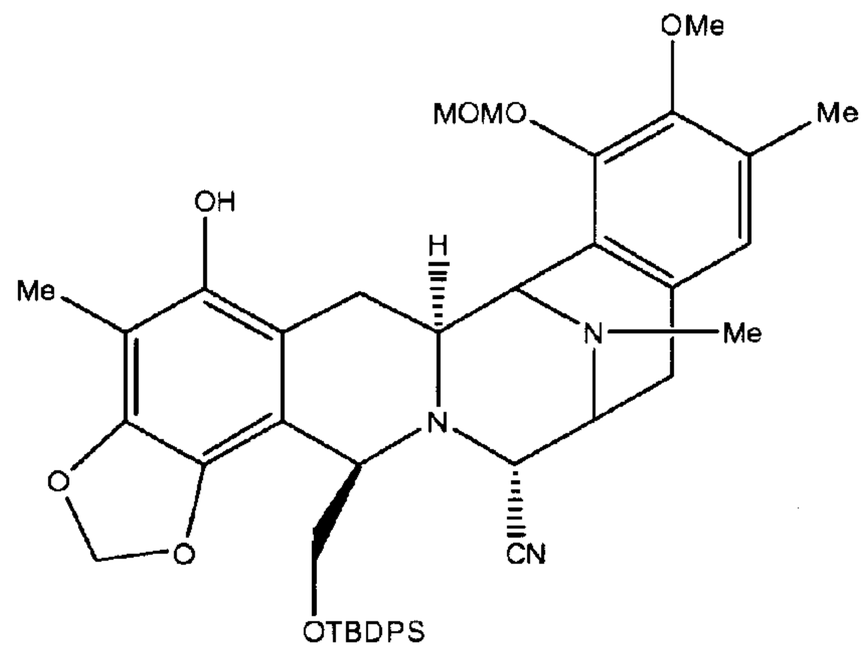
-- --.

Column 67, line 5-15: The formula of the compound int-11 reading:



“ ”

should read:



int-11

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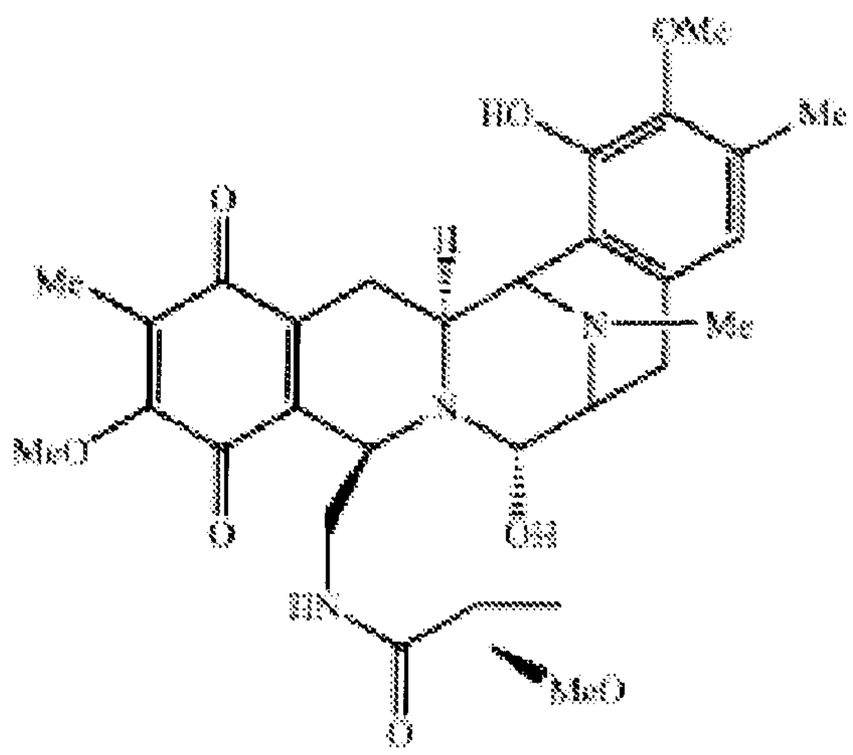
--.

