

SYNTHESIS OF EPOTHILONES, INTERMEDIATES THERETO, ANALOGUES AND USES THEREOF

BACKGROUND OF THE INVENTION

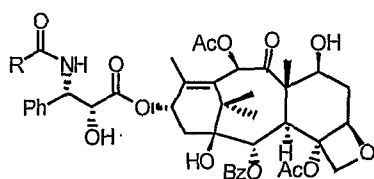
5 Epothilones A and B (**2a** and **2b**, Scheme 1) are naturally occurring cytotoxic macrolides that were isolated from a cellulose degrading mycobacterium, *Sorangium cellulosum* (Höfle *et al. Angew. Chem., Int. Ed. Engl.* **1996**, 35, 1567 and *J. Antibiot.* **1996**, 49, 560; each of which is incorporated herein by reference). Despite their vastly different structures, epothilones A and B share the same mechanism of action as

10 paclitaxel (Taxol[®]) which involves growth inhibition of tumor cells by tubulin polymerization and stabilization of microtubule assemblies (Bollag *et al. Cancer Res.* **1995**, 55, 2325; incorporated by reference). In spite of its unquestioned clinical value as a front-line chemotherapeutic agent, Taxol[®] is far from an ideal drug. Its marginal water solubility necessitates recourse to formulation vehicles such as Cremophores that

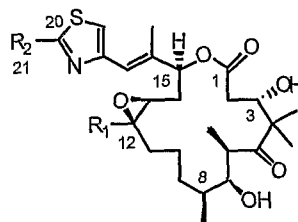
15 pose their own risks and management issues (Essayan *et al. J. Allergy Clin. Immunol.* **1996**, 97, 42; incorporated herein by reference). Moreover, Taxol[®] is vulnerable to deactivation through multiple drug resistance (MDR) (Giannakakou *et al. J. Biol. Chem.* **1997**, 272, 17118; incorporated herein by reference). However, it has also been demonstrated that epothilones A and B retain remarkable potency against MDR tumor

20 cells (Kowalski *et al. Mol. Biol. Cell* **1995**, 6, 2137; incorporated herein by reference). Additionally, the increased water solubility in comparison to paclitaxel may be useful for the formulability of epothilones. While the naturally occurring compound, epothilone B (**2b**, EpoB, in Scheme 1), is a potent member of the epothilone family of natural products, it unfortunately possesses, at least in xenograft mice, a worryingly

25 narrow therapeutic index (Su *et al. Angew. Chem. Int. Ed. Engl.* **1997**, 36, 1093; Harris *et al. J. Org. Chem.* **1999**, 64, 8434; each of which is incorporated herein by reference).



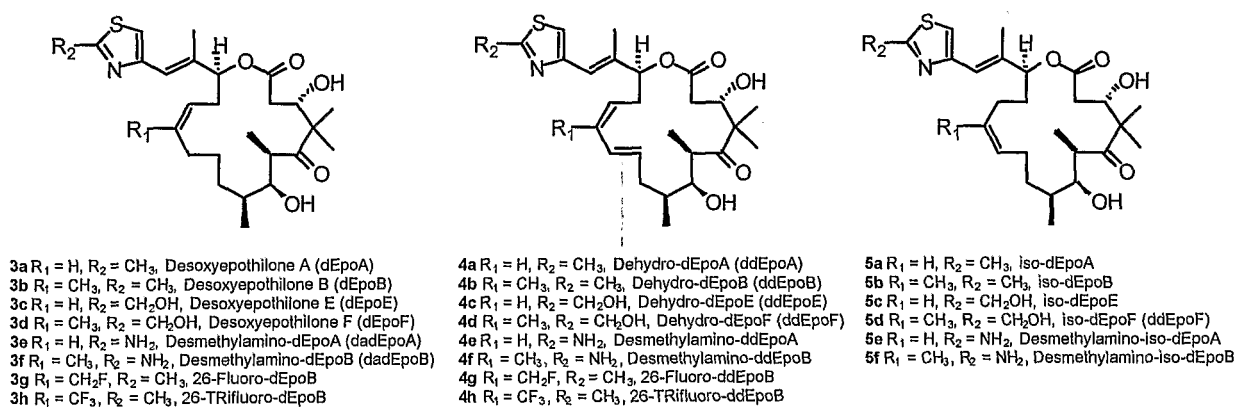
1a R = Ph, Paclitaxel (Taxol)
1b R = *t*-Bu, Docetaxel (Taxotere)



2a R₁ = H, R₂ = CH₃, Epothilone A (EpoA)
2b R₁ = CH₃, R₂ = CH₃, Epothilone B (EpoB)
2c R₁ = H, R₂ = CH₂OH, Epothilone E (EpoE)
2d R₁ = CH₃, R₂ = CH₂OH, Epothilone F (EpoF)

Scheme 1: Taxoids and Epothilones

Given the limited therapeutic index of EpoB, other epothilone analogues, in particular the 12,13-desoxyepothilones, were investigated for their ability to provide an improved therapeutic profile (see, U.S. Patent No.: 6,242,469, 6,284,781, 6,300,355, 6,369,234, 6,204,388, 6,316,630; each of which is incorporated herein by reference). *In vivo* experiments conducted on various mouse models demonstrated that 12,13-desoxyepothilone B (**3b**, dEpoB in Scheme 2) possesses therapeutic potential against various sensitive and resistant human tumors in mice xenografts (Chou *et al.* *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 9642 and 15798; incorporated herein by reference). Recently, the therapeutic superiority of these desoxyepothilones over other anticancer agents has been conclusively demonstrated by thorough comparative studies (Chou *et al.* *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 8113; incorporated herein by reference). Due to its impressive *in vivo* profile, dEpoB has been advanced through toxicology evaluations in dogs, and is now in human trials as an anticancer drug.



Scheme 2. Various Desoxyepothilone Analogues

In light of the promising therapeutic utility of the 12,13-desoxyepothilones, it would be desirable to investigate additional analogues as well as additional synthetic methodologies for the synthesis of existing epothilones, desoxyepothilones, and analogues thereof, as well as novel analogues thereof. In particular, given the interest in the therapeutic utility of this class of compounds, it would also be desirable to develop methodologies capable of providing significant quantities of any epothilones or

desoxyepothilones previously described, or those described herein, for clinical trials and for large-scale preparation.

DESCRIPTION OF THE DRAWINGS

5 *Figure 1* shows a synthesis of 12-trifluoromethyl-9,10-dehydro-desoxyepothilone D.

Figure 2 shows several methods of preparing 12-trifluoromethyl-9,10-dehydro-desoxyepothilone D from Epo A.

10 *Figure 3* shows the preparation of 9,10-dehydro-epoD from Epo 490 by isomerizing the C10-C11 double bond to the C9-C10 position.

Figure 4 depicts various epothilone analogs with modifications at the 9,10-position.

Figure 5 shows the synthesis of various 9,10-dehydro-epothilone analogs using a ring closing metathesis reaction at the 9,10-position.

15 *Figure 6* shows the results of an experiment in which an extra large MX-1 xenograft was implanted in nude mice. MX-1 tumor tissue (50mg) was implanted s.c. on day 0. On day 22 (D22) when tumor size reached $960 \pm 132\text{mg}$ (about 3.4% of body weight), Fludelone 25mg/kg, 6 hr-i.v. infusion, Q3Dx5 was given on D22, D25, D28, D31 and D34 as indicated by arrows. The second cycle of treatment, following 9
20 day rest, was given on D43, D46, D49 and D52. Tumor size changes in the vehicle treated control (●) and Fludelone treated group (□) (n = 5 each). Observation was continued Q3D up to D180 when the experiment was terminated (128 days following cessation of treatment on D52). *Figure 6A* shows the therapeutic effect as demonstrated by a reduction in tumor size. *Figure 6B* shows photographs for the nude
25 mice (one mouse each selected from the control group and the treated group) taken on D25, D31, D37, D43 and D52. No relapse was observed on D180 when the experiment was terminated. *Figure 6C* shows changes in body weight.

30 *Figure 7* shows the results of an experiment in which nude mice were implanted with human T-cell lymphoblastic leukemia (CCRF-CEM/Taxol) which is 44-fold resistant to Taxol. Tumor tissue of CCRF-CEM/Taxol (44-fold resistant *in vitro*), 50mg/mouse was implanted s.c. into nude mice on Day 0. Treatment of 6hr-i.v. infusion started on D8 with Fludelone 15mg/kg (□) (n = 3) and 30mg/kg (Δ) (n = 4), or

beginning D16, Q2Dx3 and then the dose was increased to 40mg/kg, Q2Dx3 (D22-26) and then 60mg/kg, Q2Dx3 (D28-40). *Figure 10A* shows the therapeutic effect as demonstrated by a reduction in tumor size. *Figure 10B* shows changes in body weight.

Figure 11 shows the treatment of nude mice bearing human lung carcinoma (A549/Taxol) which is 44-fold resistant to Taxol using 26-trifluoro-9,10-dehydro-dEpoB and Taxol (6 hour iv infusion, Q3Dx11). *Figure 11A* shows the therapeutic effect as demonstrated by a reduction in tumor size. *Figure 11B* shows changes in body weight.

Figure 12 shows the treatment of nude mice bearing human lung carcinoma A549/Taxol which is 44-fold resistant to Taxol using 9,10-dehydro-dEpoB. (6 hour iv infusion, Q4Dx5, x3). *Figure 12A* shows the therapeutic effect as demonstrated by a reduction in tumor size. *Figure 12B* shows changes in body weight before and after treatment.

Figure 13 shows the a comparision of 26-trifluoro-9,10-dehydro-dEpoB, dEpoB, and Taxol in the treatment of nude mice bearing human mammary carcinoma MX-1 xenografts using an oral formulation (PO, Q2Dx7, x5). *Figure 13A* shows the therapeutic effect as demonstrated by a reduction in tumor size. *Figure 13B* shows changes in body weight.

Figure 14 is a table summarizing various therapeutic and pharmacokinetic parameters of epothilone derivatives.

Figure 15 shows the treatment of nude mice bearing human lung carcinoma (A549) xenograft using 26-trifluoro-9,10-dehydro-dEpoB and dEpoB (6 hour iv infusion, (Q2Dx6)x2, x2). *Figure 15A* shows the therapeutic effect as demonstrated by reduction in tumor volume. *Figure 15B* shows changes in body weight during and after treatment.

Figure 16 shows stabilization of microtubule formation at 10 μ M drug. Microtubules formed in the presence of 10 μ M taxol was defined as 100%. Tubulin from bovine brain was a product of Sigma. The tubulin assembly assay was carried out according to the manufacturer's specifications. Tubulin (1 mg in 200 μ l) was incubated with 790 μ l of buffer (0.1 M MES, 1 mM EGTA, 0.5 mM $MgCl_2$, 0.1 mM EDTA, and 2.5 M glycerol, pH 6.5) and with 10 μ l of drug (final concentration 10 μ M). For assembly, the incubation was carried out at 35 °C for 40 minutes, and for disassembly

of the same samples, the incubation was carried out at 4 °C for 40 minutes. Absorbance at 350 nm was measured for the microtubule stabilization. Solvent blank (DMSO) was subtracted from the absorbance.

5 *Figure 17* shows the potency of epothilones against tumor cell growth *in vitro* and the relative therapeutic index.

Figure 18 is cell cycle analysis as determined by Propidium iodide DNA staining. Cell cycle was arrested in G2M phase starting from 6 hours and absolutely blocked at 24 hours for OPM-2 myeloma cells after treatment with dEpoB (upper panel) and Fludelone (lower panel). The two drugs induced the same pattern of cell
10 cycle arrest.

Figure 19 shows Annexin V staining on the RPMI8226 myeloma cell line. In early apoptotic cells, the membrane phospholipids phosphatidylserine (PS) is translocated from the inner to the outer leaflet of the plasma membrane, thereby exposing PS to the external cellular environment. Annexin V is a 35-36 kD Ca^{+2}
15 dependent phospholipid-binding protein that has a high affinity for PS, and binds to cells with exposed PS. In conjunction with a vital dye such as 7-amino-actinomycin (7-AAD) staining dead cells, this assay allows the identification of early apoptotic cells. The data showed that the majority of cells either enter apoptosis or are dead after 24 hours treatment with dEpoB or Fludelone.

20 *Figure 20* shows micrographs of myeloma and lymphoma cell lines treated with Fludelone (125 nM) for 24 hours. The characteristic ring-like structures of the cells arrested in G2/M is seen.

Figure 21 shows the cell count of RPMI8226 myeloma cells treated with Fludelone and dEpoB (125 nM). At different time points (1, 2, 4, 8, 24 hours), the drug
25 was washed out, and the cells were continued to be incubated for up to 48 hours. Note that with as little as 1 hour exposure to Fludelone the tumor cell numbers progressively decrease with the majority of cells undergoing apoptosis at 24 hours whereas with dEpoB cells continue to expand.

Figure 22 shows α -tubulin staining of RPMI8226 myeloma cells. Fludelone
30 appears to stabilize microtubules and greatly increase the microtubule polymer mass at an early stage of treatment (at 12 hours with RPMI8226 myeloma cells). After 24

hours of drug treatment, microtubules mass was decrease and disrupted while cells underwent apoptosis.

Figure 23 is the cell cycle analysis determined by Propidium iodide DNA staining. Solid lines describe the values for G1 and G2, gray shaded area depict the cells in S phase, dotted line delineates the overall curve giving the number of doublets especially evident with Taxol (upper left image). Upper row demonstrates the G2M phase arrest after 24 hours of incubation with Fludelone (10 nM), dEpoB (100 nM), and Taxol (100 nM) of the ovarian cancer cell line IGROV. The lower row demonstrate the increase cell cycle arrest of HT-29 with increased concentrations of Fludelone. The 100 nM incubation with Fludelone resulted in massive apoptosis after 24 hours hampering cell cycle analysis in several cell lines including IGROV and HT-29.

Figure 24 shows Annexin V staining of the Ovar3 ovarian cancer cell line after 24 hour incubation with 100 nM of Fludelone, Taxol, or dEpoB. Percentages given in the lower left quadrant is the percentage of Annexin V⁺/PI⁻ negative cells, which resemble cells in the early apoptotic stage.

Figure 25 is a cytospin of HT-29 colon cancer cells after 24 hours of treatment stained with HEMA 3 (200x magnification). (a) control cells treated with the dissolvent (DMSO). (b)-(d) Increased concentrations of Fludelone 1, 10, and 100 nM are shown. (e) HT-29 cells after the application of 100 nM dEpoB and (f) after 100 nM of Taxol. All three drugs produce the same phenotype after 24 hours apparent in a ring-like structure of the nucleus resulting in apoptosis.

Figure 26 shows the experimental design of a system for evaluating the action of Fludelone and dEpoB in disseminated and metastatic human tumor xenograft models.

Figure 27 shows cell proliferation and IC₅₀ (the concentration for 50% cell inhibition) assays. Cell proliferation was determined using the sodium 3'-[1-(phenylamino-carbonyl)-3,4-tetrazolium]-bis (4-methoxy-6-nitro) benzene sulfonic acid hydrate (XTT), which measured the conversion of a tetrazolium compound into formazan by a mitochondrial dehydrogenase enzyme in live cells. The amount of formazan is proportional to the number of living cells present in the assay mixture. Each data point was the average of four independent determinations. The IC₅₀ of Fludelone is about 7.6~36.67nM and dEpoB 36.67~61.34nM for myeloma cell lines

(RPMI8226, CAG). The IC₅₀ of Fludelone is about 60~80nM for lymphoma lines (SKIDLBCL and RL).

Figure 28 shows cell proliferation and IC₅₀ assays on normal stromal cells. The same method was used as described in *Figure 104*. Human marrow stromal cell lines, HS-27A and HS-5 immortalized by E6/E7 genes, have normal marrow stromal function, which support stem cell self-renewal, and proliferation. The IC₅₀ of Fludelone and dEpoB is about 90-100nM for these stromal lines with a comparable population doubling time with tumor lines.

Figure 29 shows the titration of Fludelone and dEpoB concentrations on cell cycle arrest. CAG myeloma cell lines were used. At the concentration of 31.25nM, both drugs can absolutely blocked cell cycle at G2M phase (data below 31.25nM is not shown).

Figure 30 shows the Titration of Fludelone and dEpoB concentration on cell cycle arrest. RPMI8226 myeloma cell lines were used. At the concentration of 31.25nM, both drugs can absolutely blocked cell cycle at G2M phase (data below 31.25nM is not shown).

Figure 31 shows Annexin V staining on the CAG myeloma cell lines. In early apoptotic cells, the membrane phospholipids phosphatidylserine (PS) is translocated from the inner to the outer leaflet of the plasma membrane, thereby exposing PS to the external cellular environment. Annexin V is a 35-36kD Ca²⁺ dependent phospholipids-binding protein that has a high affinity for PS, and binds to cells with exposed PS. In conjunction with a vital dye such as 7-amino-actinomycin (7-AAD) staining dead cells, this assay allows to identify early apoptotic cells. The data showed that the majority of cells either enter apoptosis or are dead after 24 hours treatment with Fludelone or dEpoB. Note that X-axis is Annexin staining and Y-axis is 7-AAD staining.

Figure 32 is a DNA fragmentation assay. A major biochemical hallmark of apoptosis nucleosome excision from chromatin. Nucleosome excision is caused by endonuclease-mediated digestion of the exposed DNA linker regions between nucleosome in chromatin. Since the 180-200 base pairs of DNA around a histone core are conformationally protected from digestion, this endonuclease-mediated nucleosome excision is observable as a DNA ladder in agarose gels. The data showed that typical DNA ladders were detected after treatment of RPMI8226 and CAG myeloma cells with

Fludelone or dEpoB for 24 hrs. The data implicated that cell apoptosis is induced by epothilones through the caspase pathway.

Figure 33 shows the alternation of body weight and the outcome after epothilone (20mg/kg) treatment of disseminated CAG xenograft myeloma mice. The data show that the control mice died in about 30 days and significant body weight loss was observed after 20 days of treatment. There is no significant difference in life span between mice treated with dEpoB and controls; however, mice treated with Fludelone showed a significant extension of survival days than either control and dEpoB groups. The number of mice used in each group was four, and mice were irradiated 300 Rad prior to injection of CAG cells.

Figure 34 shows the treatment of disseminated CAG xenograft myeloma mice at week 4. 10×10^6 CAG myeloma cells engineered with Luc-eGFP-TK fusion gene were injected intravenously into the mouse-tail vein, and the treatment was started when decent myeloma cells implantation was detected by bioluminescence imaging on day 7. The figure showed the bioluminescence images on mice treated with either control, or dEpoB, or Fludelone, respectively.

Figure 35 shows quantification of the average tumor photon emissions in CAG xenograft mice (N=4). The figure show that mice treated with Fludelone had significant decreased tumor photon emissions than mice treated with either vehicle or dEpoB alone. Tumor photon emission correlates positively with tumor burden.

Figure 36 shows alternations in body weight and the outcome after epothilone (20mg/kg) treatment of disseminated CAG xenograft myeloma mice. The figure show a similar feature as *Figure 33* in the first 30 days; however, the only survival group of Fludelone-treated mice received additional 5 dosages of Velcade (6.25 ug/mouse, I.V) boost.

Figure 37 shows the treatment of disseminated CAG xenograft myeloma mice at day 18. The figure shows the significant difference of images among groups, which reflects its tumor burden in the group.

Figure 38 shows the quantification of the average tumor photon emissions in the treatment of disseminated CAG xenograft myeloma mice at day 18. The figure shows the significant difference of images among groups, which reflects its tumor burden in the group.

Figure 39 shows the treatment of disseminated CAG xenograft myeloma mice at day 40. The figure shows the images difference, which reflects tumor burden in three groups of mice.

5 *Figure 40* shows the quantification of the average tumor photon emissions in the treatment of disseminated CAG xenograft myeloma mice at day 40. The figure shows the images difference, which reflects tumor burden in three groups of mice.

Figure 41 shows CAG xenograft myeloma mice treated with Fludelone in combination with Velcade. Mice treated with Fludelone from day 0 to 28 (20 mg/kg, Q2d) and Velcade from day 35 to 45 (6.25 ug/mouse, I.V, 3/w). The treatment started
10 on day 10 and the images are taken after 12 dosages of Fludelone and 5 dosages of Velcade at day 59. In comparison with the images on day 40, two of three mice had significantly decreased tumor burden in both femurs and invertebrate column.

Figure 42 is a Kaplan-Meier survival curve of seven xenografted NOD/SCID-mice with CAG myeloma cells. Control mice died within 40 days after transplanting
15 CAG cells, and mice treated with dEpoB died within 50 days. Except for one mouse which died in 70 days, all mice treated with Fludelone survived beyond 80 days.

Figure 43 shows the pathways of cell apoptosis.

Figure 44 shows the epothilone-induced time-dependent processing of caspase
20 3 in CAG myeloma cells, showing an increased 17 kd cleaved form of caspase 3 with drug treatment time.

Figure 45 shows immunohistochemical staining of CAG myeloma cells using cleaved caspase-3 antibody, showing cytoplasmic and perinuclear localization in apoptotic cells (low and high magnifications are shown).

Figure 46 shows Immunohistochemical staining of CAG myeloma cells using
25 cleaved caspase-3 antibody, showing cytoplasmic and perinuclear localization in apoptotic cells (low and high magnifications are shown).

Figure 47 indicates that caspase 8 activity was increased after epothilone treatment, and that this increase can be inhibited by caspase 8 specific inhibitors.

Figure 48 indicates that caspase 8 activity was increased after epothilone
30 treatment, and that this increase can be inhibited by caspase 8 specific inhibitors.

Figure 49 indicates that caspase 9 activity was increased after epothilone treatment.

Figure 50 shows Annexin V staining of CB CD34+ cells incubated with dEpoB or Fludelone in the absence or presence of KL for 24 hours. Then drugs were washed out and annexin V staining was performed. Solid area represents vehicle control and open area represents drug treated group. There is no obvious increased apoptosis of non-cycling human CD34+ cells with short period of exposure to epothilones.

Figure 51 shows the influence of epothilones on non-cycling human CD34+ cells in colony formation. There is no significant difference of progenitor cells evaluated by 2 weeks colony formation between controls and drug treatment.

Figure 52 shows the influence of epothilones on non-cycling human CD34+ cells in colony formation. There is no significant difference of progenitor cells evaluated by 2 weeks colony formation between controls and drugs treatment.

Figure 53 shows the therapeutic effects against the drug resistant human T-cell lymphoblastic leukemia CCRF-CEM/Paclitaxel xenograft by Fludelone and Paclitaxel (body weight loss). There was no toxicity death for all treatments despite marked body weight decreases.

DEFINITIONS

Certain compounds of the present invention, and definitions of specific functional groups are also described in more detail below. For purposes of this invention, the chemical elements are identified in accordance with the Periodic Table of the Elements, CAS version, Handbook of Chemistry and Physics, 75th Ed., inside cover, and specific functional groups are generally defined as described therein. Additionally, general principles of organic chemistry, as well as specific functional moieties and reactivity, are described in "Organic Chemistry", Thomas Sorrell, University Science Books, Sausalito: 1999, the entire contents of which are incorporated herein by reference. Furthermore, it will be appreciated by one of ordinary skill in the art that the synthetic methods, as described herein, utilize a variety of protecting groups. By the term "protecting group", as used herein, it is meant that a particular functional moiety, *e.g.*, O, S, or N, is temporarily blocked so that a reaction can be carried out selectively at another reactive site in a multifunctional compound. In preferred embodiments, a protecting group reacts selectively in good yield to give a

protected substrate that is stable to the projected reactions; the protecting group should be selectively removable in good yield by readily available, preferably non-toxic reagents that do not attack the other functional groups; the protecting group forms an easily separable derivative (more preferably without the generation of new stereogenic centers); and the protecting group has a minimum of additional functionality to avoid further sites of reaction. As detailed herein, oxygen, sulfur, nitrogen, and carbon protecting groups may be utilized. Exemplary protecting groups are detailed herein, however, it will be appreciated that the present invention is not intended to be limited to these protecting groups; rather, a variety of additional equivalent protecting groups can be readily identified using the above criteria and utilized in the method of the present invention. Additionally, a variety of protecting groups are described in "Protective Groups in Organic Synthesis" Third Ed. Greene, T.W. and Wuts, P.G., Eds., John Wiley & Sons, New York: 1999, the entire contents of which are hereby incorporated by reference.

It will be appreciated that the compounds, as described herein, may be substituted with any number of substituents or functional moieties. In general, the term "substituted" whether preceded by the term "optionally" or not, and substituents contained in formulas of this invention, refer to the replacement of hydrogen radicals in a given structure with the radical of a specified substituent. When more than one position in any given structure may be substituted with more than one substituent selected from a specified group, the substituent may be either the same or different at every position. As used herein, the term "substituted" is contemplated to include all permissible substituents of organic compounds. In a broad aspect, the permissible substituents include acyclic and cyclic, branched and unbranched, carbocyclic and heterocyclic, aromatic and nonaromatic substituents of organic compounds. For purposes of this invention, heteroatoms such as nitrogen may have hydrogen substituents and/or any permissible substituents of organic compounds described herein which satisfy the valencies of the heteroatoms. Furthermore, this invention is not intended to be limited in any manner by the permissible substituents of organic compounds. Combinations of substituents and variables envisioned by this invention are preferably those that result in the formation of stable compounds useful in the treatment, for example of proliferative disorders, including, but not limited to cancer.

The term “stable”, as used herein, preferably refers to compounds which possess stability sufficient to allow manufacture and which maintain the integrity of the compound for a sufficient period of time to be detected and preferably for a sufficient period of time to be useful for the purposes detailed herein.