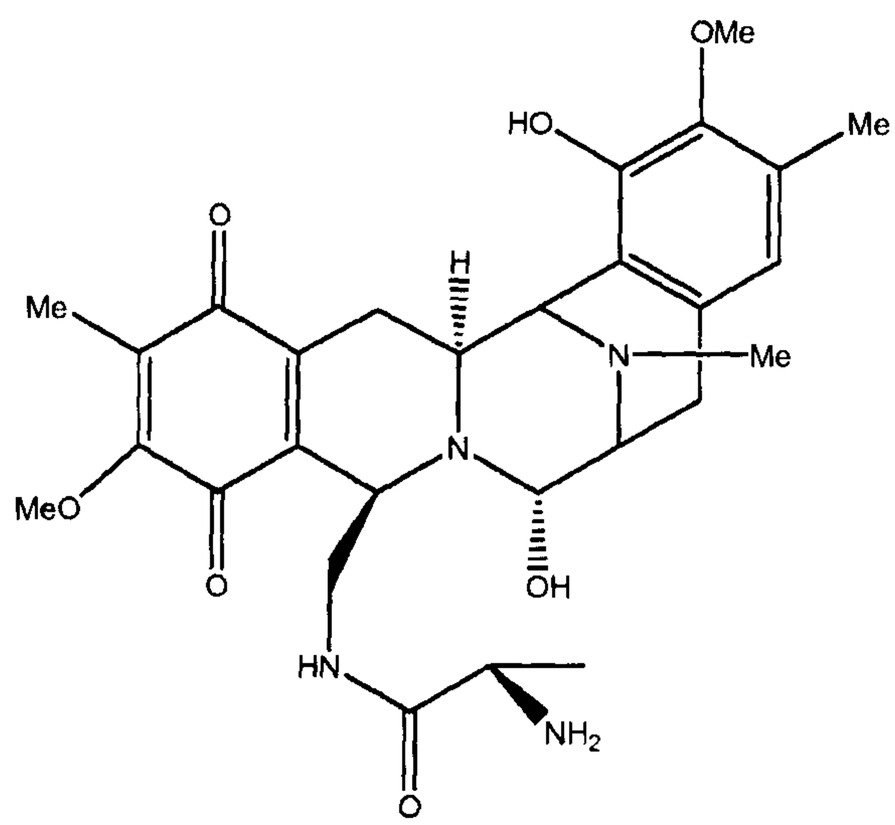
Column 67, line 25-35: the formula reading:



"

"

should read:



SAFRACIN B

--

--.

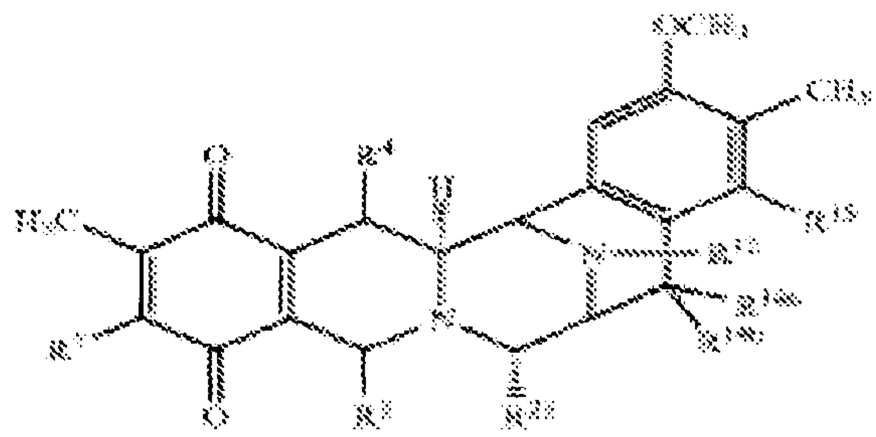
Column 68, line 6: "(X)" should read --(XXI)--.

Column 68, line 34: "-CH^2-NH-CO-$CR^{25a}R^{25b}R^{25c}$" should read -- -CH_2-NH-CO-$CR^{25a}R^{25b}R^{25c}$--.

Column 71, line 20: scheme VIII, first formula: the reactive "Phenyilsothiocyanate" should read --Phenylisothiocyanate--.