5 The term “aliphatic”, as used herein, includes both saturated and unsaturated, straight chain (*i.e.*, unbranched), branched, cyclic, or polycyclic aliphatic hydrocarbons, which are optionally substituted with one or more functional groups. As will be appreciated by one of ordinary skill in the art, “aliphatic” is intended herein to include, but is not limited to, alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, and cycloalkynyl
10 moieties. Thus, as used herein, the term “alkyl” includes straight, branched and cyclic alkyl groups. An analogous convention applies to other generic terms such as “alkenyl”, “alkynyl”, and the like. Furthermore, as used herein, the terms “alkyl”, “alkenyl”, “alkynyl”, and the like encompass both substituted and unsubstituted groups. In certain embodiments, as used herein, “lower alkyl” is used to indicate those alkyl
15 groups (cyclic, acyclic, substituted, unsubstituted, branched or unbranched) having 1-6 carbon atoms.

 In certain embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-20 aliphatic carbon atoms. In certain other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-10 aliphatic carbon
20 atoms. In yet other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-8 aliphatic carbon atoms. In still other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-6 aliphatic carbon atoms. In yet other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-4 carbon atoms. Illustrative aliphatic groups thus include, but
25 are not limited to, for example, methyl, ethyl, n-propyl, isopropyl, cyclopropyl, -CH₂-cyclopropyl, allyl, n-butyl, sec-butyl, isobutyl, tert-butyl, cyclobutyl, -CH₂-cyclobutyl, n-pentyl, sec-pentyl, isopentyl, tert-pentyl, cyclopentyl, -CH₂-cyclopentyl, n-hexyl, sec-hexyl, cyclohexyl, -CH₂-cyclohexyl moieties and the like, which again, may bear one or more substituents. Alkenyl groups include, but are not limited to, for example,
30 ethenyl, propenyl, butenyl, 1-methyl-2-buten-1-yl, and the like. Representative alkynyl groups include, but are not limited to, ethynyl, 2-propynyl (propargyl), 1-propynyl, and the like.

The term "alkoxy", or "thioalkyl" as used herein refers to an alkyl group, as previously defined, attached to the parent molecular moiety through an oxygen atom or through a sulfur atom. In certain embodiments, the alkyl group contains 1-20 aliphatic carbon atoms. In certain other embodiments, the alkyl group contains 1-10 aliphatic carbon atoms. In yet other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-8 aliphatic carbon atoms. In still other embodiments, the alkyl group contains 1-6 aliphatic carbon atoms. In yet other embodiments, the alkyl group contains 1-4 aliphatic carbon atoms. Examples of alkoxy, include but are not limited to, methoxy, ethoxy, propoxy, isopropoxy, n-butoxy, tert-butoxy, neopentoxy, and n-hexoxy. Examples of thioalkyl include, but are not limited to, methylthio, ethylthio, propylthio, isopropylthio, n-butylthio, and the like.

The term "alkylamino" refers to a group having the structure -NHR' wherein R' is alkyl, as defined herein. In certain embodiments, the alkyl group contains 1-20 aliphatic carbon atoms. In certain other embodiments, the alkyl group contains 1-10 aliphatic carbon atoms. In yet other embodiments, the alkyl, alkenyl, and alkynyl groups employed in the invention contain 1-8 aliphatic carbon atoms. In still other embodiments, the alkyl group contains 1-6 aliphatic carbon atoms. In yet other embodiments, the alkyl group contains 1-4 aliphatic carbon atoms. Examples of alkylamino include, but are not limited to, methylamino, ethylamino, iso-propylamino and the like.

Some examples of substituents of the above-described aliphatic (and other) moieties of compounds of the invention include, but are not limited to aliphatic; heteroaliphatic; aryl; heteroaryl; arylalkyl; heteroarylalkyl; alkoxy; aryloxy; heteroalkoxy; heteroaryloxy; alkylthio; arylthio; heteroalkylthio; heteroarylthio; F; Cl; Br; I; -OH; -NO₂; -CN; -CF₃; -CH₂CF₃; -CHCl₂; -CH₂OH; -CH₂CH₂OH; -CH₂NH₂; -CH₂SO₂CH₃; -C(O)R_x; -CO₂(R_x); -CON(R_x)₂; -OC(O)R_x; -OCO₂R_x; -OCON(R_x)₂; -N(R_x)₂; -S(O)₂R_x; -NR_x(CO)R_x wherein each occurrence of R_x independently includes, but is not limited to, aliphatic, heteroaliphatic, aryl, heteroaryl, arylalkyl, or heteroarylalkyl, wherein any of the aliphatic, heteroaliphatic, arylalkyl, or heteroarylalkyl substituents described above and herein may be substituted or unsubstituted, branched or unbranched, cyclic or acyclic, and wherein any of the aryl or heteroaryl substituents described above and herein may be substituted or unsubstituted.

Additional examples of generally applicable substituents are illustrated by the specific embodiments shown in the Examples that are described herein.

In general, the terms "aryl" and "heteroaryl", as used herein, refer to stable mono- or polycyclic, heterocyclic, polycyclic, and polyheterocyclic unsaturated moieties having preferably 3-14 carbon atoms, each of which may be substituted or unsubstituted. Substituents include, but are not limited to, any of the previously mentioned substituents, *i.e.*, the substituents recited for aliphatic moieties, or for other moieties as disclosed herein, resulting in the formation of a stable compound. In certain embodiments of the present invention, "aryl" refers to a mono- or bicyclic carbocyclic ring system having one or two aromatic rings including, but not limited to, phenyl, naphthyl, tetrahydronaphthyl, indanyl, indenyl, and the like. In certain embodiments of the present invention, the term "heteroaryl", as used herein, refers to a cyclic aromatic radical having from five to ten ring atoms of which one ring atom is selected from S, O, and N; zero, one, or two ring atoms are additional heteroatoms independently selected from S, O, and N; and the remaining ring atoms are carbon, the radical being joined to the rest of the molecule via any of the ring atoms, such as, for example, pyridyl, pyrazinyl, pyrimidinyl, pyrrolyl, pyrazolyl, imidazolyl, thiazolyl, oxazolyl, isooxazolyl, thiadiazolyl, oxadiazolyl, thiophenyl, furanyl, quinolinyl, isoquinolinyl, and the like.

It will be appreciated that aryl and heteroaryl groups (including bicyclic aryl groups) can be unsubstituted or substituted, wherein substitution includes replacement of one, two, three, or more of the hydrogen atoms thereon independently with any one or more of the following moieties including, but not limited to: aliphatic; heteroaliphatic; aryl; heteroaryl; arylalkyl; heteroarylalkyl; alkoxy; aryloxy; heteroalkoxy; heteroaryloxy; alkylthio; arylthio; heteroalkylthio; heteroarylthio; F; Cl; Br; I; -OH; -NO₂; -CN; -CF₃; -CH₂CF₃; -CHCl₂; -CH₂OH; -CH₂CH₂OH; -CH₂NH₂; -CH₂SO₂CH₃; -C(O)R_x; -CO₂(R_x); -CON(R_x)₂; -OC(O)R_x; -OCO₂R_x; -OCON(R_x)₂; -N(R_x)₂; -S(O)₂R_x; -NR_x(CO)R_x wherein each occurrence of R_x independently includes, but is not limited to, aliphatic, heteroaliphatic, aryl, heteroaryl, arylalkyl, or heteroarylalkyl, wherein any of the aliphatic, heteroaliphatic, arylalkyl, or heteroarylalkyl substituents described above and herein may be substituted or unsubstituted, branched or unbranched, cyclic or acyclic, and wherein any of the aryl or

heteroaryl substituents described above and herein may be substituted or unsubstituted. Additional examples of generally applicable substituents are illustrated by the specific embodiments shown in the Examples that are described herein.

The term "cycloalkyl", as used herein, refers specifically to groups having three
 5 to seven, preferably three to ten carbon atoms. Suitable cycloalkyls include, but are not limited to cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl and the like, which, as in the case of other aliphatic, heteroaliphatic or heterocyclic moieties, may optionally be substituted with substituents including, but not limited to aliphatic; heteroaliphatic; aryl; heteroaryl; arylalkyl; heteroarylalkyl; alkoxy; aryloxy; heteroalkoxy; heteroaryloxy; alkylthio; arylthio; heteroalkylthio; heteroarylthio; F; Cl; Br; I; -OH; -NO₂; -CN; -CF₃; -CH₂CF₃; -CHCl₂; -CH₂OH; -CH₂CH₂OH; -CH₂NH₂; -CH₂SO₂CH₃; -C(O)R_x; -CO₂(R_x); -CON(R_x)₂; -OC(O)R_x; -OCO₂R_x; -OCON(R_x)₂; -N(R_x)₂; -S(O)₂R_x; -NR_x(CO)R_x wherein each occurrence of R_x independently includes, but is not limited to, aliphatic, heteroaliphatic, aryl, heteroaryl, arylalkyl, or
 15 heteroarylalkyl, wherein any of the aliphatic, heteroaliphatic, arylalkyl, or heteroarylalkyl substituents described above and herein may be substituted or unsubstituted, branched or unbranched, cyclic or acyclic, and wherein any of the aryl or heteroaryl substituents described above and herein may be substituted or unsubstituted. Additional examples of generally applicable substituents are illustrated by the specific
 20 embodiments shown in the Examples that are described herein.

The term "heteroaliphatic", as used herein, refers to aliphatic moieties that contain one or more oxygen, sulfur, nitrogen, phosphorus or silicon atoms, *e.g.*, in place of carbon atoms. Heteroaliphatic moieties may be branched, unbranched, cyclic or acyclic and include saturated and unsaturated heterocycles such as morpholino,
 25 pyrrolidinyl, *etc.* In certain embodiments, heteroaliphatic moieties are substituted by independent replacement of one or more of the hydrogen atoms thereon with one or more moieties including, but not limited to aliphatic; heteroaliphatic; aryl; heteroaryl; arylalkyl; heteroarylalkyl; alkoxy; aryloxy; heteroalkoxy; heteroaryloxy; alkylthio; arylthio; heteroalkylthio; heteroarylthio; F; Cl; Br; I; -OH; -NO₂; -CN; -CF₃; -CH₂CF₃; -CHCl₂; -CH₂OH; -CH₂CH₂OH; -CH₂NH₂; -CH₂SO₂CH₃; -C(O)R_x; -CO₂(R_x); -CON(R_x)₂; -OC(O)R_x; -OCO₂R_x; -OCON(R_x)₂; -N(R_x)₂; -S(O)₂R_x; -NR_x(CO)R_x wherein each occurrence of R_x independently includes, but is not limited to, aliphatic,
 30

heteroaliphatic, aryl, heteroaryl, arylalkyl, or heteroarylalkyl, wherein any of the aliphatic, heteroaliphatic, arylalkyl, or heteroarylalkyl substituents described above and herein may be substituted or unsubstituted, branched or unbranched, cyclic or acyclic, and wherein any of the aryl or heteroaryl substituents described above and herein may be substituted or unsubstituted. Additional examples of generally applicable substituents are illustrated by the specific embodiments shown in the Examples that are described herein.

The terms "halo" and "halogen" as used herein refer to an atom selected from fluorine, chlorine, bromine and iodine.

The term "haloalkyl" denotes an alkyl group, as defined above, having one, two, or three halogen atoms attached thereto and is exemplified by such groups as chloromethyl, bromoethyl, trifluoromethyl, and the like.

The term "heterocycloalkyl" or "heterocycle", as used herein, refers to a non-aromatic 5-, 6-, or 7- membered ring or a polycyclic group, including, but not limited to a bi- or tri-cyclic group comprising fused six-membered rings having between one and three heteroatoms independently selected from oxygen, sulfur and nitrogen, wherein (i) each 5-membered ring has 0 to 1 double bonds and each 6-membered ring has 0 to 2 double bonds, (ii) the nitrogen and sulfur heteroatoms may be optionally be oxidized, (iii) the nitrogen heteroatom may optionally be quaternized, and (iv) any of the above heterocyclic rings may be fused to a benzene ring. Representative heterocycles include, but are not limited to, pyrrolidinyl, pyrazolinyl, pyrazolidinyl, imidazolinyl, imidazolidinyl, piperidinyl, piperazinyl, oxazolidinyl, isoxazolidinyl, morpholinyl, thiazolidinyl, isothiazolidinyl, and tetrahydrofuryl. In certain embodiments, a "substituted heterocycloalkyl or heterocycle" group is utilized and as used herein, refers to a heterocycloalkyl or heterocycle group, as defined above, substituted by the independent replacement of one, two or three of the hydrogen atoms thereon with but are not limited to aliphatic; heteroaliphatic; aryl; heteroaryl; arylalkyl; heteroarylalkyl; alkoxy; aryloxy; heteroalkoxy; heteroaryloxy; alkylthio; arylthio; heteroalkylthio; heteroarylthio; F; Cl; Br; I; -OH; -NO₂; -CN; -CF₃; -CH₂CF₃; -CHCl₂; -CH₂OH; -CH₂CH₂OH; -CH₂NH₂; -CH₂SO₂CH₃; -C(O)R_x; -CO₂(R_x); -CON(R_x)₂; -OC(O)R_x; -OCO₂R_x; -OCON(R_x)₂; -N(R_x)₂; -S(O)₂R_x; -NR_x(CO)R_x wherein each occurrence of R_x independently includes, but is not limited to, aliphatic, heteroaliphatic, aryl, heteroaryl,

arylalkyl, or heteroarylalkyl, wherein any of the aliphatic, heteroaliphatic, arylalkyl, or heteroarylalkyl substituents described above and herein may be substituted or unsubstituted, branched or unbranched, cyclic or acyclic, and wherein any of the aryl or heteroaryl substituents described above and herein may be substituted or unsubstituted.

5 Additional examples of generally applicable substituents are illustrated by the specific embodiments shown in the Examples which are described herein.

“Labeled”: As used herein, the term “labeled” is intended to mean that a compound has at least one element, isotope or chemical compound attached to enable the detection of the compound. In general, labels typically fall into three classes: a) isotopic labels, which may be radioactive or heavy isotopes, including, but not limited to, ^2H , ^3H , ^{32}P , ^{35}S , ^{67}Ga , $^{99\text{m}}\text{Tc}$ (Tc-99m), ^{111}In , ^{123}I , ^{125}I , ^{169}Yb , and ^{186}Re ; b) immune labels, which may be antibodies or antigens, which may be bound to enzymes (such as horseradish peroxidase) that produce detectable agents; and c) colored, luminescent, phosphorescent, or fluorescent dyes. It will be appreciated that the labels may be incorporated into the compound at any position that does not interfere with the biological activity or characteristic of the compound that is being detected. In certain embodiments of the invention, photoaffinity labeling is utilized for the direct elucidation of intermolecular interactions in biological systems (*e.g.*, to probe the epothilone binding site in a tubulin dimer). A variety of known photophores can be employed, most relying on photoconversion of diazo compounds, azides, or diazirines to nitrenes or carbenes (See, Bayley, H., *Photogenerated Reagents in Biochemistry and Molecular Biology* (1983), Elsevier, Amsterdam.), the entire contents of which are hereby incorporated by reference. In certain embodiments of the invention, the photoaffinity labels employed are o-, m- and p-azidobenzoyls, substituted with one or more halogen moieties, including, but not limited to 4-azido-2,3,5,6-tetrafluorobenzoic acid.

“Polymer”: The term “polymer”, as used herein, refers to a composition comprising chains that may be open, closed, linear, branched or cross-linked of repeating units (monomers) that may be the same or different. It will be appreciated that in certain embodiments the term polymer refers to biopolymers, which, as used herein, is intended to refer to polymeric materials found in nature or based upon those materials found in nature, including, but not limited to nucleic acids, peptides, and

mimetics thereof. In certain other embodiments, the term polymer refers to synthetic polymers, such as biodegradable polymers or other polymeric materials. It will be appreciated that polymeric solid supports are also encompassed by the polymers of the present invention. Inventive compounds can be attached to polymeric supports and thus certain synthetic modifications can be conducted on the solid phase. As used herein, the term "solid support" is meant to include, but is not limited to, pellets, disks, capillaries, hollow fibers, needles, pins, solid fibers, cellulose beads, pore-glass beads, silica gels, polystyrene beads optionally cross-linked with divinylbenzene, grafted copoly beads, poly-acrylamide beads, latex beads, dimethylacrylamide beads optionally crosslinked with N-N'-bis-acryloylethylenediamine, and glass particles coated with a hydrophobic polymer. One of ordinary skill in the art will realize that the choice of particular solid support will be limited by the compatibility of the support with the reaction chemistry being utilized. An exemplary solid support is a Tentagel amino resin, a composite of 1) a polystyrene bead crosslinked with divinylbenzene and 2) PEG (polyethylene glycol). Tentagel is a particularly useful solid support because it provides a versatile support for use in on-bead or off-bead assays, and it also undergoes excellent swelling in solvents ranging from toluene to water.

DESCRIPTION OF CERTAIN EMBODIMENTS OF THE INVENTION

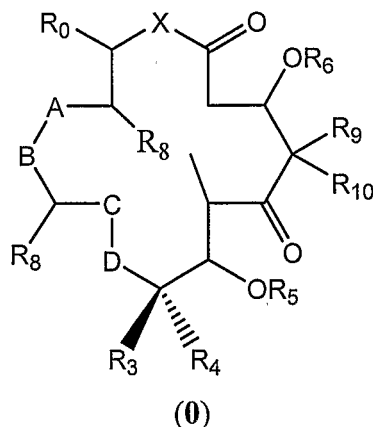
In recognition of the need to develop novel and effective cancer therapies, the present invention provides novel synthetic methodologies enabling access to macrocycles having a broad range of biological and pharmacological activity, as well as novel compounds with such activity, novel therapeutic compositions, and methods of using these compounds and compositions.

In certain embodiments, the inventive compounds are useful in the treatment of cancer. Certain compounds of the invention exhibit cytotoxic or growth inhibitory effects on cancer cells lines, exhibit an ability to polymerize tubulin and stabilize microtubule assemblies, and/or lead to shrinkage or disappearance of tumors in cancer cell xenograft models. In certain embodiments, the compounds may have reduced or minimal side effects including toxicity to vital organs, nausea, vomiting, diarrhea, alopecia, weight loss, weight gain, liver toxicity, skin disorders, *etc.* The compounds

may also be easier to formulate due to increased water solubility, decreased toxicity, increased therapeutic range, increased efficacy, *etc.*

General Description of Compounds of the Invention

- 5 Compounds of the invention include compounds of the general formula (0) as further defined below:



- wherein R_0 is a substituted or unsubstituted aryl, heteroaryl, arylalkyl, arylalkenyl, arylalkynyl, heteroarylalkyl, heteroarylalkenyl, or heteroarylalkynyl moiety; in certain embodiments, R_0 is a arylalkyl, arylalkenyl, heteroarylalkyl, or heteroarylalkenyl moiety; in other embodiments, R_0 is a heteroarylalkenyl moiety; in certain embodiments, R_0 is a heteroarylalkyl moiety; in other embodiments, R_0 is a 5-7 membered aryl or heteroaryl moiety; in yet other embodiments, R_0 is an 8-12 membered bicyclic aryl or heteroaryl moiety; in still other embodiments, R_0 is a bicyclic moiety wherein a phenyl ring is fused to a heteroaryl or aryl moiety; in other embodiments, R_0 is a bicyclic moiety wherein a phenyl ring is fused to a thiazole, oxazole, or imidazole moiety; in yet other embodiments, R_0 is a substituted or unsubstituted phenyl moiety;
- 20 R_3 and R_4 are each independently hydrogen; or substituted or unsubstituted, linear or branched, cyclic or acyclic aliphatic, heteroaliphatic, aryl, heteroaryl, arylalkyl, or heteroarylalkyl moiety, optionally substituted by one or more of hydroxy, protected hydroxy, alkoxy, carboxy, carboxaldehyde, linear or branched alkyl or cyclic acetal, fluorine, amino, protected amino, amino substituted with one or two alkyl or aryl moieties, N-hydroximino, or N-alkoxyimino; in certain embodiments, R_3 and R_4 are each independently hydrogen, fluorine, or lower alkyl; in other embodiments, R_3
- 25

and R_4 are each independently hydrogen or methyl; in still other embodiments, R_3 is methyl, and R_4 is hydrogen;

R_5 and R_6 are each independently hydrogen or a protecting group; in certain embodiments, R_5 and R_6 are both hydrogen;

5 X is O, S, $C(R_7)_2$, or NR_7 , wherein each occurrence of R_7 is independently hydrogen or lower alkyl; in certain embodiments, X is O; in other embodiments, X is NH;

10 Y is O, S, NH, $C(R_7)_2$, CH_2 , $N(R_7)$, or NH, wherein each occurrence of R_7 is independently hydrogen or lower alkyl; in certain embodiments, Y is O; in other embodiments, Y is NH; in yet other embodiments, Y is CH_2 ;

15 each R_8 is independently hydrogen; halogen, hydroxy, alkoxy, amino, dialkylamino, alkylamino, fluoro, cyano, or substituted or unsubstituted, linear or branched, cyclic or acyclic aliphatic, heteroaliphatic, aryl, heteroaryl, arylalkyl, arylalkenyl, arylalkynyl, or heteroarylalkyl, heteroarylalkenyl, heteroarylalkynyl moiety, optionally substituted by one or more of hydroxy, protected hydroxy, alkoxy, carboxy, carboxaldehyde, linear or branched alkyl or cyclic acetal, fluorine, amino, protected amino, amino substituted with one or two alkyl or aryl moieties, N-hydroximino, or N-alkoxyimino; in certain embodiments, R_8 is hydrogen; in other
20 embodiments, R_8 is hydroxy; in yet other embodiments, R_8 is fluorine; in still other embodiments, R_8 is lower alkyl such as methyl; in other embodiments R_8 is $-CF_3$, $-CF_2H$, or $-CFH_2$; in other embodiments, R_8 is perfluorinated or fluorinated alkyl group; in yet other embodiments, R_8 is halogenated or perhalogenated alkyl group;

25 R_9 and R_{10} are each independently hydrogen; or substituted or unsubstituted, linear or branched, cyclic or acyclic aliphatic, heteroaliphatic, aryl, heteroaryl, aryl, arylalkyl, arylalkenyl, arylalkynyl, heteroarylalkyl, heteroarylalkenyl, or heteroarylalkynyl moiety, optionally substituted by one or more of hydroxy, protected hydroxy, alkoxy, carboxy, carboxaldehyde, linear or branched alkyl or cyclic acetal, fluorine, amino, protected amino, amino substituted with one or two alkyl or aryl moieties, N-hydroximino, or N-alkoxyimino; in certain embodiments, one of R_9 and
30 R_{10} is methyl; in other embodiments, both R_9 and R_{10} are methyl; in yet other embodiments, one of R_9 and R_{10} is methyl, and the other is hydrogen; in other embodiments, both R_9 and R_{10} are hydrogen;

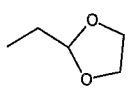
A-B represents $CR_A=CR_B$ -, $C(R_A)_2-C(R_B)_2$ -, or $-C\equiv C$ -;

C-D represents $-CR_C=CR_D$ -, $-C(R_C)_2-C(R_D)_2$ -, or $-C\equiv C$ -;

wherein each occurrence of R_A is independently hydrogen; halogen; -
 $OR_{A'}$; $-SR_{A'}$;

5 $-N(R_{A'})_2$; $-C(O)OR_{A'}$; $-C(O)R_{A'}$; $-CONHR_{A'}$; $-O(C=O)R_{A'}$; $-O(C=O)OR_{A'}$; $-$
 $NR_{A'}(C=O)R_{A'}$; N_3 ; $N_2R_{A'}$; cyclic acetal; or cyclic or acyclic, linear or branched
 aliphatic, heteroaliphatic, aryl, or heteroaryl, optionally substituted with one or
 more of hydrogen; halogen; $-OR_{A'}$; $-SR_{A'}$; $-N(R_{A'})_2$; $-C(O)OR_{A'}$; $-C(O)R_{A'}$; $-$
 $CONHR_{A'}$; $-O(C=O)R_{A'}$; $-O(C=O)OR_{A'}$; $-NR_{A'}(C=O)R_{A'}$; N_3 ; $N_2R_{A'}$; cyclic
 10 acetal; or cyclic or acyclic, linear or branched substituted or unsubstituted
 aliphatic, heteroaliphatic, aryl, or heteroaryl moiety;

R_B is, independently for each occurrence, hydrogen; halogen; $-OR_{B'}$; $-$
 $SR_{B'}$; $-N(R_{B'})_2$; $-C(O)OR_{B'}$; $-C(O)R_{B'}$; $-CONHR_{B'}$; $-O(C=O)R_{B'}$; $-$
 $O(C=O)OR_{B'}$; $-NR_{B'}(C=O)R_{B'}$; N_3 ; $N_2R_{B'}$; cyclic acetal; or cyclic or acyclic,
 15 linear or branched aliphatic, heteroaliphatic, aryl, or heteroaryl, optionally
 substituted with one or more of hydrogen; halogen; $-OR_{B'}$; $-SR_{B'}$; $-N(R_{B'})_2$; $-$
 $C(O)OR_{B'}$; $-C(O)R_{B'}$; $-CONHR_{B'}$; $-O(C=O)R_{B'}$; $-O(C=O)OR_{B'}$; $-$
 $NR_{B'}(C=O)R_{B'}$; N_3 ; $N_2R_{B'}$; cyclic acetal; or cyclic or acyclic, linear or branched
 substituted or unsubstituted aliphatic, heteroaliphatic, aryl, or heteroaryl moiety;