Column 75, line 25-35: The compound of formula (XXIIa) reading:

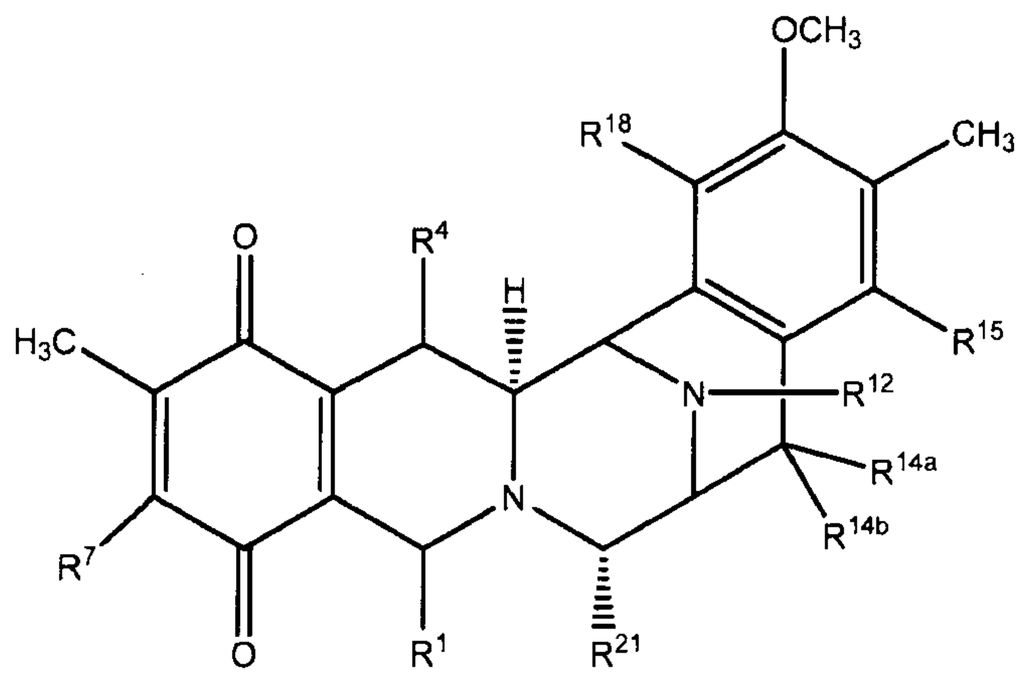
"



"

should read:

--

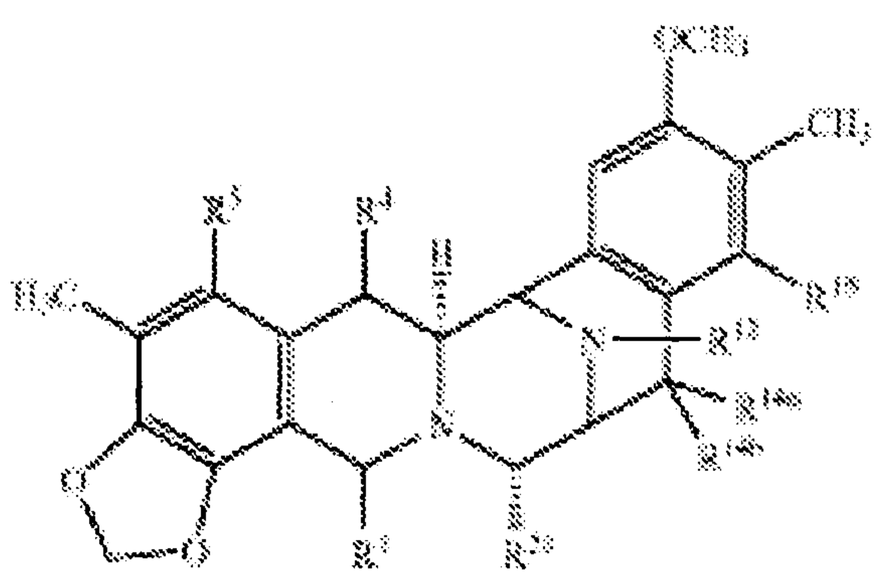


(XXIIa)

--.

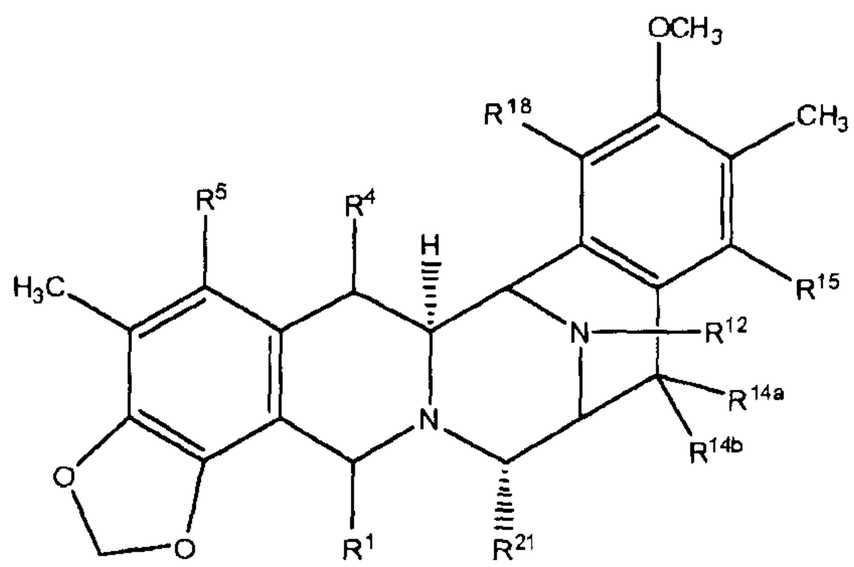
Column 75, line 50-60: The compound of formula (XXIIb) reading:

"



"

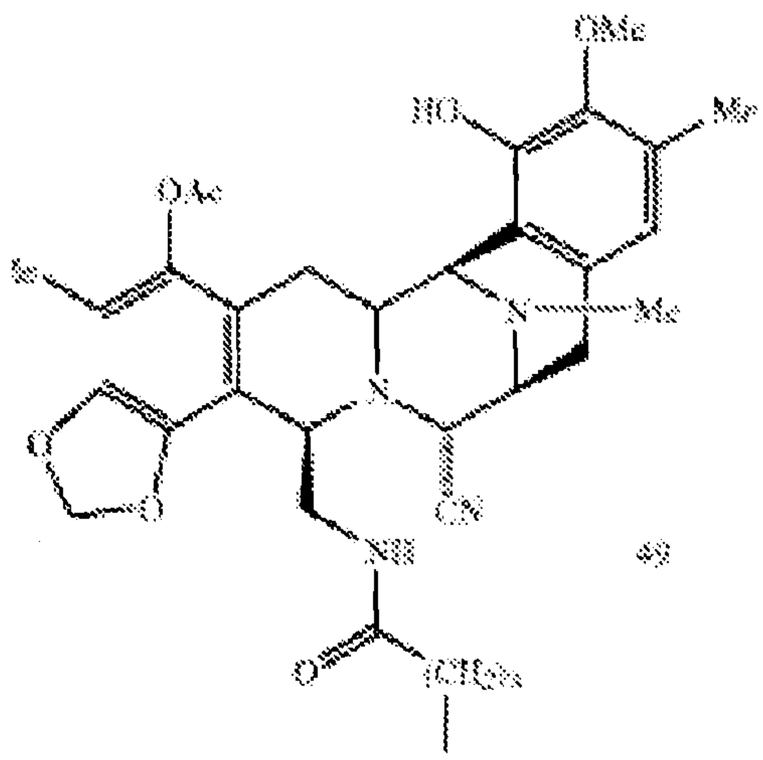
should read:



(XXIIb)

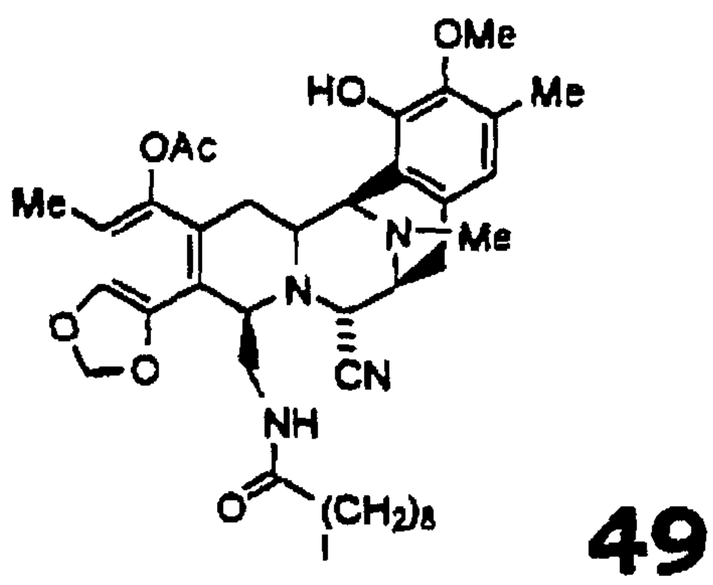
-- --.

Column 97, line 10-15: The compound 49 reading:



" "

should read:



-- --.

Column 131, line 13: the term "1, 23° C" should read --1 h, 23 ° C--.

Column 133, line 9: the reative "TroCl" should read --TrocCl--.

Column 139, line 3: "(3 x 0.10 ml)" should read --(3 x 10 ml)--.

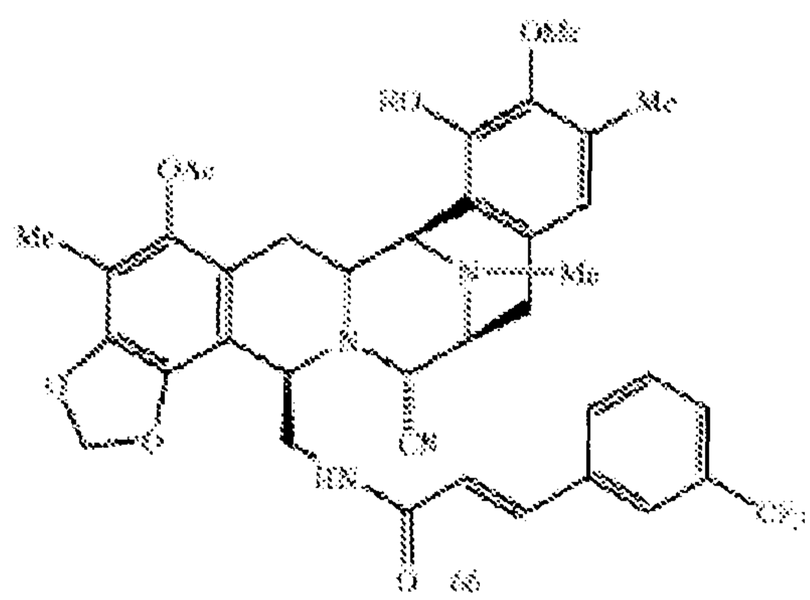
Column 145, line 67: "3.42 (d, 3=8.5 Hz, 1 H)" should read --3.42 (d, J=8.5 Hz, 1 H)--.

Column 184, line 48: "butyl chloride" should read --butyrylchloride--.

Column 184, line 66: "$C_{32}H_{38}N_{407}$" should read --$C_{32}H_{38}N_4O_7$--.

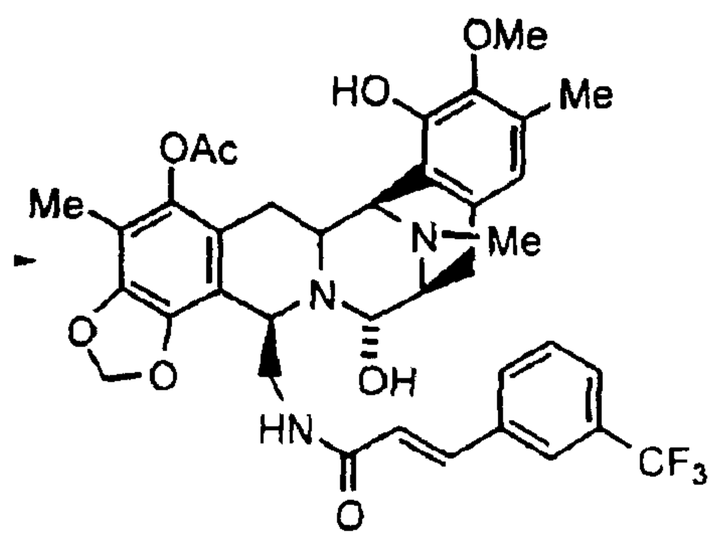
Column 185, line 18: first compound: "66" should read --50--.

Column 185, line 25-35: The compound reading:



" 66 "

should read:



-- 66 --.

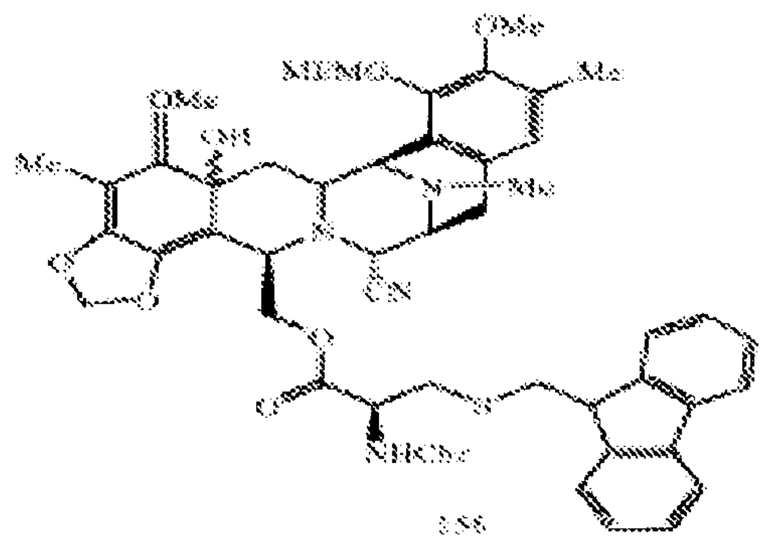
Column 195, line 66: "8175.3" should read --δ 175.3--.