20 in certain embodiments, R_B is hydrogen, , methyl, ethyl, propyl, butyl,
 pentyl, hexyl, cyclopropyl, cyclobutyl, cyclopentyl, or cyclohexyl, each
 unsubstituted or optionally substituted with one or more occurrences of halogen,
 $-OH$, $-OR_{B'}$, NH_2 , or $N(R_{B'})_2$, or any combination thereof, wherein each
 occurrence of $R_{B'}$ is independently hydrogen, alkyl, aryl, or a protecting group,
 25 in other embodiments, R_B is hydrogen, methyl, or ethyl, in still other
 embodiments, R_B is methyl, in other embodiments, $-CY_3$, $-CHY_2$, $-CH_2Y$, where
 Y is F, Br, Cl, I, $OR_{B'}$, $NHR_{B'}$, $N(R_{B'})_2$, or $SR_{B'}$; in yet other embodiments, R_B is
 $-CF_3$, $-CH_2F$, or CHF_2 ; in other embodiments, R_B is perfluorinated or
 fluorinated alkyl group; in yet other embodiments, R_B is halogenated or
 30 perhalogenated alkyl group;

R_C is, independently for each occurrence, hydrogen; halogen; $-OR_C$; $-SR_C$;
 $-N(R_C)_2$; $-C(O)OR_C$; $-C(O)R_C$; $-CONHR_C$; $-O(C=O)R_C$; $-O(C=O)OR_C$;
 $-NR_C(C=O)R_C$; N_3 ; N_2R_C ; cyclic acetal; or cyclic or acyclic, linear or
 5 branched aliphatic, heteroaliphatic, aryl, or heteroaryl, optionally substituted
 with one or more of hydrogen; halogen; $-OR_C$; $-SR_C$; $-N(R_C)_2$; $-C(O)OR_C$; $-C(O)R_C$;
 $-CONHR_C$; $-O(C=O)R_C$; $-O(C=O)OR_C$; $-NR_C(C=O)R_C$; N_3 ; N_2R_C ;
 cyclic acetal; or cyclic or acyclic, linear or branched substituted or unsubstituted
 aliphatic, heteroaliphatic, aryl, or heteroaryl moiety; in certain embodiments, R_C
 10 is halogen, alkyl, hydroxy, or amino; in other embodiments, R_C is fluorine; in
 yet other embodiments, R_C is hydroxy;

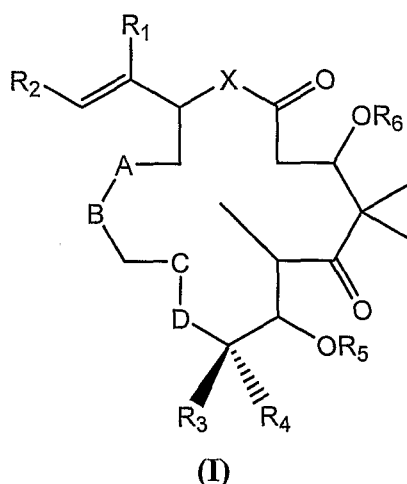
R_D is, independently for each occurrence, hydrogen; halogen; $-OR_D$; $-SR_D$;
 $-N(R_D)_2$; $-C(O)OR_D$; $-C(O)R_D$; $-CONHR_D$; $-O(C=O)R_D$; $-O(C=O)OR_D$; $-NR_D(C=O)R_D$;
 15 N_3 ; N_2R_D ; cyclic acetal; or cyclic or acyclic, linear or branched
 aliphatic, heteroaliphatic, aryl, or heteroaryl, optionally substituted with one or
 more of hydrogen; halogen; $-OR_D$; $-SR_D$; $-N(R_D)_2$; $-C(O)OR_D$; $-C(O)R_D$; $-CONHR_D$;
 $-O(C=O)R_D$; $-O(C=O)OR_D$; $-NR_D(C=O)R_D$; N_3 ; N_2R_D ; cyclic
 acetal; or cyclic or acyclic, linear or branched substituted or unsubstituted
 20 aliphatic, heteroaliphatic, aryl, or heteroaryl moiety; or

wherein any two of R_A , R_B , R_C or R_D taken together may form a cyclic
 moiety and may be linked through an oxygen, sulfur, carbon or nitrogen atom,
 or any two adjacent groups R_A , R_B , R_C , or R_D , taken together, may form a 3-6-
 membered substituted or unsubstituted aliphatic, heteroaliphatic, aryl or
 25 heteroaryl ring; in certain embodiments, R_A and R_B taken together form a 3-
 member ring linked through an oxygen, sulfur, carbon, or nitrogen atom; in
 other embodiments R_C and R_D taken together form a 3-membered ring linked
 through an oxygen, sulfur, carbon, or nitrogen atom;

wherein each occurrence of R_A , R_B , R_C and R_D is independently hydrogen; a
 30 protecting group; a linear or branched, substituted or unsubstituted, cyclic or acyclic,
 aliphatic, heteroaliphatic, aryl, heteroaryl, arylalkyl, arylalkenyl, arylalkynyl,
 arylalkenyl, arylalkynyl, or heteroarylalkyl, heteroarylalkenyl, heteroarylalkynyl

moiety; and pharmaceutically acceptable derivatives thereof; and pharmaceutically acceptable derivatives thereof.

The compounds of the invention include compounds of the general formula (I)
 5 as further defined below:

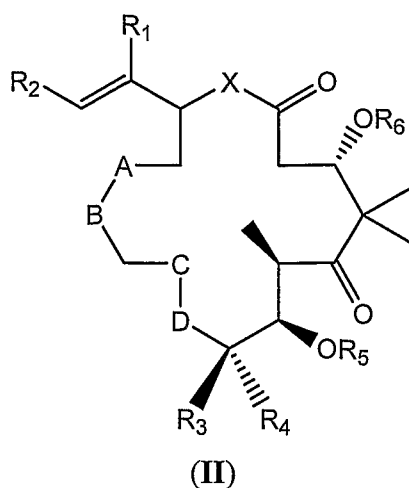


wherein R₁ is hydrogen or lower alkyl; in certain embodiments, R₁ is methyl; in certain embodiments, R₁ is -CF₃, -CF₂H, or CH₂F; in other embodiments, R₁ is
 10 perfluorinated or fluorinated alkyl group; in yet other embodiments, R₁ is halogenated or perhalogenated alkyl group;

R₂ is a substituted or unsubstituted aryl, heteroaryl, arylalkyl, or heteroarylalkyl moiety; in certain embodiments, R₂ is substituted or unsubstituted oxazole; in other embodiments, R₂ is substituted or unsubstituted thiazole; and

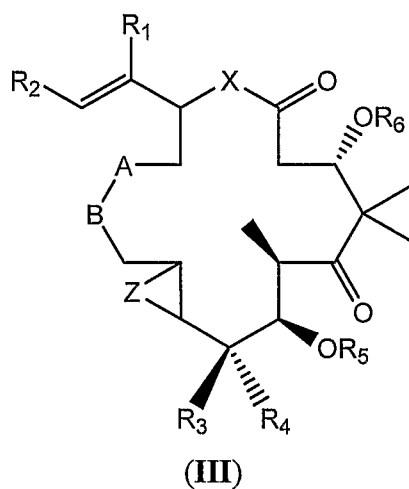
15 A, B, C, D, R₃, R₄, R₅, R₆, and X are as defined above.

In certain embodiments, the compounds of the invention include compounds of the general formula (II) with the stereochemistry defined as shown:



wherein A, B, C, D, R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , and X are as defined above.

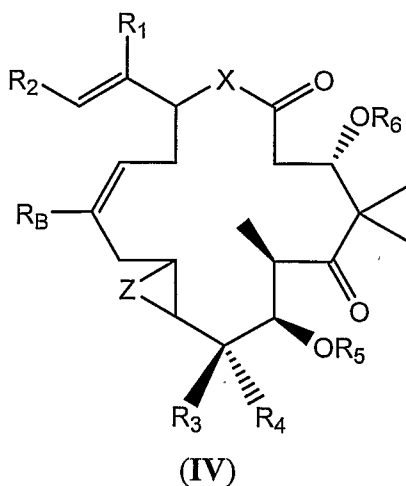
- 5 In certain embodiments, the compounds of the invention include compounds of the general formula (III) as shown:



- 10 wherein Z is an oxygen atom, a sulfur atom, $-NR_Z-$, or $-C(R_Z)_2-$; and A, B, R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , and X are as defined above. In certain preferred embodiments, Z is oxygen. In other embodiments, Z is $-NH-$. In yet other embodiments, Z is $-CH_2-$. In other embodiments, R_Z is hydrogen, alkyl, halogen, or acyl. In certain embodiments, R_Z is fluorine.

15

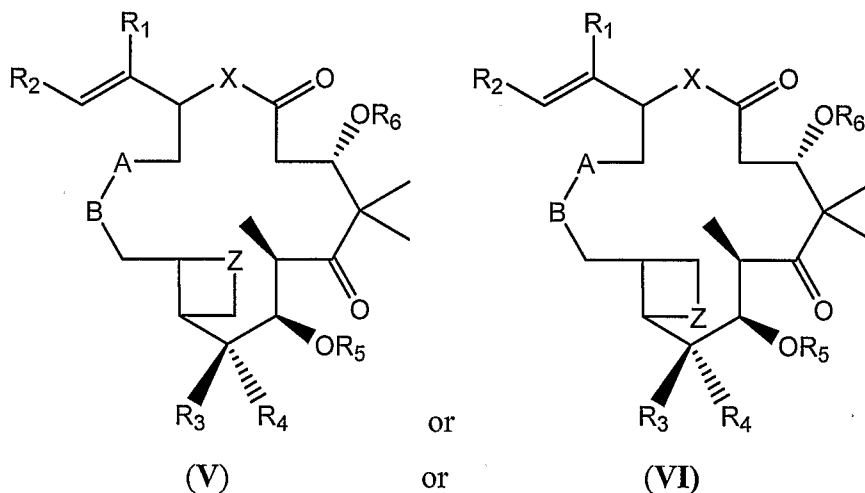
In certain embodiments, the compounds of the invention include compounds of the general formula (IV) with the stereochemistry defined as shown:



wherein A, B, R₁, R₂, R₃, R₄, R₅, R₆, X, and Z are as defined above. In certain embodiments, R_B is methyl. In other embodiments, R_B is -CF₃.

5

In certain embodiments, the compounds of the invention include compounds of the general formula (V) or (VI) as shown:

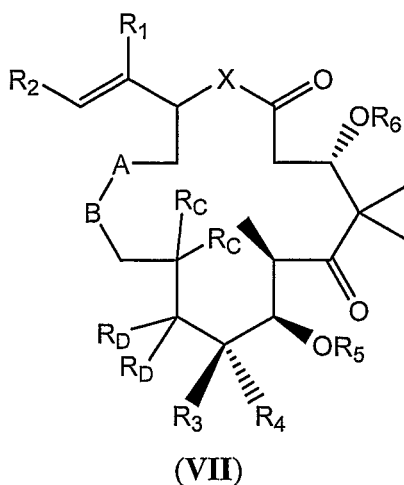


10

wherein Z is an oxygen atom, a sulfur atom, -NR_Z-, or -C(R_Z)₂-; and A, B, R₁, R₂, R₃, R₄, R₅, R₆, and X are as defined above. In certain preferred embodiments, Z is oxygen. In other embodiments, Z is -NH-. In yet other embodiments, Z is -CH₂-. In other embodiments, R_Z is hydrogen, alkyl, halogen, or acyl. In certain embodiments, R_Z is fluorine.

15

In certain embodiments, the compounds of the invention include compounds of the general formula (VII) as shown:



wherein A, B, R_C, R_D, R₁, R₂, R₃, R₄, R₅, R₆, and X are as defined above. In certain preferred embodiments, each R_C is independently hydrogen, halogen, or lower alkyl. In other embodiments, each R_D is independently hydrogen, halogen or lower alkyl.

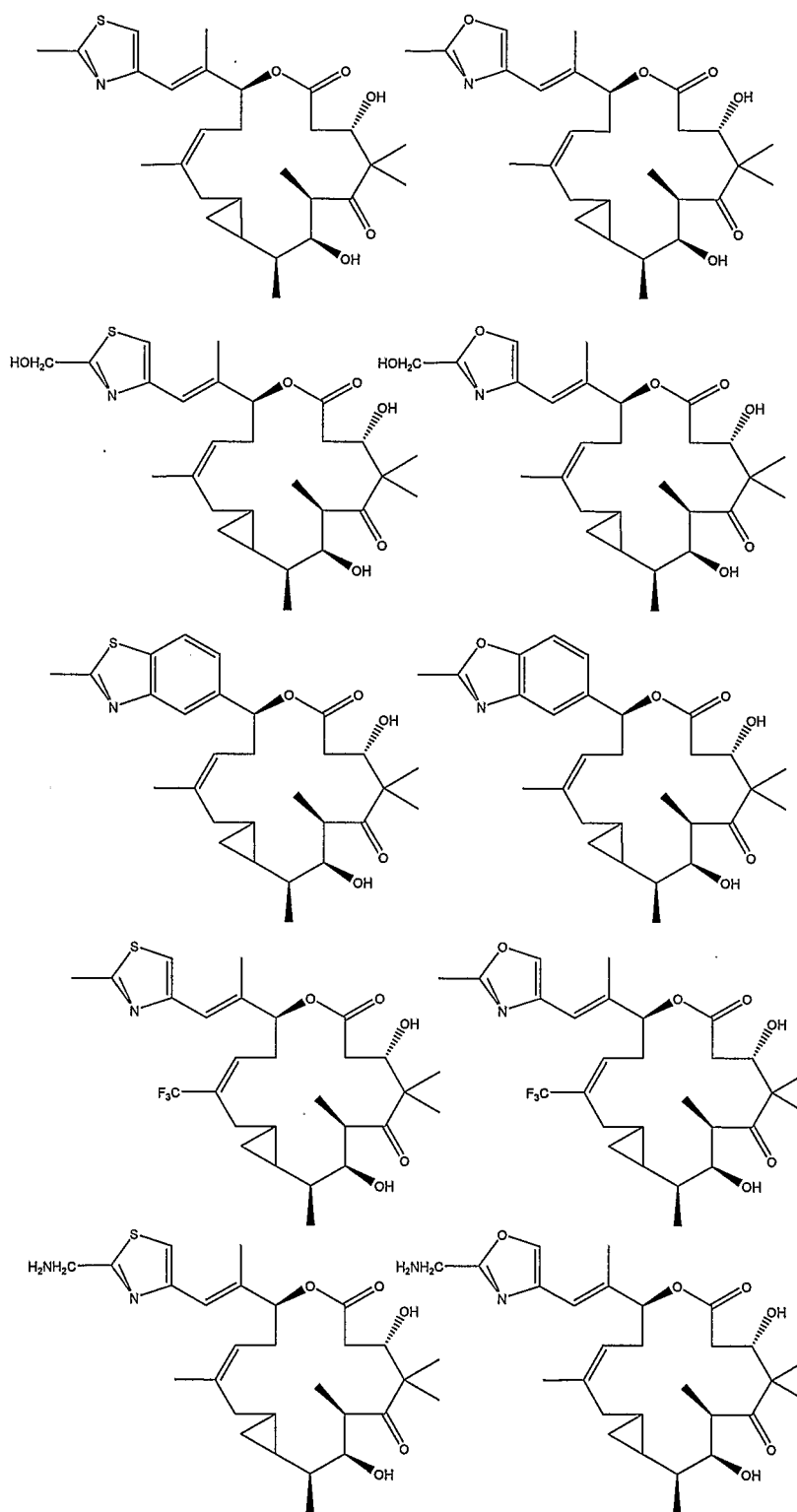
In certain embodiments, X is O. In other embodiments, X is NH. In other embodiments, X is CH₂.

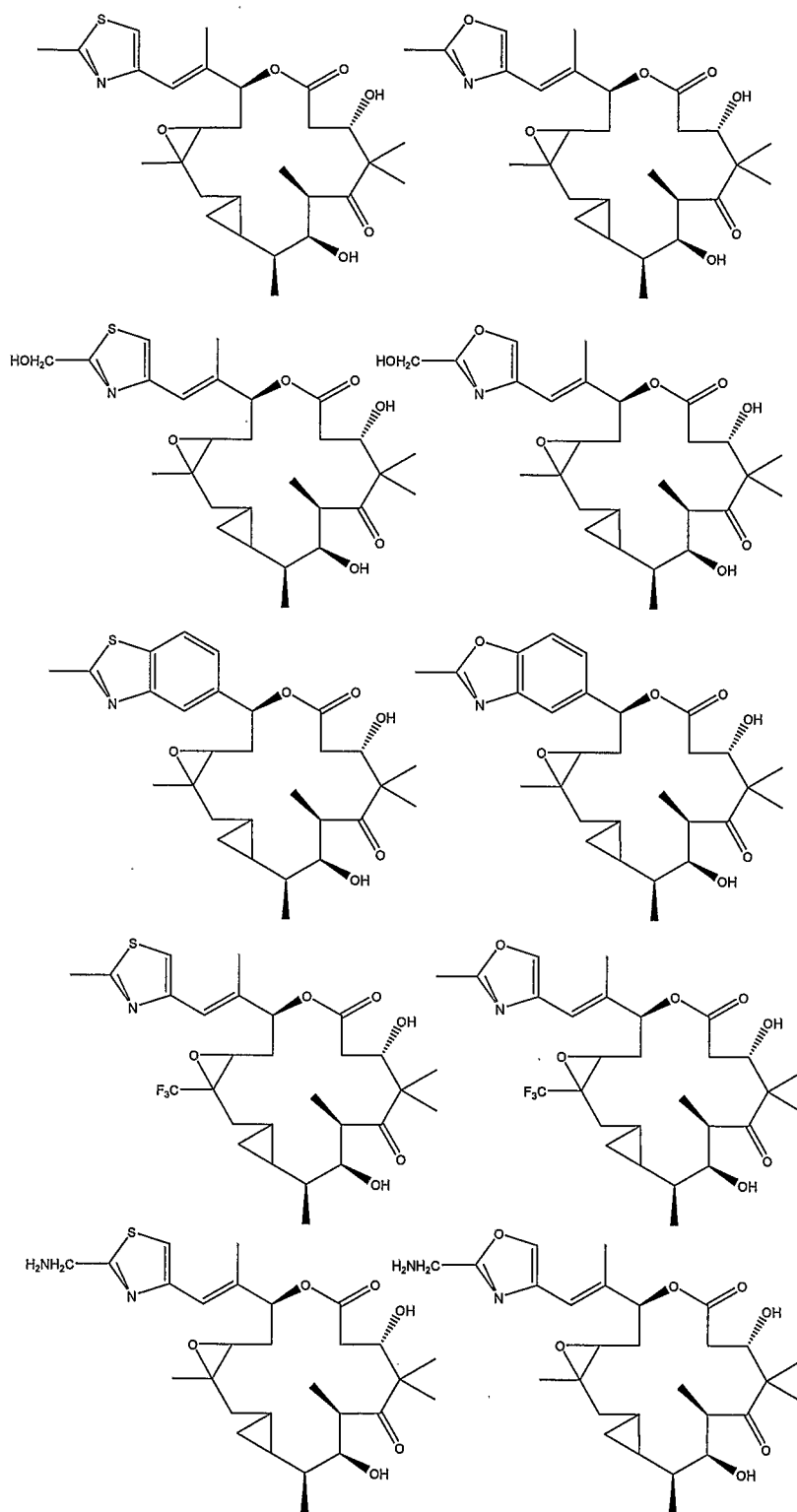
In some embodiments, R₂ is a substituted or unsubstituted thiazole. In certain embodiments, R₂ is 2-methyl-thiazo-4-yl. In other embodiments, R₂ is 2-hydroxymethyl-thiazo-4-yl. In yet other embodiments, R₂ is 2-aminomethyl-thiazo-4-yl. In other embodiments, R₂ is 2-thiolmethyl-thiazo-4-yl.

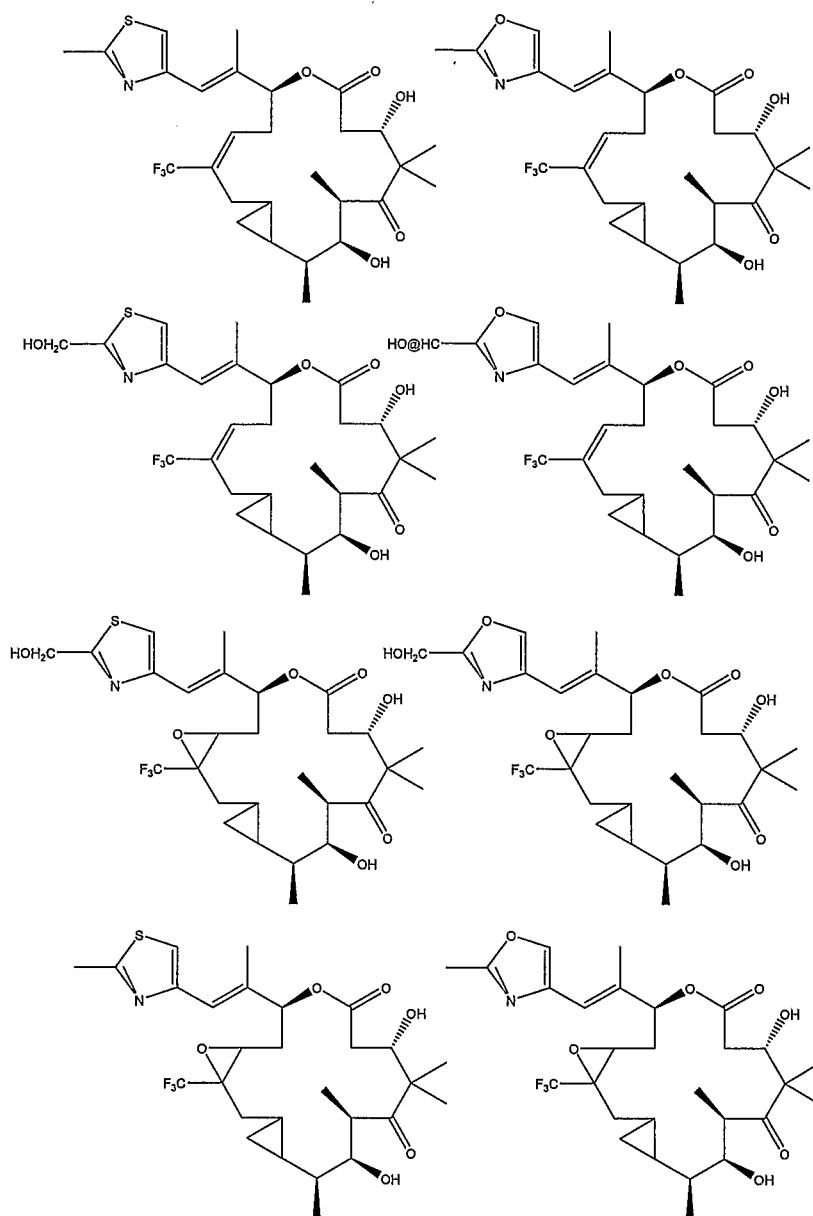
In certain embodiments R₂ is a substituted or unsubstituted oxazole. In certain embodiments, R₂ is 2-methyl-oxazo-4-yl. In other embodiments, R₂ is 2-hydroxymethyl-oxazo-4-yl. In yet other embodiments, R₂ is 2-aminomethyl-oxazo-4-yl. In other embodiments, R₂ is 2-thiolmethyl-oxazo-4-yl.

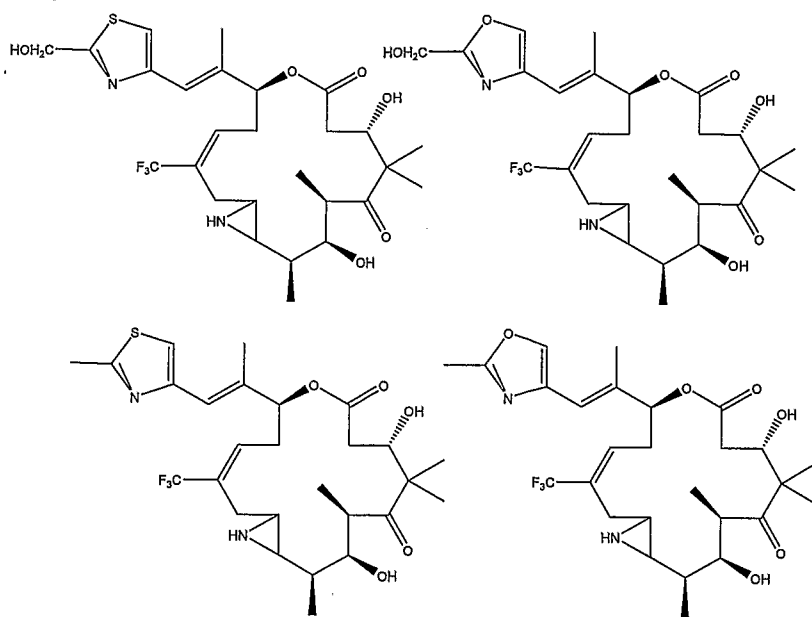
In certain embodiments, R_B is hydrogen, methyl, ethyl, -CF₃, -CH₂F, -CF₂H. In certain embodiments, R_B is methyl. In yet other embodiments, R_B is -CF₃. In certain embodiments, R_B is hydrogen. In other embodiments, R_B is ethyl.

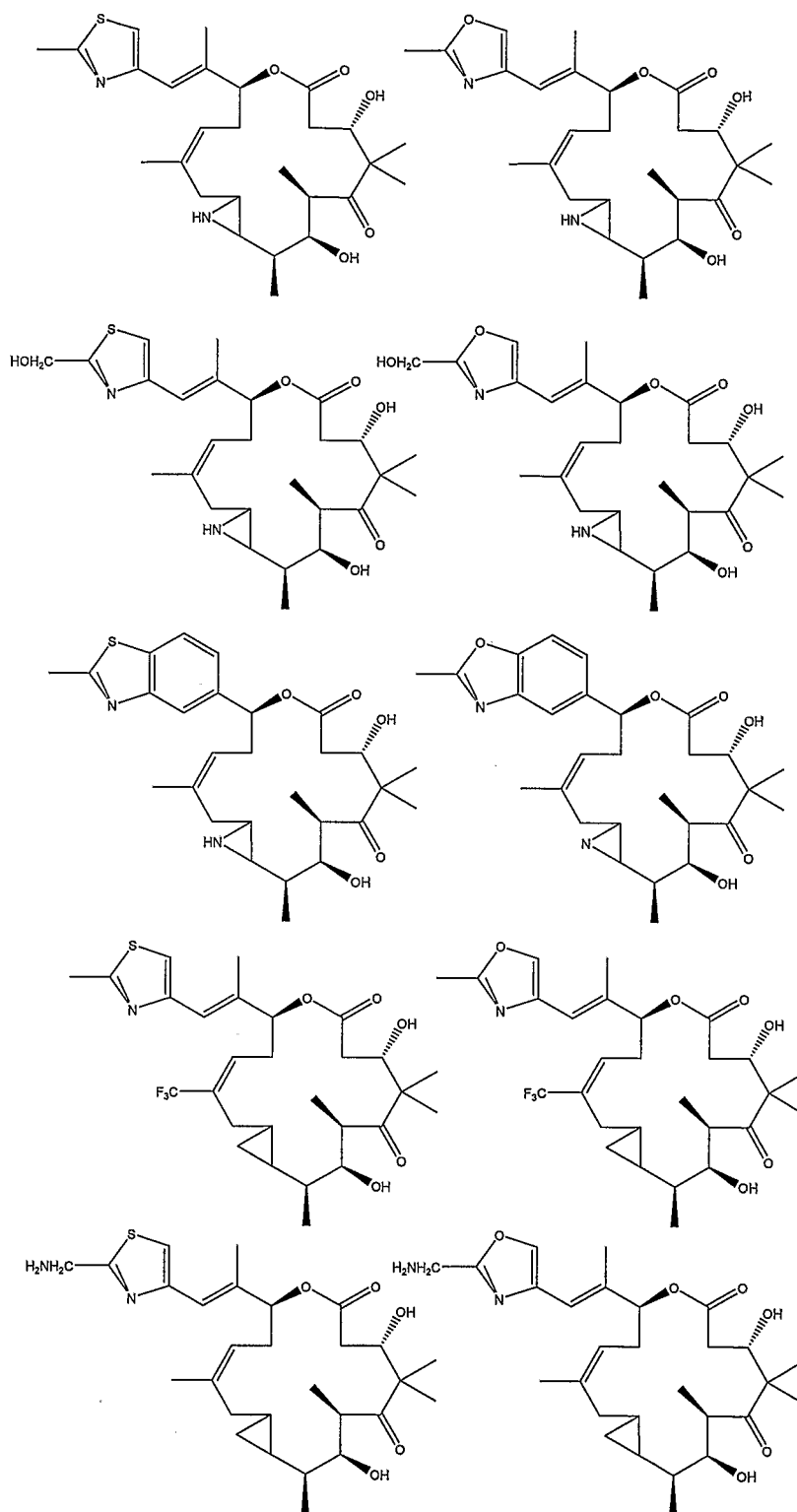
Certain preferred compounds include, for example:











Compounds of this invention include those specifically set forth above and described herein, and are illustrated in part by the various classes, subgenera, and species disclosed elsewhere herein. Without wishing to be bound by any particular

5 theory, certain compounds of the present invention have been modified at C9 and C10

to constrain the conformation of the molecule in much the same manner as a double carbon-carbon bond at the C9-C10 position would. Electronic effects, steric effects, hydrogen bonding, dipole effects, or a combination thereof may be used to create this conformational constraint. For example, cyclic ring systems such as oxiranes, cyclopropyl, and aziridine may be used to constrain the conformation of the molecule. In other embodiments 4, 5, or 6-membered rings are used to accomplish the same effect. The effect may also be accomplished by a conjugated p-orbital system such as that found in an ester, thioester, or amide. The effect may also be accomplished by an extended delocalized pi system such as those found in an aromatic ring system. In other embodiments, the steric effects of substituents around the C9 and C10 positions are used to constrain the conformation of the molecule in the same way the molecule is constrained by a C9-C10 carbon-carbon double bond.

It will be appreciated by one of ordinary skill in the art that asymmetric centers may exist in the compounds of the present invention. Thus, inventive compounds and pharmaceutical compositions thereof may be in the form of an individual enantiomer, diastereomer or geometric isomer, or may be in the form of a mixture of stereoisomers. In certain embodiments, the compounds of the invention are enantiopure compounds. In certain other embodiments, a mixtures of stereoisomers or diastereomers are provided.

It will be appreciated that some of the foregoing classes and subclasses of compounds can exist in various isomeric forms. The invention encompasses the compounds as individual isomers substantially free of other isomers and alternatively, as mixtures of various isomers, *e.g.*, racemic mixtures of stereoisomers. Additionally, the invention encompasses both (Z) and (E) double bond isomers unless otherwise specifically designated. Thus, compounds of the invention generally depicted in structures herein encompass those structures in which double bonds are (Z) or (E). In certain preferred embodiments, the double bond at the C12-C13 position is in the cis or Z configuration. In some embodiments, the double bond at the C9-C10 position is in the trans or E configuration. In still other embodiments, the double bond at the C12-C13 position is in the cis or Z configuration, and the double bond at the C9-C10 position is in the trans or E configuration. The invention also encompasses tautomers of specific compounds as described above.

- Additionally, the present invention provides pharmaceutically acceptable derivatives of the inventive compounds, and methods of treating a subject using these compounds, pharmaceutical compositions thereof, or either of these in combination with one or more additional therapeutic agents. The phrase, “pharmaceutically acceptable derivative”, as used herein, denotes any pharmaceutically acceptable salt, ester, or salt of such ester, of such compound, or any other adduct or derivative which, upon administration to a patient, is capable of providing (directly or indirectly) a compound as otherwise described herein, or a metabolite or residue thereof.
- Pharmaceutically acceptable derivatives thus include among others pro-drugs. A pro-drug is a derivative of a compound, usually with significantly reduced pharmacological activity, which contains an additional moiety that is susceptible to removal *in vivo* yielding the parent molecule as the pharmacologically active species. An example of a pro-drug is an ester that is cleaved *in vivo* to yield a compound of interest. Pro-drugs of a variety of compounds, and materials and methods for derivatizing the parent compounds to create the pro-drugs, are known and may be adapted to the present invention. Certain exemplary pharmaceutical compositions and pharmaceutically acceptable derivatives will be discussed in more detail herein below.
- Compounds of this invention which are of particular interest include those which:
- exhibit cytotoxic or growth inhibitory effect on cancer cell lines maintained *in vitro* or in animal studies using a scientifically acceptable cancer cell xenograft model;
 - exhibit an ability to polymerize tubulin and stabilize microtubule assemblies;
 - exhibit minimal levels of toxicity to vital organs;
 - lead to tumor disappearance in scientifically acceptable cancer cell xenograft models;
 - lead to tumor shrinkage in scientifically acceptable cancer cell xenograft models;
 - lead to tumor disappearance in scientifically acceptable cancer cell xenograft models and delayed/or no recurrence of the tumor after stopping treatment;

- exhibit transient and reversible body weight decreases and show therapeutic effects in scientifically acceptable cancer cell xenograft models;
- exhibit enhanced water solubility over epothilones A, B, C or D, or paclitaxel, or additionally or alternatively exhibit sufficient solubility to be formulated in an aqueous medium using reduced proportion of chremophor; and/or
- exhibit a therapeutic profile (*e.g.*, optimum safety and curative effect) that is superior to that of epothilone B, epothilone D, or paclitaxel.

A variety of epothilone analogs as described *supra* have been prepared, characterized, and tested as exemplified herein. 9,10-dehydro-epothilone analogs have been found to be useful in the treatment of cancer, and in particular compounds have been prepared and found to possess one or more of the desired characteristics listed above.

Synthetic Methodology

The synthesis of certain epothilones, desoxyepothilones and analogues thereof have been previously described (see, U.S. Patents 6,242,469, 6,284,781, 6,300,355, 6,204,388, 6,316,630, and 6,369,234; U.S. Patent Applications 09/797,027, 09/796,959, and 10/236,135; and PCT Publication Nos. WO 99/01124, WO 99/43653, and WO 01/64650, the entire contents of which are hereby incorporated by reference). In recognition of the need for improved or additional synthetic methodologies to efficiently generate epothilones, desoxyepothilones and analogues thereof in large quantities, the present invention provides an efficient and modular route for the synthesis of epothilones, desoxyepothilones and analogues thereof. Although the synthesis of certain exemplary compounds is described in the Exemplification herein, it will be appreciated that this methodology is generally applicable to the generation of analogues and conjugates as discussed above for each of the classes and subclasses described herein.

In particular, the 9,10-dehydroepothilone compounds of the present invention may be prepared in a variety of ways using synthetic methodologies useful in the synthesis of epothilones. In certain embodiments, the compounds are prepared using a convergent synthetic route. For example, the epothilone may be synthesized by preparing two or three intermediates which are brought together to yield the desired compound. In one embodiment, one of the intermediates is an acyl portion containing

carbons 1-9, and another intermediate contains carbons 10-15 and may also contain the thiazole side chain. These two roughly equal portions of the epothilone may be brought together first using an esterification reaction between C-1 and an oxygen off C-15. The macrocycle may then be closed using a carbon-carbon coupling reaction such as a
5 Suzuki coupling or ring closing metathesis reaction. In one embodiment, the final ring closing step is accomplished using a ring closing metathesis reaction to form the 9,10-double bond and close the macrocycle. The ring closing metathesis reaction is accomplished using an organometallic catalyst such as the Grubbs catalyst as shown in Scheme 8 below. In certain embodiments, the 9,10-double bond is reduced or oxidized,
10 or the 9,10-double bond may be further functionalized to prepare additional epothilone derivatives. In certain embodiments, the 9,10-double bond is converted into a cyclopropyl moiety by the treatment of the 9,10-double bond with a carbene or carbenoid reagent such as CH_2N_2 .

In certain embodiments, the 9,10-dehydroepothilone compound is prepared by
15 the isomerization of the double from the 10,11-position (*e.g.*, Epo490) to the 9,10-position. This isomerization may be catalyzed by the presence of a transition metal such as palladium.

In other embodiments, the final ring closing step is accomplished using a ring closing metathesis reaction to form the 12,13-double bond and close the macrocycle.
20 In certain embodiments, the 12,13-double bond is reduced or oxidized. In other embodiments, a macroaldolization or macrolactonization reaction is used to form the macrocycle.

Certain exemplary syntheses of the compounds of the invention are provided in the Figures and in the Examples. As would be appreciated by one of ordinary skill in
25 the art, a variety of analogs and derivatives may be prepared using the synthetic procedures described herein. For example, one could accomplish many of the synthetic steps with different protecting groups or different substituents on the 16-membered ring.

30 *Pharmaceutical Compositions*

This invention also provides a pharmaceutical preparation comprising at least one of the compounds as described above and herein, or a pharmaceutically acceptable

derivative thereof, which compounds are capable of inhibiting the growth of or killing cancer cells, and, in certain embodiments of special interest are capable of inhibiting the growth of or killing multidrug resistant cancer cells. In certain embodiments, the pharmaceutical preparation also comprises as solubilizing or emulsifying agent such as
5 Cremophor (polyoxyl 35 castor oil) or Solutol (polyethylene glycol 660 12-hydroxystearate).

As discussed above, the present invention provides novel compounds having antitumor and antiproliferative activity, and thus the inventive compounds are useful for the treatment of cancer. Accordingly, in another aspect of the present invention,
10 pharmaceutical compositions are provided, wherein these compositions comprise any one of the compounds as described herein, and optionally comprise a pharmaceutically acceptable carrier. In certain embodiments, these compositions optionally further comprise one or more additional therapeutic agents. In certain other embodiments, the additional therapeutic agent is an anticancer agent, as discussed in more detail herein.

15 It will also be appreciated that certain of the compounds of present invention can exist in free form for treatment, or where appropriate, as a pharmaceutically acceptable derivative thereof. According to the present invention, a pharmaceutically acceptable derivative includes, but is not limited to, pharmaceutically acceptable salts, esters, salts of such esters, or any other adduct or derivative which upon administration
20 to a patient in need is capable of providing, directly or indirectly, a compound as otherwise described herein, or a metabolite or residue thereof, e.g., a prodrug.

As used herein, the term "pharmaceutically acceptable salt" refers to those salts which are, within the scope of sound medical judgement, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic
25 response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, S. M. Berge, *et al.* describe pharmaceutically acceptable salts in detail in *J. Pharmaceutical Sciences*, 66: 1-19 (1977), incorporated herein by reference. The salts can be prepared in situ during the final isolation and purification of the compounds of the invention, or
30 separately by reacting the free base function with a suitable organic acid. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid, hydrobromic acid, phosphoric

acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, 5 bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, harnisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, 10 nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p-toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, 15 when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, loweralkyl sulfonate and aryl sulfonate.

Additionally, as used herein, the term "pharmaceutically acceptable ester" refers to esters which hydrolyze in vivo and include those that break down readily in the 20 human body to leave the parent compound or a salt thereof. Suitable ester groups include, for example, those derived from pharmaceutically acceptable aliphatic carboxylic acids, particularly alkanoic, alkenoic, cycloalkanoic and alkanedioic acids, in which each alkyl or alkenyl moiety advantageously has not more than 6 carbon atoms. Examples of particular esters include formates, acetates, propionates, butyrates, 25 acrylates and ethylsuccinates.

Furthermore, the term "pharmaceutically acceptable prodrugs" as used herein refers to those prodrugs of the compounds of the present invention which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals with undue toxicity, irritation, allergic response, and the 30 like, commensurate with a reasonable benefit/risk ratio, and effective for their intended use, as well as the zwitterionic forms, where possible, of the compounds of the invention. The term "prodrug" refers to compounds that are rapidly transformed in vivo

to yield the parent compound of the above formula, for example by hydrolysis in blood. A thorough discussion is provided in T. Higuchi and V. Stella, Pro-drugs as Novel Delivery Systems, Vol. 14 of the A.C.S. Symposium Series, and in Edward B. Roche, ed., Bioreversible Carriers in Drug Design, American Pharmaceutical Association and
5 Pergamon Press, 1987, both of which are incorporated herein by reference.

As described above, the pharmaceutical compositions of the present invention additionally comprise a pharmaceutically acceptable carrier, which, as used herein, includes any and all solvents, diluents, or other liquid vehicle, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents,
10 preservatives, solid binders, lubricants and the like, as suited to the particular dosage form desired. Remington's Pharmaceutical Sciences, Fifteenth Edition, E. W. Martin (Mack Publishing Co., Easton, Pa., 1975) discloses various carriers used in formulating pharmaceutical compositions and known techniques for the preparation thereof. Except insofar as any conventional carrier medium is incompatible with the anti-cancer
15 compounds of the invention, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition, its use is contemplated to be within the scope of this invention. Some examples of materials which can serve as pharmaceutically acceptable carriers include, but are not limited to, sugars such as lactose, glucose and sucrose;
20 starches such as corn starch and potato starch; cellulose and its derivatives such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; Cremophor; Solutol; excipients such as cocoa butter and suppository waxes; oils such as peanut oil, cottonseed oil; safflower oil; sesame oil; olive oil; corn oil and soybean oil; glycols; such a propylene glycol; esters such as ethyl
25 oleate and ethyl laurate; agar; buffering agents such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol, and phosphate buffer solutions, as well as other non-toxic compatible lubricants such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, releasing agents, coating agents, sweetening, flavoring and perfuming
30 agents, preservatives and antioxidants can also be present in the composition, according to the judgment of the formulator.

Uses of Compounds and Pharmaceutical Compositions

The invention further provides a method for inhibiting tumor growth and/or tumor metastasis. In certain embodiments of special interest, the invention provides a method of treating cancers by inhibiting tumor growth and/or tumor metastasis for
5 tumors multidrug resistant cancer cells. The method involves the administration of a therapeutically effective amount of the compound or a pharmaceutically acceptable derivative thereof to a subject (including, but not limited to a human or animal) in need of it. In certain embodiments, specifically for treating cancers comprising multidrug resistant cancer cells, the therapeutically effective amount is an amount sufficient to kill
10 or inhibit the growth of multidrug resistant cancer cell lines. In certain embodiments, the inventive compounds are useful for the treatment of solid tumors.

The compounds and pharmaceutical compositions of the present invention may be used in treating or preventing any disease or conditions including proliferative diseases (*e.g.*, cancer), autoimmune diseases (*e.g.*, rheumatoid arthritis), and infections
15 (*e.g.*, bacterial, fungal, *etc.*). The compounds and pharmaceutical compositions may be administered to animals, preferably mammals (*e.g.*, domesticated animals, cats, dogs, mice, rats), and more preferably humans. Any method of administration may be used to deliver the compound or pharmaceutical compositions to the animal. In certain embodiments, the compound or pharmaceutical composition is administered
20 parenterally.

In yet another aspect, according to the methods of treatment of the present invention, tumor cells are killed, or their growth is inhibited by contacting the tumor cells with an inventive compound or composition, as described herein. Thus, in still another aspect of the invention, a method for the treatment of cancer is provided
25 comprising administering a therapeutically effective amount of an inventive compound, or a pharmaceutical composition comprising an inventive compound to a subject in need thereof, in such amounts and for such time as is necessary to achieve the desired result. In certain embodiments of the present invention a “therapeutically effective amount” of the inventive compound or pharmaceutical composition is that amount
30 effective for killing or inhibiting the growth of tumor cells. The compounds and compositions, according to the method of the present invention, may be administered using any amount and any route of administration effective for killing or inhibiting the

growth of tumor cells. Thus, the expression "amount effective to kill or inhibit the growth of tumor cells", as used herein, refers to a sufficient amount of agent to kill or inhibit the growth of tumor cells. The exact amount required will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity
5 of the infection, the particular anticancer agent, its mode of administration, and the like. The anticancer compounds of the invention are preferably formulated in dosage unit form for ease of administration and uniformity of dosage. The expression "dosage unit form" as used herein refers to a physically discrete unit of anticancer agent appropriate for the patient to be treated. It will be understood, however, that the total daily usage of
10 the compounds and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular patient or organism will depend upon a variety of factors including the disorder being treated and the severity of the disorder; the activity of the specific compound employed; the specific composition
15 employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed; and like factors well known in the medical arts.

Furthermore, after formulation with an appropriate pharmaceutically acceptable
20 carrier in a desired dosage, the pharmaceutical compositions of this invention can be administered to humans and other animals orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, or drops), buccally, as an oral or nasal spray, or the like, depending on the severity of the infection being treated. In certain embodiments of the invention, the inventive compounds as
25 described herein are formulated by conjugating with water soluble chelators, or water soluble polymers such as polyethylene glycol as poly (1-glutamic acid), or poly (1-aspartic acid), as described in U.S. Patent 5,977,163, the entire contents of which are hereby incorporated by reference. In certain embodiments, the compounds of the invention may be administered orally or parenterally at dosage levels sufficient to
30 deliver from about 0.001 mg/kg to about 100 mg/kg, from about 0.01 mg/kg to about 50 mg/kg, preferably from about 0.1 mg/kg to about 40 mg/kg, preferably from about 0.5 mg/kg to about 30 mg/kg, from about 0.01 mg/kg to about 10 mg/kg, from about

0.1 mg/kg to about 10 mg/kg, and more preferably from about 1 mg/kg to about 25 mg/kg, of subject body weight per day, one or more times a day, to obtain the desired therapeutic effect. The desired dosage may be delivered as delivered every other day, every third day, every week, every two weeks, every three weeks, or every four weeks.

- 5 In certain embodiments, the desired dosage may be delivered using multiple administrations (*e.g.*, two, three, four, five, six, seven, eight, nine, or ten administrations).

Liquid dosage forms for oral and parenteral administration include, but are not limited to, pharmaceutically acceptable emulsions, microemulsions, solutions,
10 suspensions, syrups and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cottonseed,
15 groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents. In certain embodiments for parenteral administration, the compounds of the
20 invention are mixed with solubilizing agents such as Cremophor, alcohols, oils, modified oils, glycols, polysorbates, cyclodextrins, polymers, and combinations thereof. In certain embodiments, the compound is mixed with an alcohol, such as ethanol, and Cremophor (polyethoxylated castor oil).

Injectable preparations, for example, sterile injectable aqueous or oleaginous
25 suspensions may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution, suspension or emulsion in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, U.S.P. and
30 isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil can

be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid are used in the preparation of injectables.

The injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, or by incorporating sterilizing agents in the form of sterile
5 solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium prior to use.

In order to prolong the effect of a drug, it is often desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material
10 with poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle. Injectable depot forms are made by forming microencapsule matrices of the drug in biodegradable
15 polymers such as polylactide-polyglycolide. Depending upon the ratio of drug to polymer and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissues.

20 Compositions for rectal or vaginal administration are preferably suppositories which can be prepared by mixing the compounds of this invention with suitable non-irritating excipients or carriers such as cocoa butter, polyethylene glycol or a suppository wax which are solid at ambient temperature but liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the active compound.

25 Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound is mixed with at least one inert, pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid, b) binders such as, for example,
30 carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidone, sucrose, and acacia, c) humectants such as glycerol, d) disintegrating agents such as agar--agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate,

e) solution retarding agents such as paraffin, f) absorption accelerators such as quaternary ammonium compounds, g) wetting agents such as, for example, cetyl alcohol and glycerol monostearate, h) absorbents such as kaolin and bentonite clay, and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents.

Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

The active compounds can also be in micro-encapsulated form with one or more excipients as noted above. The solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings, release controlling coatings and other coatings well known in the pharmaceutical formulating art. In such solid dosage forms the active compound may be admixed with at least one inert diluent such as sucrose, lactose or starch. Such dosage forms may also comprise, as is normal practice, additional substances other than inert diluents, e.g., tableting lubricants and other tableting aids such as magnesium stearate and microcrystalline cellulose. In the case of capsules, tablets and pills, the dosage forms may also comprise buffering agents. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes.

Dosage forms for topical or transdermal administration of a compound of this invention include ointments, pastes, creams, lotions, gels, powders, solutions, sprays, inhalants or patches. The active component is admixed under sterile conditions with a pharmaceutically acceptable carrier and any needed preservatives or buffers as may be required. Ophthalmic formulation, ear drops, and eye drops are also contemplated as being within the scope of this invention. Additionally, the present invention contemplates the use of transdermal patches, which have the added advantage of providing controlled delivery of a compound to the body. Such dosage forms can be made by dissolving or dispensing the compound in the proper medium. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate can be controlled by either providing a rate controlling membrane or by dispersing the compound in a polymer matrix or gel.

As discussed above, the compounds of the present invention are useful as anticancer agents, and thus may be useful in the treatment of cancer, by effecting tumor cell death or inhibiting the growth of tumor cells. In general, the inventive anticancer agents are useful in the treatment of cancers and other proliferative disorders, including, but not limited to breast cancer, brain cancer, skin cancer, cervical cancer, colon and rectal cancer, leukemia, lung cancer, melanoma, multiple myeloma, non-Hodgkin's lymphoma, ovarian cancer, pancreatic cancer, prostate cancer, and gastric cancer, to name a few. In certain embodiments, the inventive anticancer agents are active against leukemia cells and melanoma cells, and thus are useful for the treatment of leukemias (e.g., myeloid, lymphocytic, promyelocytic, myelocytic and lymphoblastic leukemias, whether acute or chronic forms) and malignant melanomas. In still other embodiments, the inventive anticancer agents are active against solid tumors and also kill and/or inhibit the growth of multidrug resistant cells (MDR cells). In certain embodiments, the inventive anticancer agents are active against cancers which are resistant to other known anti-neoplastic agents or which have been found not to respond clinically to other known anti-neoplastic agents. In other embodiments, the inventive anticancer agents are active against cancer which are resistant to other anti-neoplastic microtubule-stabilizing agents (e.g., paclitaxel).

It will also be appreciated that the compounds and pharmaceutical compositions of the present invention can be employed in combination therapies, that is, the

compounds and pharmaceutical compositions can be administered concurrently with, prior to, or subsequent to, one or more other desired therapeutics or medical procedures. The particular combination of therapies (therapeutics or procedures) to employ in a combination regimen will take into account compatibility of the desired therapeutics and/or procedures and the desired therapeutic effect to be achieved. It will also be appreciated that the therapies employed may achieve a desired effect for the same disorder (for example, an inventive compound may be administered concurrently with another anticancer agent), or they may achieve different effects (e.g., control of any adverse effects).

For example, other therapies or anticancer agents that may be used in combination with the inventive anticancer agents of the present invention include surgery, radiotherapy (in but a few examples, γ -radiation, neutron beam radiotherapy, electron beam radiotherapy, proton therapy, brachytherapy, and systemic radioactive isotopes, to name a few), endocrine therapy, biologic response modifiers (interferons, interleukins, and tumor necrosis factor (TNF) to name a few), hyperthermia and cryotherapy, agents to attenuate any adverse effects (e.g., antiemetics), and other approved chemotherapeutic drugs, including, but not limited to, alkylating drugs (mechlorethamine, chlorambucil, Cyclophosphamide, Melphalan, Ifosfamide), antimetabolites (Methotrexate), purine antagonists and pyrimidine antagonists (6-Mercaptopurine, 5-Fluorouracil, Cytarabine, Gemcitabine), spindle poisons (Vinblastine, Vincristine, Vinorelbine, Paclitaxel, Docetaxel), podophyllotoxins (Etoposide, Irinotecan, Topotecan), antibiotics (Doxorubicin, Bleomycin, Mitomycin), nitrosoureas (Carmustine, Lomustine), inorganic ions (Cisplatin, Carboplatin), enzymes (Asparaginase), and hormones (Tamoxifen, Leuprolide, Flutamide, and Megestrol), to name a few. For a more comprehensive discussion of updated cancer therapies see, <http://www.nci.nih.gov/>, a list of the FDA approved oncology drugs at <http://www.fda.gov/cder/cancer/druglistframe.htm>, and The Merck Manual, Seventeenth Ed. 1999, the entire contents of which are hereby incorporated by reference.