Column 198, line 30: "40° C" should read -- - 40° C--.

Column 200, line 4: Please insert --Route 2-- before Example 71.

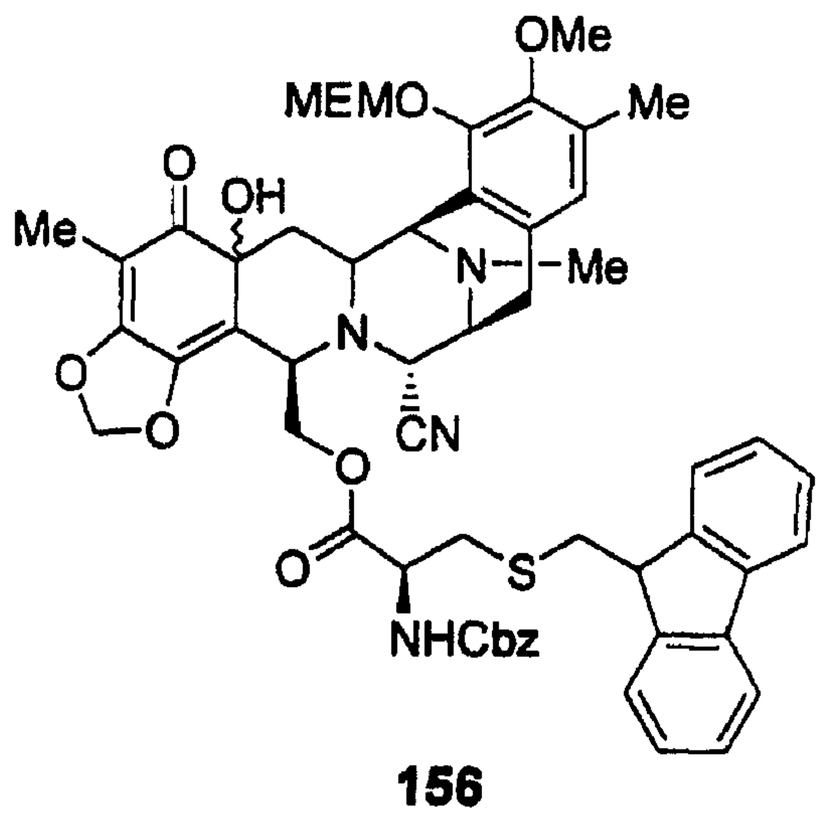
Column 200, line 38: "THP" should read --THF--.

Column 214, line 15-25: The compound 156 reading:



" "

should read



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UNITED STATES PATENT AND TRADEMARK OFFICE

(12) CERTIFICATE EXTENDING PATENT TERM
UNDER 35 U.S.C. § 156

(68)	PATENT NO.	:	7,420,051
(45)	ISSUED	:	September 2, 2008
(75)	INVENTOR	:	Andrés Francesch et al.
(73)	PATENT OWNER	:	Pharma Mar, S.A.
(95)	PRODUCT	:	YONDELIS® (trabectedin)

This is to certify that an application under 35 U.S.C. § 156 has been filed in the United States Patent and Trademark Office, requesting extension of the term of U.S. Patent No. 7,420,051 based upon the regulatory review of the product YONDELIS® (trabectedin) by the Food and Drug Administration. According to United States Patent and Trademark Office records, the original expiration date of the patent as of the date of issuance of this certificate is January 11, 2022. Because it appears that the requirements of the law have been met, this certificate extends the term of the patent for the period of

(94) 1,471 days

subject to the payment of maintenance fees as provided by law, with all rights pertaining thereto as provided by 35 U.S.C. § 156.

I have caused the seal of the United States Patent and Trademark Office to be affixed this 9th day of December 2019

Andrei Iancu
Under Secretary of Commerce for Intellectual Property and
Director of the United States Patent and Trademark Office