In still another aspect, the present invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention, and in certain embodiments, includes

an additional approved therapeutic agent for use as a combination therapy. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceutical products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

EQUIVALENTS

The representative examples which follow are intended to help illustrate the invention, and are not intended to, nor should they be construed to, limit the scope of the invention. Indeed, various modifications of the invention and many further embodiments thereof, in addition to those shown and described herein, will become apparent to those skilled in the art from the full contents of this document, including the examples which follow and the references to the scientific and patent literature cited herein. It should further be appreciated that the contents of those cited references are incorporated herein by reference to help illustrate the state of the art. The following examples contain important additional information, exemplification and guidance which can be adapted to the practice of this invention in its various embodiments and equivalents thereof.

EXAMPLES

Example 1: Oral and Parenteral Administration of 26-trifluoro-9,10-*trans*-dehdyro-epothilone D in the Treatment of Human Tumors implanted in Nude Mice

In this Example, it is demonstrated that structurally designed 16-membered macrolide microtubule-stabilization agent, 26-trifluoro-9,10-*trans*-dehdyro-epothilone D, shrinks tumors, renders tumor disappearance, and achieves long term non-relapse when given by 6 hr. – i.v. infusion or orally. Mice bearing large size tumor (Figure 6) or Taxol-resistant tumor xenografts (Figure 7) are shown to be curable with 26-trifluoro-9,10-*trans*-dehdyro-epothilone D as a single agent monotherapy. The curative therapeutic spectrum encompasses leukemia as well as breast, colon, and lung

carcinomas (Figures 6, 9, and 13). All chemotherapeutic experiments *in vivo* reported here were carried out using human tumor xenografts in immunodeficient nude mice. This animal model is commonly used in evaluating anti-tumor compounds prior to clinical trials in cancer patients.

5 The MX-1 and HCT-116 experiments lasted 5.5 and 6.5 months, respectively (Figures 6 and 9). There was no tumor relapse in both experiments with 3.8 and 5.2 months tumor free, respectively, following the cessation of treatment. For the HCT-116 experiment (Figure 9), both paclitaxel and 26-trifluoro-9,10-*trans*-dehydro-epothilone D at 20mg/kg were used and both achieved tumor disappearance. However,
10 the paclitaxel treated group relapsed at 1.1 months after stopping treatment, whereas 26-trifluoro-9,10-*trans*-dehydro-epothilone D treated animals were tumor free for over 5.2 months. Assuming a tumor doubling time of 4 days (based on the vehicle treated control), the paclitaxel treatment of HCT-116 tumor resulted in 99.7% tumor suppression or 2.56-log cell kill, whereas the cell kill by 26-trifluoro-9,10-*trans*-
15 dehydro-epothilone D in MX-1 and HCT-116 experiments would be >8.5-log and >11.6 log, respectively, when the experiments were terminated. Based on the healthy, active conditions of the treated mice with body weight returned to pretreatment levels, it is expected that "cure" can be ensured throughout the 2 year life span of the mice if prolonged treatment or additional cycles of treatment were enforced. This result should
20 be achievable despite the fact that "cure" would be more difficult to attain in immunodeficient mice than in the immuno-competent mice. To our knowledge, this is the longest xenograft therapeutic studies carried out in nude mice in biomedical literature, and the longest complete remission that has been reported either with parenteral or oral administration for a single antitumor agent. It is pertinent to state that
25 it is relatively easy to find a compound that suppresses tumor growth. But it is relatively rare to find a compound that achieves tumor shrinkage. The finding with our compound, 26-trifluoro-9,10-*trans*-dehydro-epothilone D, that achieves complete tumor disappearance in all mice with no relapse for as long as 5.2 months to our knowledge has never been reported previously, indicating the great potential of 26-
30 trifluoro-9,10-*trans*-dehydro-epothilone D for therapeutic development.

Additional significant benefit of oral therapy is that the use of Cremophor formulation that can cause severe allergic reactions can be avoided. It is well known

that the use of Cremophor in the formulation in Taxol, desoxy-EpoB, and 15-aza-Epo13 induced troublesome allergic reactions which make pretreatment with antihistamine and/or steroid necessary.

Oral effectiveness of 26-trifluoro-9,10-*trans*-dehdyro-epothilone D is consistent with its remarkable metabolic stability in mouse plasma and in human liver microsomal S9 fraction *in vitro*. This metabolic stability is attributable to the trifluorination at C-26 position of the epothilone molecule (Figure 14). Introducing the double bond at C9-C10 also increased metabolic stability (Figure 14). The closeness of the optimal dose of 26-trifluoro-9,10-*trans*-dehdyro-epothilone D for i.v. infusion (20-30mg/kg, Q2D) and for the oral administration (20mg/kg, QD or 30mg/kg Q2D) suggests that the compound is well absorbed and has excellent bioavailability *in vivo*.

It is worthy of noting that many of our *in vivo* therapeutic studies on Fludelone (Figures 13, 7, 9, 10, 11, and 16) against xenografts were carried out in parallel with Taxol, which is one of the most important cancer therapeutic agents currently in use in clinics. The profound findings with Fludelone, in comparison with Taxol, indicate the promising potential of this compound for the treatment of cancer.

Materials and Methods

Chemicals: All epothilones were synthesized as indicated herein. Paclitaxel (Taxol[®]) and vinblastine sulfate (VBL) were purchased from Sigma. All these compounds were dissolved in dimethylsulfoxide for the *in vitro* assays, (except VBL in saline). For *in vivo* studies, all epothilones and paclitaxel were dissolved in Cremophor/ethanol (1:1) vehicle and then diluted with saline for iv infusion for 6hrs via tail vein using a custom-designed mini-catheter and a programmable pump (Chou *et al.* (1998) *Proc. Natl. Acad. Sci. USA* **95**, 15798-15802; Chou *et al.* (2001) *Proc. Natl. Acad. Sci. USA* **98**, 8113-8118; each of which is incorporated herein by reference). Oral administration of the drugs were prepared by dissolving the compound in ethanol and suspended with equal volume of Tween-80 and the suspension was diluted with 5 volumes of saline prior to administration to nude mice. The gavage was carried out using a 1 ml syringe and a gauge #22 ball-tipped animal feeding needle (Popper & Sons, Inc. New Hyde Park, NY).

Tumor and Cell Lines: The CCRF-CEM human lymphoblastic leukemia cells and their vinblastine resistant subline (CCRF-CEM/VBL₁₀₀, 720-fold resistance) were obtained from Dr. William Beck of the University of Illinois, Chicago, and CCRF-CEM/Taxol (44-fold resistance) by exposing CCRF-CEM cells to increasing sublethal

5 concentration (IC₅₀-IC₉₀) of paclitaxel for six months. The degree of resistance are shown in Figure 14. Human mammary carcinoma (MX-1), human lung carcinoma cells (A549), and human colon carcinoma (HCT-116) were obtained from American Type Culture Collection (ATCC, Rockville, MD).

10 Animals: Athymic nude mice bearing the nu/nu gene were obtained from NCI, Frederick, MD and used for all human tumor xenografts. Male nude mice 6 weeks or older weighing 20-22 g or more were used. Drugs were administered via the tail vein for 6 hours by iv infusion using a homemade infusion mini-catheter and containment tube. A programmable Harvard PHD2000 syringe pump with multitrack was used for
15 iv infusion. A typical 6 hour infusion volume for each drug in Cremophor/ethanol (1:1) was 100 µl in 2.0 ml of saline. For oral administration, both Fludelson and Taxol were dissolved in ethanol and the diluted 5-fold with Tween-80. The Taxol solution should be used within 5min to avoid precipitation. Tumor volume was assessed by measuring length x width x height (or width) by using a caliper. For tumor-bearing nude mice
20 during the course of experiment, the body weight refers to total weight minus the weight of the tumor. All animal studies conducted in accordance with the guidelines for the National Institute of Health Guide for the Care and Use of Animals and the protocol approved by the Memorial Sloan-Kettering Cancer Center's Institutional Animal Care and Use Committee.

25

Cytotoxicity Assays: In preparation for *in vitro* cytotoxicity assays, cells were cultured at an initial density 2-5 x 10⁴ cells per milliliter. They were maintained in a 5% CO₂-humidified atmosphere at 37°C in RPMI medium 1640 (GIBCO/BRL) containing penicillin (100 units/mL), streptomycin (100 µg/mL, GIBCO/BRL), and 5% heat-
30 inactivated FBS. For solid tumor cells growing in a monolayer (such as HCT-116 and A549), cytotoxicity of the drug was determined in 96-well microtiter plates by using the sulforhodamine B method (Skehan *et al.* (1990) *J. Natl. Cancer Inst.* **82**, 1107-

1112; incorporated herein by reference). For cells grown in suspension (such as CCRF-CEM and its sublines), cytotoxicity was measured, in duplicate, by using the 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-5-carboxanilide)-2H-terazodium hydroxide (XTT) microculture method (Scudiero *et al.* (1988) *Cancer Res.* **48**, 4827-4833; incorporated
5 herein by reference) in 96-well microtiter plates. For both methods, the absorbance of each well was measured with a microplate reader (Power Wave XS, Bio-Tek, Winooski, VT). Dose-effect relationship data from 6 to 7 concentrations of each drug, in duplicate, were analyzed with the median-effect plot by using a computer program (Chou *et al.* (1997) *CalcuSyn for Windows* (Biosoft, Cambridge, U.K.); each of which
10 is incorporated herein by reference).

Stability of epothilones in mouse and in human liver S9 fraction: The stability study was carried out with a fully automated HPLC system which consisted of a Prospekt-2 (Spark Holland, Netherlands) sample preparation system and an Agilent 1100 HPLC
15 system. Briefly, the Prospekt 2 picked up a C8 extraction cartridge and washed it with acetonitrile and water. The Agilent autosampler, set at 37 °C, picked up 20 µl of the sample, loaded it onto the cartridge, washed it with water, then the Prospekt-2 diverted the mobile phase stream through the extraction cartridge onto the analytical column, Reliance Stable Bond C8 4x80mm with guard column (MacMod, Chadds Ford, PA)
20 and the eluent was monitored at 250 nm. The mobile phase consists of 53 or 65% acetonitrile/0.1% formic acid at 0.4ml/min, so that the retention time of the compound of interest is about 6 minutes. Sample preparation involves the addition of equal volumes of plasma to PBS for a total volume of 400 µl, filtered, and the addition of 0.5-
25 2 µl of the substrate (20 mM) to achieve about 30-50 mAU at 250nm in the HPLC analysis. For pooled human liver microsome S9 fraction (Xeno Tech, Lenex, KS), 20 µl (400 µg) or S9 fraction was mixed with 280 µl of PBS then proceeds as above. The sampling period was controlled by the autosampler and peak area data were collected to compare the rate of disappearance of the parent compound.

30 *Results*

Structure-Activity Relationships in Vitro against Human Leukemic, Taxol- and Vinblastin-resistant Leukemic Cells and Solid Tumor Cells. The potency in terms of IC₅₀ (in µM) for twelve representative epothilones against the growth of

human leukemic CCRF-CEM cells and their sublimes resistant to vinblastine CCRF-CEM/VBL and resistant to paclitaxel, CCRF-CEM/Taxol were listed in the table in Figure 17. Also listed are the IC₅₀ values for two human solid tumor cell lines: lung carcinoma A549 and colon carcinoma HCT-116. It is shown that deH-EpoB replaces
5 EpoB as the most potent epothilone known. It is also shown that i) 9,10-dehydro-modification on dEpoB, dEpoF, or F₃-dEpoF always resulted in marked increase in potency, whereas trifluorination on the C-26 position somewhat reduced the potency, however metabolic stability is greatly increased by the trifluorination (Figure 14); and
10 ii) Most epothilones are not cross-resistant with vinblastine, typical substrate for the P-glycoprotein of multi-drug resistance (MDR) nor cross-resistant with Taxol. Taxol is a good substrate for MDR phenotype but Taxol resistance may also be generated by mutation in tubulin gene. The exceptions are 15-aza-EpoB, 15-aza-deH-dEpoB which shows considerable cross-resistance to both vinblastine and paclitaxel. dEpoF and its derivative showed some cross-resistance with vinblastine but not paclitaxel. iii)
15 Leukemic CCRF-CEM cells and solid-tumor cells A549 and HCT-116 are almost equally susceptible to epothilones. iv) F₃-deH-dEpoB, deH-dEpoB, and deH-EpoB, which are our new lead compounds for further pharmacologic evaluations, are not appreciably cross-resistant to vinblastine nor to paclitaxel.

In earlier studies (Schiff *et al.* (1979) *Nature (London)* **277**, 665-667; Meng *et al.* (1997) *J. Am. Chem. Soc.* **119**, 2733-2734; each of which is incorporated herein by
20 reference) it was concluded that epothilone molecules can be dissected into three zones. Thus, in the C-1~8 acyl sector, structural changes are not tolerated in terms of *in vitro* cytotoxicity and microtubule stabilizing ability. This is in contrast to the C-9~15 O-alkyl sector and the C-15 pendant aryl sectors wherein considerable modification of
25 structures is tolerated (Haar *et al.* (1996) *Biochemistry* **35**, 243-250; Kowalski *et al.* (1997) *J. Biol. Chem.* **272**, 2534-2541; each of which is incorporated herein by reference).

Epothilones with 12,13-epoxyl moiety, such as EpoB, and deH-EpoB, although the most potent agents in the epothilone series but they possessed rather poor
30 therapeutic index at their maximal tolerated doses. This hypothesis is illustrated with the therapeutic data given in the table in Figure 14.

Physico-chemical, Metabolic, Pharmacological Properties and Therapeutic Results of Epothilone Derivatives.

Inter-relating different properties of a series of epothilones facilitates the understanding of factors that contribute to the therapeutic end-results for the lead compounds. The table in Figure 14 summarizes the profiles of nine epothilone derivatives. The structure-cytotoxic activity relationship *in vitro* provides potency-based initial evaluation. For example, new class of 9,10-dehydro modification greatly increased potency *in vitro* and *in vivo*. In these preselected compounds, it is more difficult to correlate the structure-microtubule stabilization potency because the potency are all quite high (*i.e.*, similar to the paclitaxel potency). Water solubility and lipophilicity play varied role in the therapeutic effect and may be important for the design of formulation. DeH-dEpoF, F₃-deH-dEpoF, and deH-dEpoB greatly increased water solubility relative to other epothilones. The finding that F₃-deH-dEpoB is orally efficacious reduces the concern of water-insolubility in the drug formulation and the usage of allergy causing Cremophor can be avoided.

In vitro, deH-EpoB, deH-dEpoF, EpoB and deH-dEpoB, in this order have sub-nanomolar IC₅₀'s whereas F₃-deH-dEpoF, dEpoF, F₃-deH-dEpoB, dEpoB and F₃-dEpoB, in this order, have IC₅₀'s range of 1.3 to 9.3nM (Figure 17). DeH-EpoB and EpoB are the most potent epothilones known *in vitro* and *in vivo* but they do not yield the best therapeutic index. Apparently, epoxyl moiety on C-12~13 of EpoB and deH-EpoB contribute greatly to the toxicity toward the host as evident by lower value in the maximal-body weight percent drop without a death (Figure 14). By contrast, F₃-deH-dEpoB and dEpoB tolerated the highest values of body weight dropped and they showed excellent therapeutic results such as complete tumor remission in all animals. In general, *in vitro* potency and the optimal therapeutic dose revealed good correlations with 6hr- i.v. infusion of treatment. Of special interest is that F₃-deH-dEpoB (Fludelone) possesses the widest curative therapeutic dose range (10-30 mg/kg) (Chou *et al.* (2003) *Angew Chem. Int. Ed. Engl.* **42**, 4762-4767; incorporated herein by reference) the excellent metabolic stability and the best overall therapeutic results among the epothilones listed (Figure 14). Furthermore, F₃-deH-dEpoB provides curative therapeutic effect via oral route of administration. It is concluded that the most potent epothilones does not necessarily perform better as antitumor agents and that F₃-deH-dEpoB is the lead candidate for the therapeutic development.

Therapy on Extra Large MX-1 Tumor Xenografts. As shown in Figure 6A, treatment of MX-1 xenografts as large as 3.4% of body weight with F₃-deH-dEpoB, 25mg/kg, 6 hr- i.v. infusion Q3Dx5 beginning D22 after tumor implantation, led to marked tumor shrinkage (>97.4%). During the resting period of 9 days without treatment (D34-D43), the tumor size continued to shrink (>99.3%) and tumor disappeared in 2 out of 5 mice studied, whereas body weight of the treated group recovered to the pretreatment level during the same resting period (Figure 6B). Resumption of treatment on D43, Q3Dx4, led to tumor disappearance on the remaining 3 mice on D50, D50 and D51. After D52 (*i.e.*, date of the last dose), animals were observed every three days until D165 when the experiment was terminated. There was no tumor relapse in all 5 animals on D165 (*i.e.*, over 3.7 months after cessation of treatment).

The photographs of the nude mice of this experiment taken on D25, D31, D37, D43, and D52 from one mouse each for the control and treated groups are shown in Figure 6B.

Curative Cancer Therapy of MX-1 Xenografts by Fludelone via Oral Administration. As shown in Figure 13A, F₃-deH-dEpoB given orally at 30mg/kg every two days for 7 times led to MX-1 tumor shrinkage and tumor disappearance in 2 out of 4 mice. Additional two doses (after skipping on dose) led to tumor disappearance in 4 out of 4 mice. By contrast, oral treatment of Taxol at the same dose and the same schedule of suppressed MX-1 tumor growth but did not lead to tumor shrinkage. Two days after the last dose of Taxol (D36), it induced tumor suppression of 66.9%. F₃-deH-dEpoB treatment induced moderate yet persistent decreases in body weight with maximal drop of 15% of the body weight (Figure 13B). Taxol treatment induced little changes in body weight suggesting that oral administration of Taxol was not a suitable treatment apparently due to drug metabolic inactivation or poor bioavailability. In contrast to oral route of administration, our previous report (Chou *et al.* (2001) *Proc. Natl. Acad. Sci. USA* **98**, 8113-8118; incorporated herein by reference), Taxol treatment of MX-1 tumor at 20 mg/kg via 6 hr i.v.-infusion could lead to tumor shrinkage and tumor disappearance.

Curative Therapeutic Effect of Fludelone against Taxol-Resistant CCRF-**CEM/Taxol Xenografts.** In our recent report (Chou *et al.* (2003) *Angew Chem. Int.**Ed. Engl.* **42**, 4762-4767; incorporated herein by reference), we showed that F₃-deH-

5 dEpoB at 20 mg/kg, Q2Dx7, 6 hr-i.v. infusion against Taxol-resistant human lung carcinoma A549/Taxol (44- fold resistance to paclitaxel *in vitro*) yielded complete tumor growth suppression but failed to achieve tumor disappearance. We now use another paclitaxel- resistant xenograft, CCRF-CEM/Taxol (44- fold resistance to paclitaxel *in vitro*). As shown in Figure 7, Fludelone at 30 mg/kg Q2Dx7, 6 hr-i.v. infusion led to tumor disappearance in 2 out of 4 mice. Additional Q2Dx5 (after skipping one dose) lead to tumor disappearance in 3 out of 4 mice, with the final tumor suppression of 99.8% (Figure 7A). At the reduced dose of 15 mg/kg, tumor disappearance occurred in only 1 out of 4 mice on the 5th day after the two cycle treatment. The final tumor suppression on D34 was 98.8%. The parallel experiment with Taxol at 20mg/kg yielded tumor growth suppression but with little or no tumor shrinkage. The final tumor suppression on D34 was 75.6%.

Both F₃-deH-dEpoB (at 15 mg/kg and 30 mg/kg) and Taxol (20mg/kg) persistently reduced the body weight during the first cycle of 7 treatments via 6 hr- i.v. infusion every other day. Skipping one treatment on D22 led to immediate gaining of body weight in all mice. The second cycle of treatment of Q2Dx5 again persistently reduced the body weight but without lethality to all mice tested (Figure 7B).

Curative Therapy against Human Colon Carcinoma HCT-116 Xenografts by**Fludelone.** As shown in Figure 9A, F₃-deH-dEpoB at 20 mg/kg and 30 mg/kg or

25 Taxol at 20 mg/kg, Q2Dx4, 6hr-i.v. infusion for 3 cycles all led to tumor disappearance in 4 out of 4 mice. The treatment started on D9 after tumor implantation. There was a one-dose-skip on D17 between the 1st and 2nd cycles and there was a two-dose-skip on D27 and D29 between the 2nd and 3rd cycles. D9-D37 was the 3-cycle therapy period and D37-D200 was the follow-up period. The experiment lasted 200 days which represent more than a quarter of the average life-span of the mice.

For F₃-deH-dEpoB at 30mg/kg, tumor disappeared on D21, 23, 33, and 41 and at 20 mg/kg, tumor disappeared on D31, 35, 41, and 45. For both F₃-deH-dEpoB

doses, there was no tumor relapse in 4 out of 4 mice on D200 when experiment was terminated. For Taxol at 20 mg/kg, tumor disappeared on D33, 33, 41, and 45 which were similar to the observation with F₃-deH-dEpoB at 20mg/kg. However, in the Taxol treated group, tumor relapse occurred on D71, 75, 81 and 81.

5 The schedule of the treatment including the rest period is dictated by the body weight decreases and the physical conditions of the mice. For the F₃-deH-dEpoB treatment at 30 mg/kg dose, the maximal body weight loss was 30% but without lethality which occurred at the end of the 2nd cycle of treatment (*i.e.*, D29) (Figure 9B). The body weight decreases and recovery for Taxol 20 mg/kg and F₃-deH-dEpoB 20
10 mg/kg were similar in both pattern and magnitude.

Example 2: Determination of the Mechanism of Action of Fludelone (F₃-deH-dEpoB) and How it Differs from that of EpoD; Therapeutic Implications of these Differences

15 *Drug sensitivity in human Multiple Myeloma (MM) and Non-Hodgkins Lymphoma (NHL) Tumor Cell Lines*

MM accounts for 1% of all cancers and 10% of hematological malignancies, with 15,500 new cases diagnosed and > 15,000 deaths in 2002. Treatment of MM with
20 conventional chemotherapy is not curative, with a median survival of about 3 years (Barlogie *et al.* "Treatment of multiple myeloma" *Blood* 2004; 103:20-32; incorporated herein by reference). Although high-dose chemotherapy with hematopoietic stem cell support increases the rate of complete remission and event-free survival, almost every patient relapses, mandating the crucial need for salvage therapy options. Paclitaxel has
25 been used to treat multiple myeloma and non-Hodgkin's lymphoma (Miller *et al.* "Paclitaxel as the initial treatment of multiple myeloma: an Eastern Cooperative Oncology Group Study (E1A93)" *Am. J. Clin. Oncol.* 1998; 21:553-556; Jazirehi *et al.* "Resveratrol modifies the expression of apoptotic regulatory proteins and sensitizes non-Hodgkin's lymphoma and multiple myeloma cell lines to paclitaxel-induced
30 apoptosis" *Mol. Cancer Ther.* 2004; 3:71-84; each of which is incorporated herein by reference). However, the application was limited due to its high toxicity and multi-drug resistance. We have evaluated Fludelone and dEpoB against a panel of human

MM and NHL lines that have been used in a number of recent studies in NOD/SCID xenograft models for evaluation of novel therapies, including 10-propargyl-10-deazaminopterin (PDX) (Wang *et al.* "Activity of a novel anti-folate (PDX, 10-propargyl-10-deazaaminopterin) against human lymphoma is superior to methotrexate and correlates with tumor RFC-1 gene expression" *Leukemia & Lymphoma* 2003, 44:1027-1035), anti-telomerase (Wang *et al.* "Telomerase inhibition with an oligonucleotide telomerase template antagonist: *in vitro* and *in vivo* studies in multiple myeloma and lymphoma" *Blood* 2004;103:258-66; incorporated herein by reference) and anti-VEGFR Mab (Wang *et al.* "Targeting autocrine and paracrine VEGF receptor pathways regresses human lymphoma xenographs *in vivo*" *Blood*, in press; incorporated herein by reference). Both Fludelone and dEpoB can inhibit myeloma and lymphoma cell proliferation significantly. Myeloma cell lines are very sensitive to Fludelone and dEpoB with extremely low IC50s, while two NHL lines were inhibited at doses of Fudalone that were 5-10 fold higher than were effective in MM (Table 2-1).

15

Table 2-1. Cell growth inhibition and IC50 of Fludelone and dEpoB against a panel of normal human tumors and normal human cell populations as determined by the XTT tetrazonium assay.

		IC50 (nM)	IC50(nM)
Tumor cell line	Histology	dEpoB	Fludelone
RPMI8226	Myeloma	36.67±2.0	7.6±1.2
CAG	Myeloma	61.34±4.2	12.04±1.8
OPM-2	Myeloma	38.89±3.3	8.2±2.2
H929	Myeloma	42.75±4.5	9.2±1.9
MOLP-5	Myeloma	68.56±5.5	14.4±2.6
RL	Lymphoma	90±11	80±11
SKI-DLBCL	Lymphoma	72±9.8	60±4.2
HS-27A	Marrow stroma	100±10	102±8
HS-5	Marrow stroma	100±8	96±7
MRC-5	Embryonic lung fibroblasts (human)	8.2±4.3	7.4±2.7
HT-29	Colon Ca	7.2±2.2	4±1.7

HCT-116	Colon Ca	7.5±3.1	3.6±1.3
MDA MB435	Breast Ca	7.8±4.2	5.8±2.8
IGROV	Ovarian Ca	15±3.8	2±1.2
SKOV3	Ovarian Ca	13±4.7	1.6±0.5
Ovcar-3	Ovarian Ca	14±3.6	1.1±0.4
Ovcar-4	Ovarian Ca	16±2.5	1.8±0.7

Normal bone marrow stromal cells (HS-27A and HS-5 lines) showed relative resistance to these compounds, indicating that Fludelone and dEpoB have a safe therapeutic window in MM. (Table 2-1). Fludelone has ~5 fold greater potency than dEpoB on MM cell lines while both drugs had a comparable toxicity to normal marrow stromal cells. Fetal human lung fibroblasts (MRC-5) were sensitive to dEpoB and Flu but a clear therapeutic window was evident with Flu even with these very sensitive normal cells. Fludelone and dEpoB cause myeloma and lymphoma cells to arrest at G2M phase (Figure 18) and induced tumor cell apoptosis (Figures 19 and 20). We evaluated the duration of drug exposure *in vitro* necessary to cause apoptosis in the myeloma cells. Pulse exposure for 1, 2, 4, 8, and 24 hours was followed by a washout of the drug and continued incubation for up to 48 hours. Exposure of cells to either Fludelone or dEpoB for 24 hrs resulted in all cells being dead by 48 hrs. (Figure 21). With dEpoB, incubation for 4-8 hrs. slowed cell expansion whereas the same duration of exposure to Fludelone decreased the number of cells from input by ~50%. A one hour exposure to dEpoB reduced but did not prevent tumor cell expansion over 48 hrs whereas with Flu, tumor numbers decreased by 50% (Figure 21). Fludelone and dEpoB shared with paclitaxel the capacity to enhance microtubule bundle formation in tumor cells without appreciably changing the mass of microtubules in the cell shortly after exposure (Figure 22). At a later time (~24 hrs) microtubules are disrupted and cell apoptosis occurs. In a comparison of Fludelone and dEpoB on primary CD138 MM cells obtained from patient marrow we showed that Fludelone but not dEpoB induced tumor apoptosis within 24 hrs.

Drug sensitivity in human Solid Tumor Cell Lines

The IC₅₀s of Fludelone and dEpoB were determined on a panel of human solid tumor lines (colon, breast and ovarian) (Table 2-1). In every instance the Fludelone IC₅₀ was lower than that of dEpoB. Ovarian cancer lines were particularly sensitive to Fludelone and 4/5 of the ovarian lines had a mean IC₅₀ of 1.6 nM compared to a mean IC₅₀ of 16.5 nM with dEpoB. Both drugs caused tumor cells to arrest in G2M phase (Figure 23) and rapidly induced apoptosis (Figures 24 and 25).

Global gene expression of the RPMI-8226 myeloma cell line at 6 and 18 hrs after treatment with dEpoB or Fludelone at x10 of their respective IC₅₀s

Global gene expression (Affymetric chip—AU133 2.0) comparison of differential gene expression in the RPMI-8226 human multiple myeloma cell line was undertaken at 6 or 18 hrs after treatment with dEpoB or Fludelone (at x10 IC₅₀ dose). In the comparison of Fludelone-treated RPMI-08226 with control untreated cells, at 6 hours 5 genes were upregulated (Table 2-2) and at 18 hrs 48 genes were upregulated (Table 2-3). JUN was upregulated at both times (+3.25, +2.64). At 6 hrs 3 genes were downregulated (Table 2-2) and at 18 hrs 16 genes were downregulated (Table 2-3). HNRPD (heterogeneous nuclear ribonucleoprotein D-like) was downregulated at both times (-2.46, -3.25). In the comparison of dEpoB-treated RPMI-08226 with control untreated cells at 6hrs 21 genes were upregulated (Table 2-2) and at 18 hrs. 26 genes were upregulated (Table 2-3). JUN (+5.66, +3.73) and Tubulin α 3 (+2.64, +2.30) were upregulated at both times. At 6 hrs., three genes were downregulated (Table 2-2), and at 18 hrs., sixteen genes were downregulated (Table 2-3). HNRPD was downregulated both times (-4.92, -2.00). We then compared gene expression altered by Fludelone versus dEpoB (Table 2-2 and 2-3).

Table 2-2: Global gene expression (Affymetrix chip—AU133 2.0) comparison of differential gene expression in RPMI-8226 (human multiple myeloma cell line). Comparison of control with cells treated with Fludelone or dEpoB (at 10 IC₅₀ dose) for 6hrs.

Fludelone 6hr	Fold change	Desoxyepothilone B 6hrs	Fold Change
JUN	+3.25	IFI27 (Interferon- α inducible protein)	+18.38
SKIL (SKI-Like-)	+2.83	JUN	+5.66
BASP1. (Brain abundant membrane attached signaling.)	+2.83	G1P3 * (Interferon- α inducible protein)	+5.28
APP (Amyloid β precursor protein)	+2.14	IFITM1* (Interferon-induced transmembrane protein1)	+4.92
HMGCS1 (3-OH-3-methylglutaryl-Co-enzyme synthase)	+200	APP (Amyloid β precursor protein)	+2.83
		CCL5 (Rantes chemokine)	+2.64
		TUBA3 (Tubulin α 3)	+2.64
		INSIG1 (Insulin induced gene)	+2.46
		TRIM22 (Tripartite motif-containing 22)	+2.30
		HLA-DPA1* (Histocompatibility class II DP α 1)	+2.30
		IFIT1* (Interferon-induced protein with tetratricopeptide repeats)	+2.30
		HMGCS1 (3-OH-3-methylglutaryl Coenzyme A)	+2.30
		MARCKS (Meristoylated alanine-rich PKC substrate)	+2.14
		ABCG1 (ABC transporter activity)	+2.14
		MX1* (Myxovirus resistance, Interferon-inducible p78)	+2.00
		OAS1* (2',5'-oligoadenylate synthetase)	+2.00
		PLSCR1 (Phospholipid scramblase)	+2.00
		STS (Steroid sulfatase)	+2.00
		DUSP4 (Dual specificity phosphatase)	+2.00
		AP1S1 (Adaptor-related protein complex-1)	+2.00
		ARHE (Ras homology family E)	+2.00
			+2.00
HNRPD Heterogeneous nuclear	-2.46	HNRPD (Heterogeneous nuclear ribonuclear protein)	-4.92

ribonucleoprotein D). SUV39 H2. (Suppressor of variegation homolog).	-2.00	D) HMOX1 (Heme oxygenase 1)	-2.30
HIST1. (Histone 1)	-2.00	HASA1A (heat shock 70kDa protein)	-2.14
		MAT2A (Methionine adenosyltransferase II α)	-2.00

Underlined:- genes up- or down-regulated in both Fludelone or dEpoB-treated cells at 6hrs.

* Interferon-inducible genes.

- 5 **Table 2-3: Global gene expression (Affymetrix chip – AU133 2.0) comparison of differential gene expression in RPMI-8226 (human multiple myeloma cell line). Comparison of control with cells treated with Fludelone or dEpoB (at x10 IC50 dose) for 18hrs.**

Fludelone 18hr	Fold change	Desoxyepothilone B 18hrs	Fold Change
PRKDC (double-stranded break repair)	+21.11	REPS1 (RALBP1 associated Eps domain)	+9.85
Fibrillin 2	+12.3	PKD1 (Polycystic kidney disease 1)	+5.6
REPS1 (RALP1 associated Eps domain)	+9.85	JUN	+3.73
PKD1 (Polycystic kidney disease 1)	+7.46	SEMA3D (Ig sema domain)	+3.73
TRIO (Triple functn domain guanyl NEF)	+4.29	PRKCBP1 (Protein kinase C binding)	+3.03
FLJ20241 (NFkB activating protein)	+3.73	PCNX (Pecanex homology)	+3.03
CCL3 (Chemokine MIP-1 α)	+3.48	GTF2H2 (General transcription factor IIH)	+2.83
PCNX (Pecanex homolog)	+3.48	KIAA1025 protein	+2.83
KIAA1025 protein	+3.25	TRIO (Triple functn domain guanyl NEF)	+2.83
TRA2A (transformer 2 α , mRNA splicing)	+3.03	CCL3 (Chemokine MIP-1 α)	+2.64
GTF2H2 (General transcription factor IIH)	+3.03	VEZATIN (transmembrane protein)	+2.46
SLC13A1 (Sodium ion transporter)	+3.03	TRA2A (transformer 2 α , mRNA splicing)	+2.46
PHC3 (Polyhomeotic like)	+2.83	APRIN (Androgen induced proliferin inhi)	+2.46
SEMA3D (Ig sema domain)	+2.83	KIAA0191 protein	+2.46
JUN	+2.64	TUBA3 (Tubulin α 3)	+2.30
PRKCBP1 (Protein kinase C binding)	+2.64	TMEM1 (transmembrane protein 1)	+2.30
GA17 (Dendritic cell protein.	+2.64	PHC3 (Polyhomeotic like)	+2.30
SEC24D (SEC24 related	+2.64	SMA3 (hydrolase activity)	+2.30
		AKAP9 (PRKA kinase anchor	+2.14

gene family D)		protein)	
SMA3 (hydrolase activity)	+2.46	INADL (PDZ, signaling cascade)	+2.14
ETS2 (v-ets oncogene homologue)	+2.46	EIFA1 (Translation initiation factor 4A-1)	+2.14
DNAJB1 (Hsp40 homolog subfamily B)	+2.30	GAPCENA (rab6 GTPase activating)	+2.14
CCT8 (Chaperonin containing TCP1)	+2.30	MGC10526 (Sugar phosphotransferase)	+2.00
EIFA1 (Translation initiation factor 4A-1)	+2.30	ATP8B1 (ATPase Class 1)	+2.00
TCS2 (Tuberous sclerosis 2)	+2.30	RPL23 (Ribosomal protein L23)	+2.00
CD72 (cell adhesion)	+2.30	RC3 (rabconnectin 3)	+2.00
HSPA1A (Heat shock protein 70kDa 1A)	+2.14		
ZFY (Zinc finger protein-Y-linked)	+2.14		
AIP1 (Atropin-1 interacting protein)	+2.14		
RPL23 (Ribosomal protein L23)	+2.14		
TMEM1 (transmembrane protein-1)	+2.14		
KIAA0191 protein	+2.14		
HSPA1B (Heat shock protein 70kDa 1B)	+2.00		
VEZATIN (transmembrane protein)	+2.00		
SERPINH1 (cysteine protease inhibitor)	+2.00		
MADH1 (mothers against decapentaplegic)	+2.00		
BAX (Bcl-2 associated protein)	+2.00		
APRIN (Androgen induced proliferin inhi)	+2.00		
APBB2 (amyloid β precursor protein-binding)	+2.00		
AKAP9 (PRKA kinase anchor protein)	+2.00		
APG16L (APG16 autophagy 16-like)	+2.00		
CENPC1 (Centromere protein C1)	+2.00		
ZF (HCF-binding transcription factor)	-6.50	ZF (HCF-binding transcription factor)	-6.06
CLorf29 (Chromosome 1 open reading frame)	-6.06	Cyclin E2	-3.03
FLJ20130 (hypothetical	-5.66	MARS (Methionine-tRNA synthetase)	-2.83

protein)		<u>POU4F1</u> (Pou domain transcription factor)	-2.46
F11 (Coagulation factor X1)	-4.00		
G1P3 (Interferon α inducible protein)	-3.73	<u>NRTN</u> (Neurturin)	-2.14
<u>HNRPD</u> (heterogeneous nuclear ribonucleoprotein D-like)	-3.25	PABPN1 Poly(A) binding protein, nuclear)	-2.00
<u>Cyclin E2</u>	-2.64	<u>HNRPD</u> (heterogeneous nuclear ribonucleoprotein D-like)	-2.00
FLJ20045 (hypothetical protein)	-2.46		
CEB1 (Cyclin E binding, ubiquitin ligase)	-2.30		
FBXO5 (F box only protein 5)	-2.30		
<u>NRTN</u> (Neurturin)	-2.3		
DUSP6 (Dual specificity phosphatase)	-2.14		
<u>POU4F1</u> (Pou domain transcription factor)	-2.14		
<u>MARS</u> (Methionine-tRNA synthetase)	-2.00		
HDAC9 (histone deacetylase 9)	-2.00		
CENPC1 (centromere protein C 1)	-2.00		

Underlined:- genes up- or down-regulated in both Fludalone or dEpob treated cells at 18 hrs.

* Interferon-inducible genes.

At 6 hrs., five genes were upregulated by Fludelone, 21 by dEpoB, and three genes were upregulated by both while three genes were downregulated by Fludelone, four by dEpoB, and no genes by both. At 18 hrs., 48 genes were upregulated by Fludelone, 26 by dEpoB and 18 genes were upregulated by both while 16 genes were downregulated by Fludelone, 7 by dEpoB and 6 genes were downregulated by both. The proposal is designated to test the hypothesis that the increased antitumor efficacy of Fludelone over dEpoB and earlier epithelones was due to some additional tumor targeting mechanism in addition to microtubule stabilization. We therefore determined what genes were differentially up or downregulated by Flu when compared to dEpoB-treated MM cells (Table 2-4).

Table 2-4: Global gene expression (Affymetrix chip – AU133 2.0) comparison of differential gene expression in RPMI-8226 (human multiple myeloma cell line) treated with dEpoB or Fludelone (at x10 IC50 dose) for 6 or 18 hrs. Genes shown were those changed selectively by Fudelone.

6hr Gene difference	Fold Change	18 hr gene difference	Fold Change
BCL3	+3.25	HMT-1 (Methyltransferase-like-1)	+11.3
Heme oxygenase	+2.30		
IFI 27* (Interferon (IFN) α -inducible)	-7.46	IFI 27* (Interferon (IFN) α -inducible)	-5.28
GIP3* (IFN α -inducible).	-6.06	GIP3* (IFN α -inducible).	-5.28
T RIM34 (Tripartite motif-containing)	-5.66	IFITM1* (IFN-induced transmembrane)	-3.25
IFTM1* (IFN-induced transmembrane)	-4.00	MX1* (Myxovirus resistance, IFN inducible).	-2.30
HLA-II DPα*	-3.73	DUSP4 (Dual specificity phosphatase)	-2.14
AIM2* (Absent in melanoma)	-2.64	PPAP2C (Phosphatidic acid phosphatase)	-2.14
PTPL (Tyrosine phosphatase-like)	-2.46	PLSCR1* (Phospholipid scramblase)	-2.00
TRIM22 (Tripartite motif-containing)	-2.46	IFIT1* (IFN-induced protein tetra-tricopeptide repeats.)	-2.00
CA4 (Carbonic anhydrase)	-2.14	OAS2* (2'-5'-oligoadenylate synthetase-2)	-2.00
MX1* (Myxovirus resistance, IFN inducible).	-2.00	TRIM22 (Tripartite motif)	-2.00

		containing-22) CEB1 (Cyclin-E binding protein)	-2.00
--	--	---	--------------

Underlined:- genes downregulated in both Fludalone or dEpoB-treated cells at both 6 and 18 hrs.

* Interferon-inducible genes.

- 5 At 6 hrs., two genes were upregulated, and at 18 hrs., one gene was upregulated. At 6 hrs., ten genes were downregulated, and at 18hrs., eleven genes were downregulated and 4 genes were downregulated at both times (*IFI27*, -7.46, -5.28), *GIP3* (-6.06, -5.28), *IFITM1* (-4.00, -3.25), *MX-1* (-2.00, -2.30). These are all are Interferon-inducible genes. Gene expression profiles of ovarian 1A9 and 1A9PTX22 ovarian xenografts
- 10 have been reported to show diminished expression of IFN response genes (*GIP3*, *IFI27*, *IFITM1*, *IFI6*, *ISG15*) 24 hrs after treatment with paclitaxel (Bani *et al.* "Gene expression correlating with response to paclitaxel in ovarian carcinoma xenografts" *Mol. Cancer Thera.* 2004;3:111-121; incorporated herein by reference). Three of these genes (*GIP3*, *IFI27*, *IFITM1*) were strongly overexpressed at 6 hrs after dEpoB
- 15 treatment of the RPMI-8226 cells but not with Fludalone, and these were also the genes whose expression most strongly distinguished the Fludalone and dEpoB gene profiles. Overexpression of IFN-responsive genes (*e.g.*, *IFR9*) has recently been associated with resistance to paclitaxel in tumor cell lines and findings support the possible involvement of mediators of IFN signaling other than IFN itself, in responsiveness to
- 20 paclitaxel (Luker *et al.* "Overexpression of IRF9 confers resistance to antimicrotubule agents in breast cancer cells" *Cancer Res.* 2001;61:6540-7; incorporated herein by reference). This novel IFN-independent role of *IRF9* in the development of resistance to antimicrotubule agents was reported in approximately one half of breast and uterine cancers (Luker *et al.* "Overexpression of IRF9 confers resistance to antimicrotubule
- 25 agents in breast cancer cells" *Cancer Res.* 2001;61:6540-7; incorporated herein by reference). Microarray analysis of tumor lines that were eEpoA or Taxol-resistant showed that the majority of genes which were highly expressed in EpoA-resistant tumors but not in Taxol-resistant tumors encoded interferon-inducible genes (Atadja *et al.* "Gene expression profiling of epothilone A-resistant cells" *Novartis Found Symp.* 2002;243:119-32; incorporated herein by reference). Remarkably, we found that 8/21
- 30 (38%) of all genes upregulated following dEpoB treatment of RPMI-8226 were IFN-

inducible and none of these were changed by Flu treatment (Table 2-2 through 2-4). The following IFN-inducible genes were shown to be upregulated in 8226 MM cells following 6 hrs or 18 hrs of dEpoB treatment and not changed with Fludelone treatment. In order of degree of upregulation following dEpoB they were 1.) Interferon (IFN) α -inducible *IFI 27* (+18.83 increase). This belongs to a family of small
5 interferon- α -inducible genes of unknown function that are upregulated in inflammatory skin disease, epithelial cancers and wound repair (Suomela *et al.* "Interferon alpha-inducible protein 27 (IFI27) is upregulated in Psoriatic skin and certain epithelial cancers" *J Invest. Dermatol.* 2004;122:717-721; incorporated herein by reference). 2.) IFN α -inducible protein (clone IFI-6-16) GIP3 (Bani *et al.* "Gene expression correlating with response to paclitaxel in ovarian carcinoma xenografts" *Mol. Cancer. Thera.* 2004;3:111-121; incorporated herein by reference) (+5.28 increase). 3.) IFN-induced transmembrane protein-1, IFTM1 (Bani *et al.*) (+4.92 increase). 4.) Myxovirus resistance, IFN inducible, MX1 (+2 increase at 6 hrs and +2.3 at 18 hrs). The MX1
15 genes confer selective innate resistance to influenza virus (Staeheli, *Ad. Viral Res.* 38:147, 1990; incorporated herein by reference). They may also serve basic cellular functions possibly as GTP-binding proteins (Arnheiter H and Meier E. "Mx proteins: antiviral proteins by chance or necessity?" *New Biol.* 1990; 90 851; incorporated herein by reference). 5.) Phospholipid scramblase 1 gene PLSCR1. Transcriptional control of
20 the gene is entirely regulated by a single IFN-stimulated response element located at the first exon (Zhou *et al.* "Transcriptional control of the human plasma membrane phospholipid scramblase 1 gene is mediated by interferon-alpha" *Blood* 2000;95:2593-2599; incorporated herein by reference). PLSCR1 was implicated in remodeling of plasma membrane phospholipids and mobilization of phosphatidylserine to the cell
25 surface. It may contribute to the rapid trans-bilayer movement of plasma membrane phospholipids that is observed in injured or apoptotic cells that are exposed to elevated intracellular Ca^{++} . Studies show it can also translocate to nucleus and may act as a transcription factor (Ben-Efraim *et al.* "Phospholipid Scramblase 1 is imported into the nucleus by a receptor-mediated pathway and interacts with DNA" *Biochem.* 2004;43:35181; incorporated herein by reference). 6.) Interferon-induced protein with tetrcopeptide repeats, IFITI (+2.3 increase). It has been associated with systemic
30 lupus erythematosus and protein-protein studies show that it may activate Rho proteins

by interaction with Rho/Rac guanidine nucleotide exchange factors (Ye *et al.* "Protein interaction for an interferon-inducible system-3526mic lupus associated gene, IFIT1" *Rheumatology* 2003;42:1155-63; incorporated herein by reference). 7.) 2'-5'-oligoadenylate synthetase-2, OAS2 (+2 increase). This family (OAS1, OAS2, OAS3, OAS4) of interferon-induced genes are implicated in stress responses and are important for the anti-viral activity of interferon (Chebath, *Nature* 330:587, 1987; Meurs *et al.* *J. Virol.* 66:5804, 1992; each of which is incorporated herein by reference). OAS2 catalyzes the synthesis of adenosine oligomers (2-5A). These then activate RNase L, an endoribonuclease latent in most mammalian cells, that in turn can inactivate viral (picornaviruses *e.g.* mengovirus) and cellular RNA (Anderson *et al.* *Eur. J. Biochem.* 2004;271:628-36; incorporated herein by reference). 8.) Histocompatibility class II DP α 1, HLA-DPA1 (+2 increased). MHC-Class II are known mediators of the biological functions of IFN (Tissot *et al.*, "Molecular cloning of a new Interferon-induced factor that represses human immunodeficiency virus Type 1 long terminal repeat expression" *J. Biol. Chem.* 1995; 270:14891-14898; incorporated herein by reference).

Summary

The CAG myeloma cell line displays many features of clinical multiple myeloma with both phenotype (CD38+CD138+ CD45-) and *in vivo* engraftment. A xenograft NOD-SCID myeloma mouse model was established by intravenously injection of 10~15 million of CAG cells engineered with fusion HSV-TK-eGFP-Luciferase gene. Therefore whole animal imaging by luciferase bioluminescence can be applied to evaluate tumor burden and drug efficacy in real-time, non-invasively reporting in living mice. The myeloma mouse exhibits bone marrow infiltration and pathological osteolytic bone lesion after 7 to 20 days tumor injection. After implantation of tumor cells, the mice were randomized and divided into vehicle control, dEpoB and Fludelone treatment groups. The dose of both dEpoB and Fludelone was 20 mg/kg, and the drugs were administered by intraperitoneal route. The average number of dose was 10 for dEpoB and 12 for Fludelone during the first 30 days of treatment. In addition, five doses of Velcade (6.25 ug/mouse, i.v) were administered in 3 out of 7 mice initially treated with Fludelone between 35 to 45 days. The results

showed that myeloma mice treated with Fludelone had significantly decreased tumor burden evaluated by bioluminescence imaging versus mice treated with dEpoB and controls. All control mice died within 30 days after initiation of treatment, however, Fludelone treated mice had at least doubled the survival time than controls. There was
5 no difference in survival between control and dEpoB treated mice. In combination with the proteasome inhibitor, Velcade, the tumors localized in vertebrate column and femurs can be greatly reduced, indicating that this combination can attack myeloma cells even under marrow microenvironment protection.

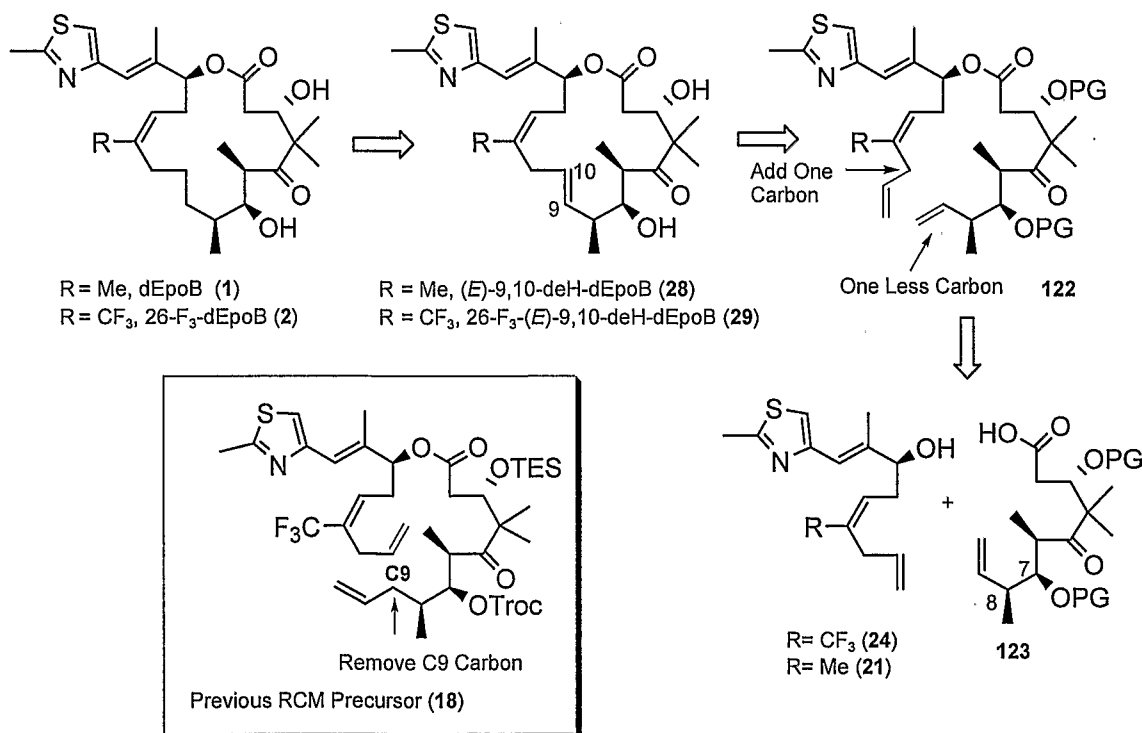
Previous data has shown that myeloma cells treated with epothilones exhibited
10 typical apoptosis characteristics. It is not clear whether caspase is involved in this process. Firstly, we investigated the activation of caspase 3, an effector caspase in the caspase pathway, in myeloma cells, by Western blot. With either dEpoB or Fludelone treatment, the myeloma cells showed increased 17 kd cleaved caspase 3, which correlates with its activity. Immunohistochemical staining of CAG myeloma cells
15 using antibody specific to cleaved caspase-3 was used to show cytoplasmic and perinuclear localization features in apoptotic cells. These data confirmed that caspase was activated in epothilone treated cells. Secondly, we attempted to assay the activity of caspase 8 and 9, initiator caspase in the caspase pathway, by fluometric method. Again both caspase 8 and 9 activities were found to be increased in myeloma cells.
20 Note that increased caspase 9 activity was more prominent than caspase 8. The signals triggering the activation of caspase 8 or 9 are actively under investigation.

Drug cytotoxicity to hematopoietic stem cells is always a major concern. Human CD34+ stem cells were isolated from umbilical cord blood and incubated with either dEpoB or Fludelone alone for 24 hours. After washing out the drugs, apoptosis
25 assays and 2 weeks progenitor assays were performed to evaluate the influence of epothilone to stem cells. Our data shows that there is no significant toxicity of dEpoB or Fludelone on non-cycling stem cells.

Example 3: (E)-9,10-Dehydroepothilones: A New Class of Microtubule
30 **Stabilizing Antitumor Agents with Highly Promising Characteristics in Murine Xenograft Models**

The synthesis of 26-F₃-[16]dEpoB (**2**) containing the usual 16-membered ring can be accomplished via a highly convergent strategy, related to that employed in the synthesis of 27-F₃-[17]ddEpoB (**19**). This strategy envisioned the formation of a *E*-9,10-olefin via a ring-closing metathesis reaction shown below (For an early instance of macrocyclization in a complex setting via a RCM see: Sinha, S. C.; Sun, J. *Angew. Chem. Int. Ed.* **2002**, *41*, 1381; incorporated herein by reference). We anticipated that chemoselective reduction of the *E*-9,10-olefin of **28** and **29** would furnish dEpoB (**1**) and the desired 26-F₃-dEpoB (**2**). The RCM precursor would be prepared by the union of the two fragments (**21** or **24**) and **123** through an esterification reaction. Coupling partner **123** would be constructed by deletion of the methylene spacer group (found in our earlier routes cf. compound **18**) between the secondary methyl group at C8. As noted above, the *in vitro* level findings with the 17-membered epothilones containing the skipped diene arrangement (see **18**), underscored the need for a corresponding investigation of the biological consequences of such a diene in the familiar 16-membered lactone setting. Given the presence of the *cis* 12,13-olefin in a dEpoB context, such a skipped diene would necessarily contain a 9,10-double bond.

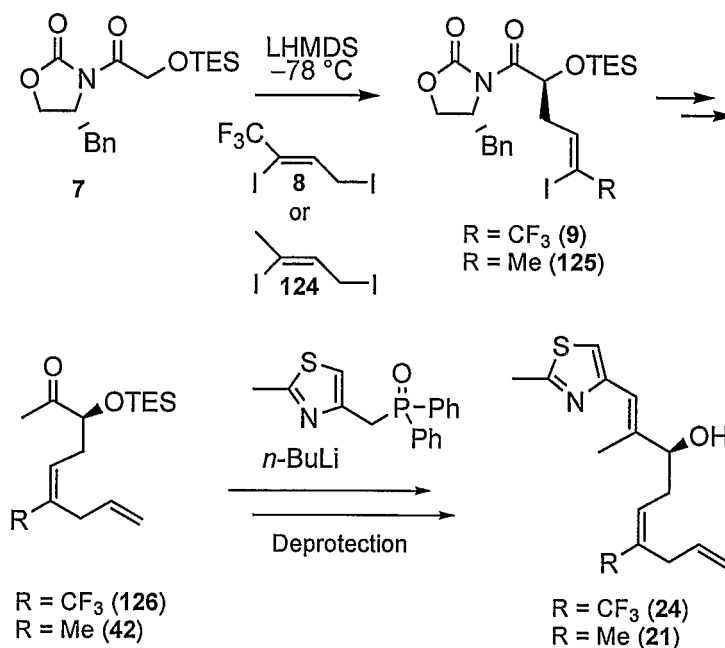
Retrosynthesis of the 9,10-dehydroepothilones and 26-F₃-dEpoB



Alkylation of the oxazolidinone **7** (Lee *et al.*, *J. Am. Chem. Soc.* **2001**, *123*, 5249; incorporated herein by reference) with the readily synthesized trifluoro and methyl allyl iodides (**8** and **124**, respectively) allowed for the C15 stereo center to be set in the appropriate absolute configuration (Evans, D. A.; Morrissey, M. M.; Dorow, R. L. *J. Am. Chem. Soc.* **1985**, *107*, 4346; Paterson, I.; Bower, S.; McLeod, M. D. *Tetrahedron Lett.* **1995**, *36*, 175; each of which is incorporated herein by reference) with high diastereomeric access, (see products **9** or **125**). The latter were converted to their corresponding Weinreb amides (Nahm *et al. Tetrahedron Lett.* **1981**, *22*, 3815; Levin *et al. Synth. Commun.* **1982**, *12*, 989; each of which is incorporated herein by reference) and thence to **126** and **42** en route to **24** and **21** by nucleophilic methylation (MeMgBr) and appropriate Horner Wittig olefinations (Lythgoe *et al. Tetrahedron Lett.* **1975**, *40*, 3863; Lythgoe, *Chem. Soc. Rev.* **1981**, 449; Toh *et al. J. Org. Chem.* **1983**, *48*, 1414; Baggiolini *et al. J. Org. Chem.* **1986**, *51*, 3098; each of which is incorporated herein by reference). While the use of such oxazolidinones as chiral auxiliaries had been pioneered by Evans and associates (Evans *et al. J. Am. Chem. Soc.* **1985**, *107*, 4346; Paterson *et al. Tetrahedron Lett.* **1995**, *36*, 175; each of which is incorporated herein by reference), its application to the synthesis of optically defined glycolates by alkylation (rather than by hydroxylation) had not been developed.

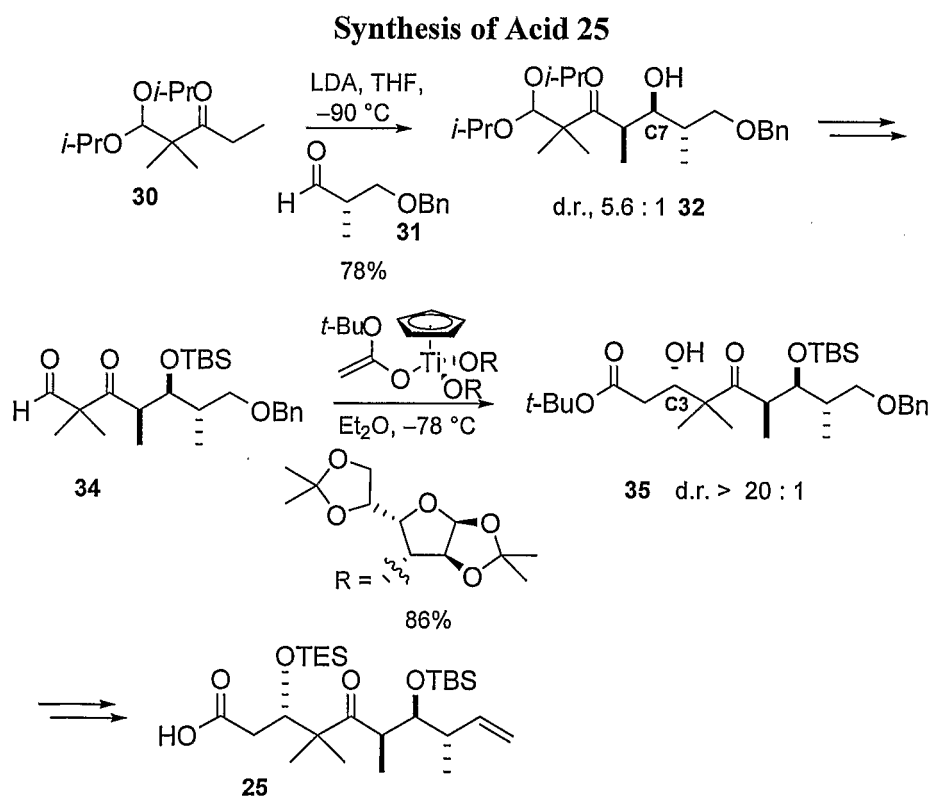
20

Synthesis of Fragments 21 and 24



The synthesis of the polypropionate fragment **25** was enabled by two critical aldol reactions, which established the relative configuration of the C3, C6, and C7 stereocenters. The first aldol reaction involved reaction of the Z-enolate of the ethyl ketone **30** with Roche aldehyde **31** (Cohen *et al. Org. Chem.* **1976**, *41*, 3505; Nagaoka *et al. Tetrahedron* **1981**, *37*, 3873; Roush *et al. J. Am. Chem. Soc.* **1990**, *112*, 6348; each of which is incorporated herein by reference) to provide the desired **32** with high diastereoselectivity. Recourse to **31**, allowed by this synthesis, is a significant advantage over the use of earlier aldehydes which required resolution for the attainment of enantiomerically pure starting materials.

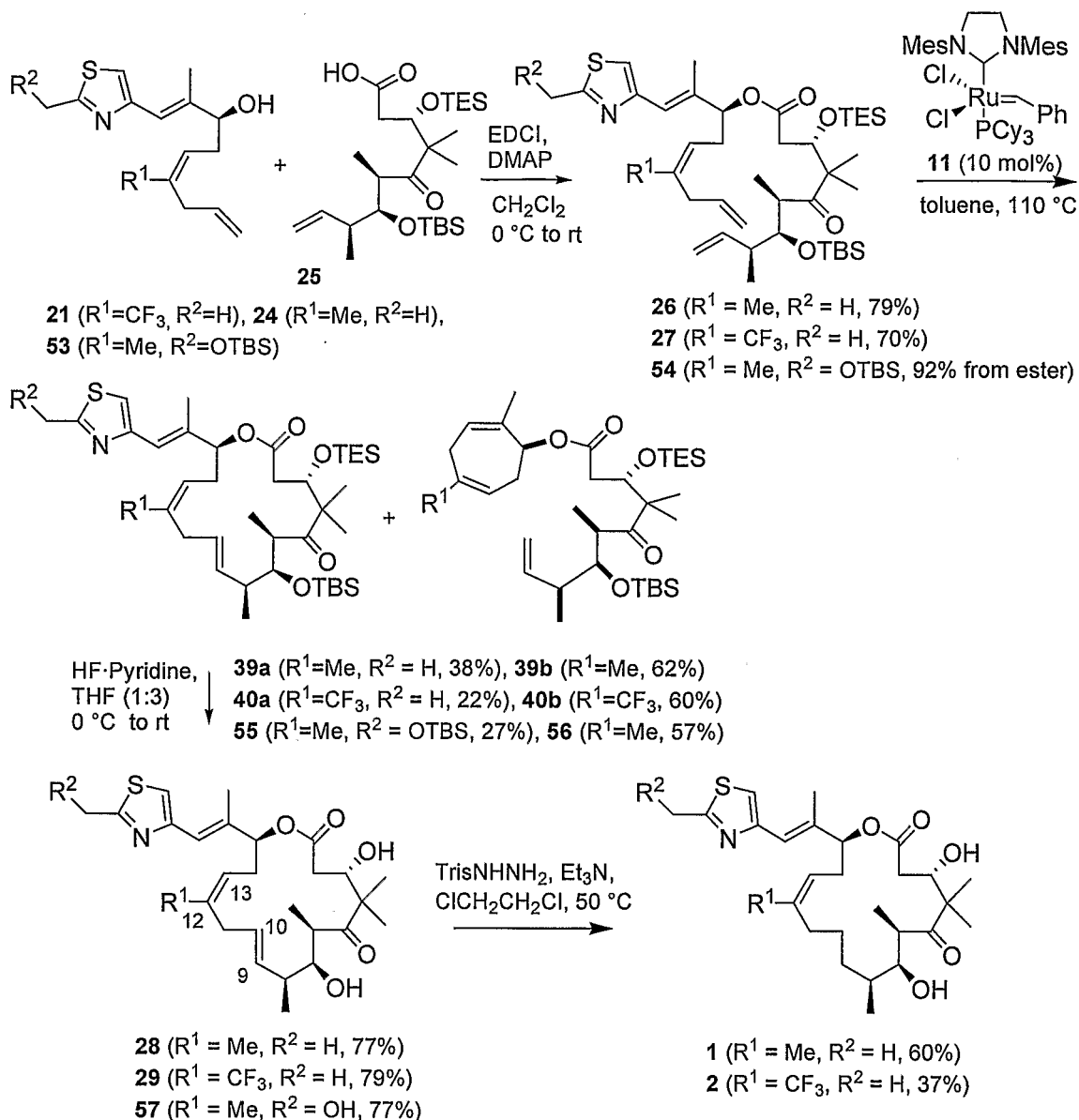
Protection of the C7-alcohol followed by hydrolysis of the acetal provided the desired aldehyde **34** and set the stage for the second aldol reaction. Reaction of **34** with Duthaler's DAG (diacetone glucose) Ti-enolate (Duthaler *et al. Angew. Chem. Int. Ed. Engl.* **1989**, *28*, 495; incorporated herein by reference) afforded the desired hydroxy tert butyl ester **35** with very high (>95%) diastereoselectivity. The latter was then converted to the desired acid **25** in straightforward steps.



The allylic alcohols **24**, **21**, and the C₁-C₉ acid fragment **25** were united through an EDCI esterification protocol, thus providing the RCM precursors **26** and **27**, respectively. Ring-closing metathesis reactions of **26** and **27** were carried out using an RCM catalyst (Scholl *et al. Tetrahedron Lett.* **1999**, *40*, 2247; Grubbs *et al. Acc. Chem. Res.* **1995**, *28*, 446; Trnka *et al. Acc. Chem. Res.* **2001**, *34*, 18; *Alkene Metathesis in Organic Chemistry* Ed.: Fürstner, A.; Springer, Berlin, **1998**; Fürstner, A. *Angew. Chem. Int. Ed. Engl.* **2000**, *39*, 3012; Schrock, *Top. Organomet. Chem.* **1998**, *1*, 1; each of which is incorporated herein by reference) in toluene. These reactions did indeed provide the *trans* isomers **39a** and **40a**. However, the major products were **39b** and **40b** which had arisen from the obvious alternate RCM pathway. These unwanted RCM isomers predominated over the desired **39a** and **40a** by ratios of ca. 3:1. Finally, deprotection of the silyl ethers of **39a** and **40a** with HF-pyridine led to the desired *E*-9,10-dehydro-epothilones **28** (White *et al. J. Am. Chem. Soc.* **2001**, *123*, 5407; White *et al. J. Am. Chem. Soc. (Addition/Correction)* **2003**, *125*, 3190; each of which is incorporated herein by reference. With compound **28** of rigorously proven structure in hand, we were surprised to find that its spectral properties were not congruent with those previously reported for a compound presumed to be the same entity. The actual structure of the compound previously assigned as **28** has now been re-evaluated. In retrospect, it is clear that **28** had not been previously prepared and, in fact the whole family of (*E*)-9,10-dehydroepothilones reported here is a new genus.) and **29**. As planned, the latter were converted to dEpoB (**1**) and 26-trifluoro-dEpoB (**2**) via diimide reduction of the *E*-9,10-olefin (Corey *et al. Tetrahedron Lett.* **1961**, 347; Pasto *et al. Org. React.* **1991**, *40*, 91; each of which is incorporated herein by reference).

By corresponding methodology, we synthesized the 9,10-dehydro-dEpoF (**57**) see below. The selective di-imide reductions of the *E*-9,10-olefins validated the structures of the various synthetic intermediates described above, thereby prompting re-evaluation of previous assignments in the literature.

Synthesis of 9,10-Dehydroepothilones



5 Examination of synthetic analogs (**28**, **29**, and **57**), in cell culture settings (see Supplemental Edition), revealed them to exert stronger inhibitory effects on various sensitive and MDR tumor cell lines than are exhibited by our current clinical candidate, dEpoB (**1**). The impressive cell growth inhibition exhibited by epothilones **28**, **29**, and **57** across a range of various drug-resistant tumors, prompted determination of the blood

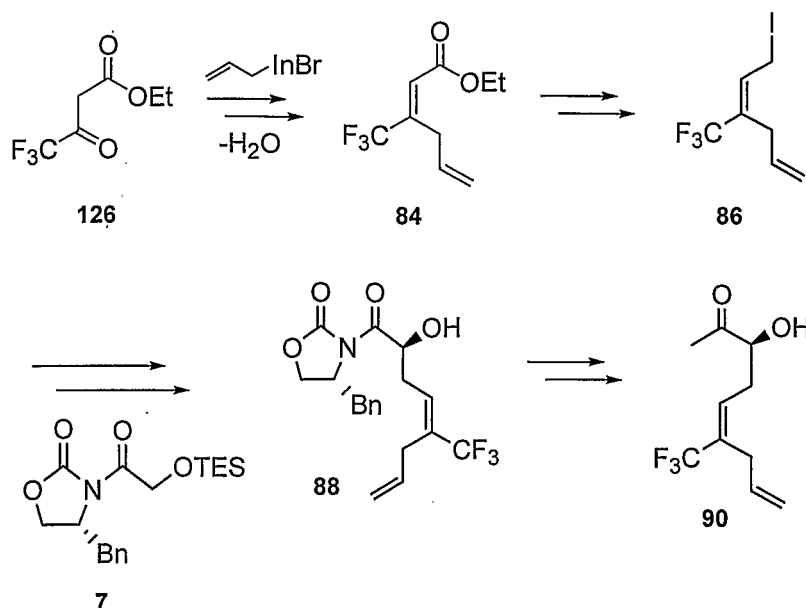
10 plasma stability of these new (*E*)-9,10 congeners. We recall that (*E*)-10,11-dehydro-dEpoB (Epo490) exhibits very poor plasma stability. Indeed, it was this plasma instability which had blocked further development of Epo490 (**6**). By contrast, on

exposure of **28**, **29**, and **57** to murine plasma, we observed a much slower drug degradation as compared to dEpoB (**1**) (by a factor of *ca.* 7). This stability constitutes a substantial advance, from a drug availability perspective, relative to dEpoB (**1**), not to speak of epo 490 (**6**). Based on the preliminary cell culture and pharmacokinetic data
5 of the (*E*)-9,10-dehydro derivatives **28** and **29**, it would be appropriate to advance them for *in vivo* investigations. Such studies are, of course, rather more intensive of drug availability than *in vitro* measurements (Rivkin *et al.* *J. Am. Chem. Soc.* **2003**, *125*, 2899; Chou *et al.* *Angew. Chem., Int. Ed.* **2003**, *42*, 4761-4767; Yoshimura *et al.* *Angew. Chem., Int. Ed.* **2003**, *42*, 2518-2521; each of which is incorporated herein by
10 reference. For recent examples of alternative potent epothilone analogs, see: Altmann *et al.* *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2765; Nicolaou *et al.* *Chem. Biol.*, **2000**, *7*, 593; each of which is incorporated herein by reference).

This requirement, and indeed the possible eventual need to prepare multigram quantities of 9,10-dehydro derivatives for further development, prompted a significant
15 reassessment of our total synthesis route. Of course, the single most serious problem was that the RCM reaction on **26**, **27**, and **54** which produced **39b**, **40b**, and **56** only as minor products. The major pathway involved an RCM reaction which was strictly confined to the *O*-alkyl sector of **26**, **27**, and **54**, leading primarily to the undesired **39b** and **40b**. Accordingly, it was decided to attempt to defer introduction of the thiazole
20 (by olefination) subsequent to the RCM (*vide infra*).

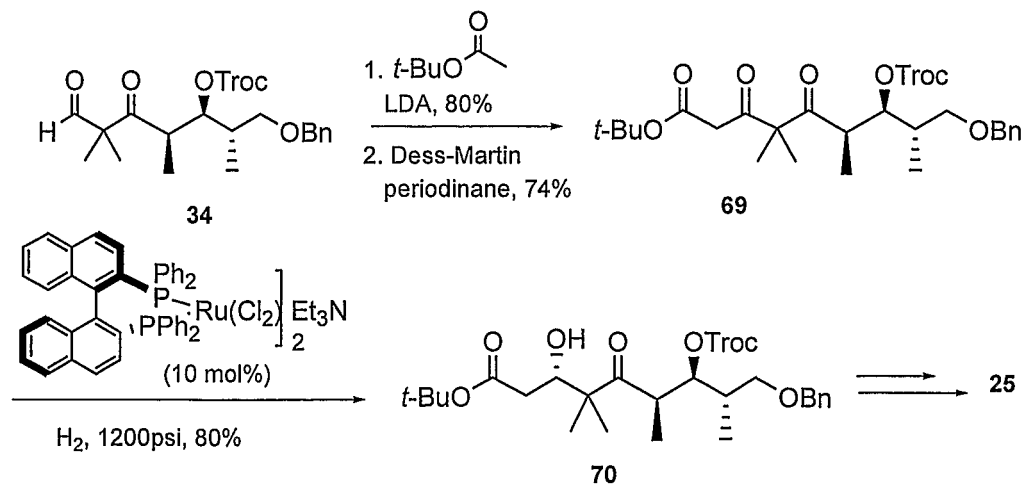
With eventual processibility in multigram scales as our goal, the syntheses of the alkyl and acyl fragments entering into the RCM reaction were re-structured. Compound **86** was readily synthesized as shown. It was used to alkylate oxazolidinone **7** in high diastereomeric excess. Following deprotection of the OTES group, and
25 nucleophilic methylation, compound **90** was in hand. This α -hydroxyketone would serve as the acyl group acceptor in formation of the central ester to prepare the all critical RCM. An obvious concern was the possible vulnerability of such a hydroxyketone as an acyl acceptor to partial racemization or diversion to regioisomeric α -ketols.

30 **Processible Synthesis Fragment 90**



A serious problem in our earlier synthesis of the acid fragment **25** was the very expensive and technically demanding Duthaler chemistry (Duthaler *et al. Angew. Chem. Int. Ed. Engl.* **1989**, 28, 495; incorporated herein by reference) to generate the desired *S* stereochemistry at the C3. To circumvent this problem, the aldol reaction was carried out without any chiral auxiliary to provide a 1:1 mixture of the corresponding β -hydroxy ketone. Reagent controlled asymmetric reduction of the derived keto function (see compound **69**), using Noyori conditions (Noyori *et al. J. Am. Chem. Soc.* **1987**, 109, 5856; incorporated herein by reference) generated the desired *S* stereochemistry at the C3 in high diastereomeric excess. The now available β -hydroxy ester **70** was transformed to acid **25** in several steps following earlier protocols (Chou *et al. Proc. Natl. Acad. Sci. U.S.A.* **2001**, 98, 8113; incorporated herein by reference).

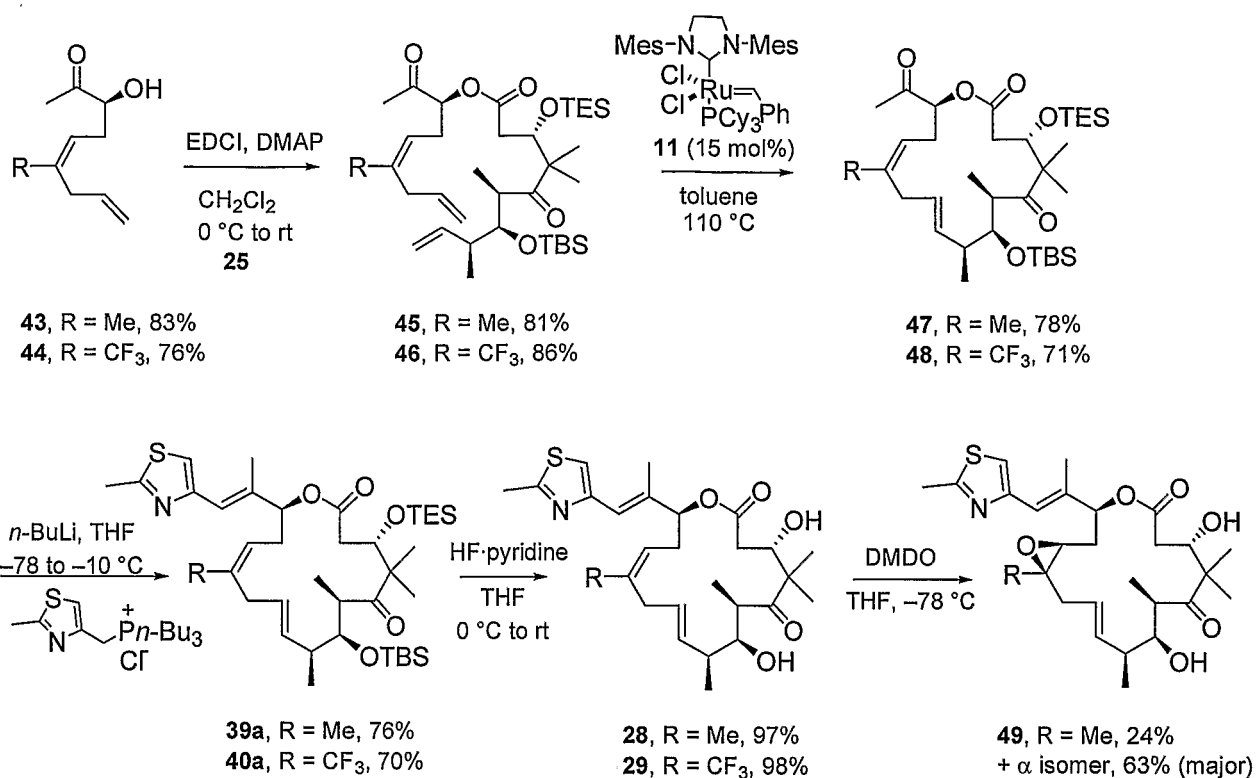
Processible Synthesis of Acid 25



Remarkably, esterification of the resultant hydroxyketones **43** and **44** with the C₁-C₉ acid fragment **25** provided the corresponding RCM cyclization precursors **45** and **46** without noticeable racemization at C15, or loss of integrity of the initial α -ketol linkage. The ring-closing metathesis reaction of **45** and **46** was carried out using an RCM catalyst (Scholl *et al. Tetrahedron Lett.* **1999**, *40*, 2247; Grubbs *et al. Acc. Chem. Res.* **1995**, *28*, 446; Trnka *et al. Acc. Chem. Res.* **2001**, *34*, 18; *Alkene Metathesis in Organic Chemistry* Ed.: Fürstner, A.; Springer, Berlin, **1998**; Fürstner, A. *Angew. Chem. Int. Ed. Engl.* **2000**, *39*, 3012; Schrock, *Top. Organomet. Chem.* **1998**, *1*, 1; each of which is incorporated herein by reference) in toluene. This reaction, now uncomplicated by alternate metathesis pathways, provided exclusively the *trans* isomers **47** and **48** in high yields. Installation of the thiazole moiety via a Wittig reaction proceeded with high E/Z selectivity and yield, to provide **28** and **29** following deprotection of the two silyl ethers (Hindupur *et al. Tetrahedron Lett.* **2000**, *2*, 7341; incorporated herein by reference. Attempts to apply Avery's protocol for the installation of the thiazole gave the desired product in low yield and with poor E/Z selectivity). This route meets the standards of amenability toward large scale synthesis.

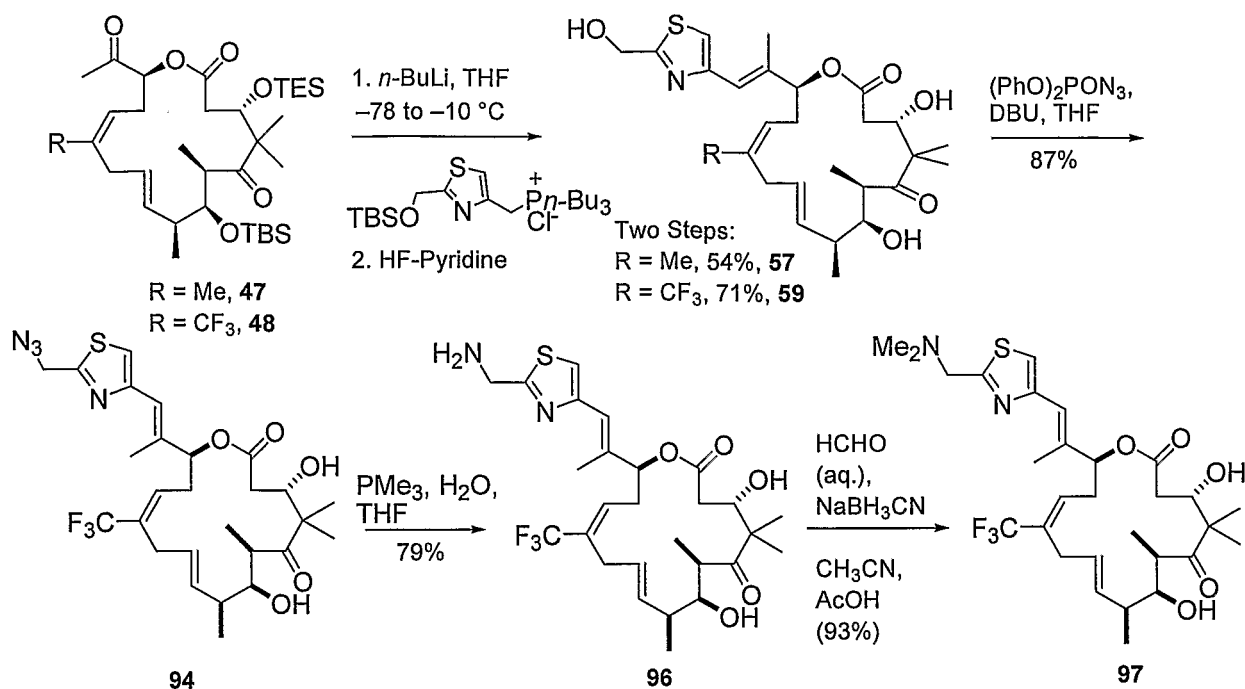
We considered whether the incorporation of C₉-C₁₀ olefin in epothilone B (**51**, EpoB) would alter its biological profile in the same direction as was the case with its 12,13 desoxy counterparts. Toward this end, we studied the epoxidation of **28** with 2,2'-dimethyldioxirane (DMDO). The reaction indeed proceeded with high chemoselectivity at the more substituted C12-C13 olefin. There was obtained an 87% yield of a 1:2.6 ratio of the (*E*)-9,10-dehydroepothilone B (**49**) and its diastereomer bearing the α -12,13-oxirane (structure not shown). *In vitro* studies with **49** (whose configurations at C12 and C13 was established by its reduction to afford Epo B) revealed it to be roughly 2 - 4 fold more potent than the parent EpoB (**51**) in various cell lines. While compound **49** proved to be the most potent epothilone we encountered in our program, its narrow therapeutic index in xenografts, as well as its difficult accessibility (*vide supra*) served to discourage further its pre-clinical development. Interestingly, the unnatural α -oxirane was virtually devoid of activity.

Processible Synthesis of (*E*)-9,10-Dehydroepothilones



Another advantage of the restructured (second generation) synthesis, described above is that a variety of heterocycles can be installed via ketone intermediates **39a** and **40a**. This point is well highlighted by the synthesis of 9,10-dehydro-dEpoF. Wittig reaction of the ketone with the appropriate phosphonium ylides afforded the desired 9,10-dehydro-dEpoF compounds **57** and **59** in high yield and with high E/Z selectivity. Furthermore, we were able to efficiently convert the 21-hydroxy **59** to derivatives of the type **96** and **97**, containing amino functionality at C21 in several steps as shown below.

Diversification of C-21 of (*E*)-9,10-Dehydroepothilones



The fully synthetic epoxythilone analogs have been evaluated against a variety of cell types to determine their antitumor potential.

5 The most salient features of our findings, are as follow: One can expect a loss of ca. an order of magnitude in replacement of the C12-C13 β-epoxide by a E-12,13 double bond (compare EpoB and dEpoB in the sensitive CCRF-CEM cell line). Another expectation, is that inclusion of an E-9,10 double bond, in addition to the Z12,13-olefin leads to a significant increase in cytotoxicity across several cell lines.

10 Still another instructive trend was seen in comparing 12-trifluoro-*E*-9,10-dehydro-dEpoB (**29**) (Fludelone), with the corresponding *E*-9,10-dehydro compound **28**. Inclusion of the three fluorine atoms at C26, attenuates cytotoxicity by up to a factor of 4, relative to (**1**). This attenuation effect of the 12-trifluoromethyl function is also seen in compounds lacking the 9,10-unsaturation (compare dEpoB (**1**) and 12-trifluoro-dEpoB (**2**)).

15 Given these data, and given the accessibility of these 9,10-dehydro compounds (including 12-trifluoro congeners) through chemical synthesis, we were in a position to initiate in vivo experiments on our most promising compounds. We describe here some particularly striking and promising results with compound **29** (Fludelone), which has emerged as a most exciting possibility for advancement to clinical evaluation. *In vivo* experiments were carried out using human tumor xenografts in immunodeficient nude

20

mice. For all their imperfections, such models in oncology are widely used in evaluating (Fiebig, H. H.; Berger, D. P.; Preclinical Phase II trials. In: Boven, E. and Winograd, B., Editors, *The Nude Mouse in Oncology Research*, CRC Press, Boca Raton (1995), 318; incorporated herein by reference) the potential of anti-tumor lead
5 compounds en route to clinical development.

Remarkably, treatment of MX-1 xenografts with 30 mg/kg dosages of Fludelone, resulted in complete tumor disappearance and the absence of any relapse for over two months after suspension of treatment (See Figure 6A). Most importantly, these therapeutic successes can be achieved either by 6 hr – i.v. infusion or by *oral*
10 administration (See Figures 6B and 10A). On the other hand, treatment of the MX-1 xenografts by oral administration of taxol did not affect the tumor (See Figure 6B and 10A). It goes without saying that if translatable to the human clinical setting, achievement of oral activity could be of significant advantage.

Taxol-resistant tumor xenografts (Figure 7A) as well as human colon carcinoma
15 (HCT-116, Figure 9A) can also be cured with Fludelone by i.v. infusion. The experiments using human mammary carcinoma (MX-1) and human colon carcinoma (HCT-116) xenografts in nude mice lasted 6.0 and 6.6 months, respectively. There was no tumor relapse in either experiment during 4.3 and 5.3 months, respectively, following the cessation of treatment. For the HCT-116 experiment, taxol and
20 Fludelone were compared at 20 mg/kg and both achieved tumor disappearance. The taxol treated group, relapsed 1.1 months after treatment was discontinued, whereas Fludelone-treated animals were tumor free for over 5.3 months.

These results have involved a particularly long and thorough therapeutic study using xenografts and report remarkably long periods of complete remission with
25 parenteral or oral administration of a single antitumor agent.

Experimentals:

General Pharmacology Methods:

Tumor and Cell Lines. The CCRF-CEM human lymphoblastic leukemia cells and their
30 vinblastine resistant subline (CCRF-CEM/VBL₁₀₀, 720-fold resistance) were obtained from Dr. William Beck of the University of Illinois, Chicago, and CCRF-CEM/Taxol (44-fold resistance *in vitro*) were produced by exposing CCRF-CEM cells to increasing

sublethal concentration (IC₅₀-IC₉₀) of paclitaxel during the six months. Human mammary carcinoma (MX-1), human lung carcinoma cells (A549) and human colon carcinoma (HCT-116) cells were obtained from American Type Culture Collection (ATCC, Rockville, MD).

5

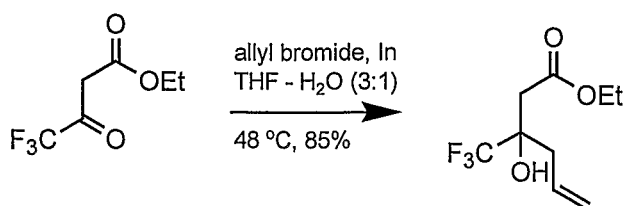
Animals. Athymic nude mice bearing the nu/nu gene were obtained from NCI, Frederick, MD and used for all human tumor xenografts. Except otherwise indicated for the female nude mice, male nude mice 6 weeks or older weighing 20-22g or more were used. Drugs were administered via the tail vein for 6 hours by iv infusion using a
10 homemade infusion mini-catheter and restrainer. A programmable Harvard PHD2000 syringe pump with multitrack was used for the iv infusion. A typical 6hr infusion volume for each drug in Cremophor/ethanol (1:1) was 100μl in 2.0ml of saline. For oral administration, both Fludelone and Taxol were dissolved in ethanol and diluted 5-fold with Tween-80. The Taxol solution should be used within 5min to avoid precipitation.
15 Tumor volume was assessed using a caliper to measure the length x width x height (or width). For tumor-bearing nude mice, the body weight during the course of experiment refers to the total weight minus the weight of the tumor. All animal studies were conducted in accordance with the guidelines for the National Institute of Health Guide for the Care and Use of Animals and the protocol approved by the Memorial Sloan-
20 Kettering Cancer Center's Institutional Animal Care and Use Committee.

Cytotoxicity Assays. In preparation for *in vitro* cytotoxicity assays, cells were cultured at an initial density 2-5 x 10⁴ cells per milliliter. They were maintained in a 5% CO₂-humidified atmosphere at 37 °C in RPMI medium 1640 (GIBCO/BRL) containing
25 penicillin (100 units/mL), streptomycin (100μg/mL, GIBCO/BRL), and 5% heat-inactivated FBS. For solid tumor cells growing in a monolayer (such as HCT-116 and A549), cytotoxicity of the drug was determined in 96-well microtiter plates by using the sulforhodamine B method (Skehan *et al. J. Natl. Cancer Inst.* **1990**, 82, 1107; incorporated herein by reference). For cells grown in suspension (such as CCRF-CEM
30 and its sublines), cytotoxicity was measured, in duplicate, by using the 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-5-carboxanilide)-2H-terazodium hydroxide (XTT) microculture method (Scudiero *et al. Cancer Res.* **1988**, 48, 4827; incorporated herein

by reference) in 96-well microtiter plates. For both methods, the absorbance of each well was measured with a microplate reader (Power Wave XS, Bio-Tek, Winooski, VT). Dose-effect relationship data from 6 to 7 concentrations of each drug, in duplicate, were analyzed with the median-effect plot by using a computer program (Chou, T.-C. & Talalay, P.T. *Adv. Enzyme Reg.* **1984**, 22, 27; Chou, T.-C. & Hayball, M. *CalcuSyn for Windows* (Biosoft, Cambridge, U.K.) (1997); each of which is incorporated herein by reference).

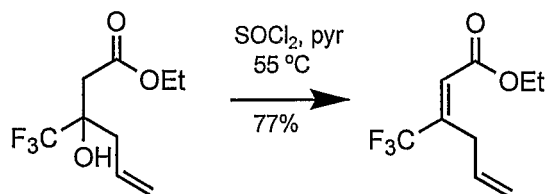
General Chemical Methods: Reagents obtained from commercial suppliers were used without further purification unless otherwise noted. The following solvents were obtained from a dry solvent system (passed through a prepacked column of alumina) and used without further drying: tetrahydrofuran, methylene chloride, diethyl ether, benzene, and toluene. Triethylamine, *N,N*-diisopropylethylamine, diisopropylamine, pyridine, and 2,6-lutidine were distilled from calcium hydride. All air and water sensitive reactions were performed in flame-dried glassware under a positive pressure of prepurified nitrogen gas or argon gas. NMR (^1H and ^{13}C) spectra were recorded on Bruker AMX-400 MHz or Bruker Advance DRX-500 MHz as noted individually, referenced to CDCl_3 (7.27 ppm for ^1H and 77.1 ppm for ^{13}C). Infrared spectra (IR) were obtained on a Perkin-Elmer FT-IR model 1600 spectrometer. Optical rotations were obtained on a JASCO model DIP-370 digital polarimeter. Low resolution (electrospray) mass spectra were recorded on PE SCIEX API 100. Analytical thin-layer chromatography was performed on E. Merck silica gel 60 F254 plates. Compounds which were not UV active were visualized by dipping the plates in ethanolic *para*-anisaldehyde solution, ethanolic phosphomolybdic acid, or ceric ammonium molybdate or and heating. Preparative thin layer chromatography was performed using the indicated solvent on Whatman[®] (LK6F Silica gel 60A) TLC plate. Silica gel chromatography was performed using the indicated on Davisil[®] (grade 1740, type 60A, 170-400 mesh) silica gel.

Chemical shifts are reported in δ values relative to chloroform (δ 7.24 for proton and δ 77.0 for carbon NMR).



To a solution of ethyl 4,4,4-trifluoroacetoacetate (24.0 mL, 0.164 mol) in THF-water (3:1 = V:V, 320 mL) at room temperature was added allyl bromide (20.0 mL, 1.4 equiv) followed by indium (powder, -100 mesh, 25 g, 1.3 equiv) and the resulting mixture was stirred at 48 °C for 15 h. The reaction mixture was cooled to room temperature, quenched with 2 N aq. HCl (400 mL) and extracted with CH₂Cl₂ (400 mL, 2 x 200 mL). Combined organics were dried (MgSO₄), filtered, and concentrated *in vacuo*. Flash chromatography (hexanes → hexanes-ether 10:1 → 8:1 → 6:1 → 4:1) gave the alcohol as clear oil (31.64 g, 85% yield): IR (film) 3426 (br m), 2986 (m), 1713 (s), 1377 (m), 1345 (m), 1301 (m), 1232 (m), 1173 (s), 1095 (m), 1023 (m), 927 (m) cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 5.82 (m, 1 H), 5.15 (m, 3 H), 4.17 (m, 2 H), 2.59 (m, 1 H), 2.58 (d, *J* = 3.4 Hz, 2 H), 2.29 (dd, *J* = 14.2, 8.6 Hz, 1 H), 1.24 (t, *J* = 7.2 Hz, 3 H); ¹³C NMR (100 MHz, CDCl₃) δ 172.08, 130.89, 125.65 (q, *J* = 280 Hz), 120.27, 73.79 (q, *J* = 28 Hz), 61.55, 38.97, 35.65, 13.82; high resolution mass spectrum *m/z* 227.0895 [(M+H)⁺; calcd for C₉H₁₄O₃F₃: 227.0895].

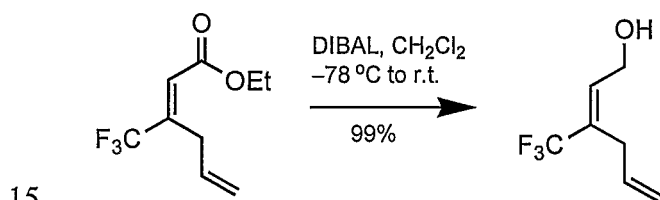
The alcohol is volatile. After the column chromatography, the alcohol was not completely concentrated. The yield was determined from overall weight and ratio between product and solvents obtained by integration of NMR.



A mixture of alcohol (16.71 g, 0.07386 mol) and pyridine (15.0 mL, 2.5 equiv) was cooled to -10 °C and treated with thionyl chloride (11.3 mL, 2.1 equiv) slowly over 11 min (yellow precipitate). The resulting mixture was warmed to 55 °C (heating at 75 °C for 17 h gave complex mixture of chlorinated products) and stirred for 12 h. The reaction mixture was cooled to -5 °C, quenched with water (200 mL) and extracted

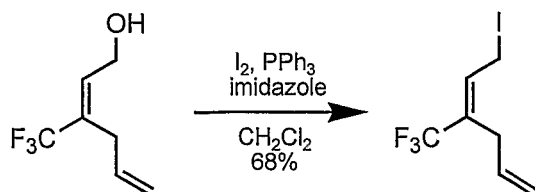
with CH₂Cl₂ (2 x 200 mL, 2 x 150 mL). Combined organics were washed with saturated NaHCO₃ (2 x 200 mL), and brine (200 mL), dried (MgSO₄), and concentrated *in vacuo*. Flash chromatography (pentane:ether 15:1) afforded the ester (11.90 g, 77% yield) as yellow oil: IR (film) 2986 (w), 1731 (s), 1308 (s), 1265 (w), 1227 (m), 1197 (s), 1133 (s), 1025 (m), 920 (w), 896 (w) cm⁻¹; ¹H NMR (400MHz, CDCl₃) δ 6.36 (s, 1 H), 5.79 (ddt, *J* = 16.9, 10.2, 6.6 Hz, 1 H), 5.15 (dd, *J* = 17.1, 1.5 Hz, 1 H), 5.08 (dd, *J* = 10.0, 1.4 Hz, 1 H), 4.22 (q, *J* = 7.1 Hz, 2 H), 3.44 (d, *J* = 6.5 Hz, 2 H), 1.29 (t, *J* = 7.1 Hz, 3 H); ¹³C NMR (100 MHz, CDCl₃) δ 164.22, 143.37 (q, *J* = 29 Hz), 132.71, 123.21 (q, *J* = 274 Hz), 122.60 (q, *J* = 6 Hz), 117.32, 60.85, 30.54, 13.85; high resolution mass spectrum *m/z* 209.0788 [(M+H)⁺; calcd for C₉H₁₂O₂F₃: 209.0789].

The ester is volatile. After the column chromatography, the ester was not completely concentrated.



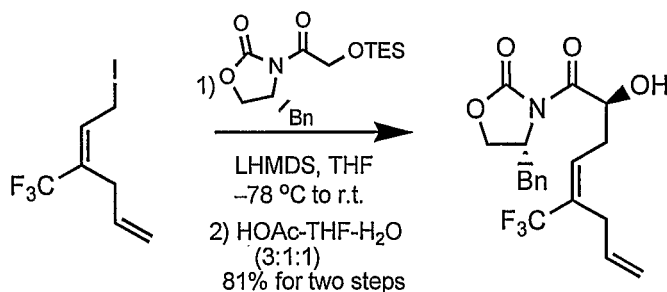
To a cooled (-75 °C) solution of the ester (7.12 g, 0.0342 mol) in CH₂Cl₂ (120 mL) was added a solution of DIBAL-H (75 mL, 2.2 equiv) in CH₂Cl₂ (1.0 M) over 35 min and the resulting mixture was warmed to room temperature over 3 h. The reaction mixture was cooled to 0 °C, quenched with saturated NH₄Cl (12 mL) and stirred at room temperature for 20 min. The reaction mixture was diluted with ether (200 mL), dried (MgSO₄), and concentrated *in vacuo*. Flash chromatography (pentane:ether 3:1 → 1:1) provided the alcohol (5.68 g, 99%) as clear oil: IR (film) 3331 (br s), 2929 (m), 1642 (m), 1445 (m), 1417 (w), 1348 (s), 1316 (s), 1217 (s), 1175 (s), 1119 (s), 1045 (m), 985 (s), 921 (m), 831 (w) cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 6.33 (td, *J* = 6.1, 1.6 Hz, 1 H), 5.75 (ddt, *J* = 17.2, 10.0, 6.2 Hz, 1 H), 5.07 (m, 2 H), 4.29 (ddd, *J* = 6.3, 4.3, 2.1 Hz, 2 H), 2.95 (d, *J* = 6.2 Hz, 2 H); ¹³C NMR (100 MHz, CDCl₃) δ 134.45 (q, *J* = 6 Hz), 133.38, 127.97 (q, *J* = 29 Hz), 123.76 (q, *J* = 271 Hz), 116.25, 57.87, 29.79

Alcohol **56** is volatile. After the column chromatography, **56** was not completely concentrated.



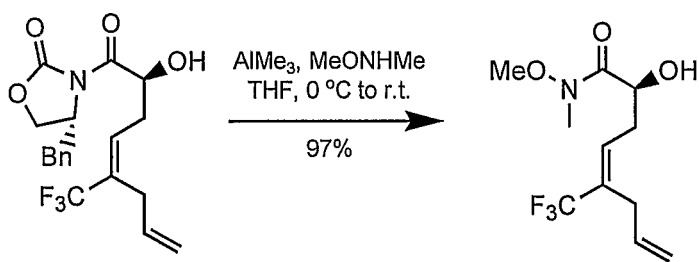
A cooled (0 °C) solution of alcohol (5.97 g, 0.0358 mol) in CH₂Cl₂ (50 mL) was treated with PPh₃ (11.17 g, 1.2 equiv), imidazole (3.55 g, 1.5 equiv) and I₂ (9.10 g, 1.1 equiv) (I₂ addition was the last) and the resulting (yellow cloudy) mixture was stirred at 0 °C for 10 min. The reaction mixture was quenched with saturated Na₂S₂O₃-saturated NaHCO₃ (1:1 = V:V, 200 mL) and extracted with pentane (3 x 200 mL). Combined organics were washed with saturated Na₂S₂O₃-saturated NaHCO₃ (1:1 = V:V, 200 mL), and brine (100 mL), dried (MgSO₄), and concentrated *in vacuo*. Flash chromatography (pentane) gave the iodide (6.69 g, 68%) as pale red oil (stored in -78 °C freezer): (IR Ifilm) 3083 (w), 2982 (w), 1636 (w), 1558 (w), 1456 (w), 1367 (w), 1317 (s), 1216 (m), 1181 (s), 1151 (s), 1120 (s), 989 (m), 921 (m), 896 (m) cm⁻¹; ¹H NMR (400MHz, CDCl₃) δ 6.45 (td, *J* = 8.9, 1.5 Hz, 1 H), 5.79 (ddt, *J* = 16.8, 10.3, 6.2 Hz, 1 H), 5.12 (m, 2 H), 3.85 (ddd, *J* = 8.9, 2.9, 1.4 Hz, 2 H), 3.00 (dt, *J* = 6.1, 1.4 Hz, 2 H); ¹³C NMR (100 MHz, CDCl₃) δ 132.42, 131.64 (q, *J* = 6 Hz), 129.63 (q, *J* = 29 Hz), 123.64 (q, *J* = 272 Hz), 117.00, 29.32, -4.27; low resolution mass spectrum *m/z* 298.7 [(M+Na)⁺; calcd for C₇H₈F₃INa: 299.0].

The allyl iodide is volatile. After the column chromatography, the allyl iodide was not completely concentrated.



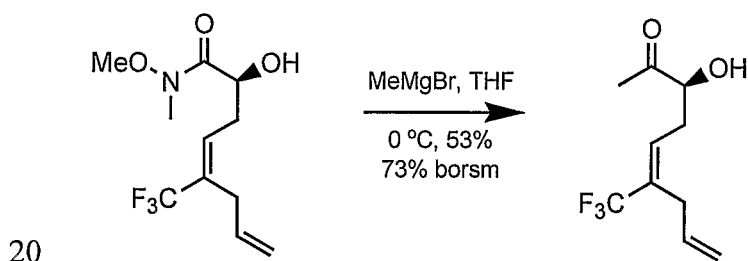
α-Hydroxyoxazolidinone. To a cooled (-78 °C) solution of TES protected 4-benzyl-3-hydroxyacetyl-oxazolidin-2-one (16.28 g, 1.92 equiv) in THF (160 mL) was added a solution of LHMDS (42.0 mL, 1.73 equiv) in THF (1.0 M) dropwise over 51 min and the resulting mixture was stirred at -78 °C for 35 min. The reaction mixture was

treated with a solution of the allyl iodide (6.69 g, 24.2 mmol) in THF (10 mL) over 15 min and the resulting mixture was allowed to warm to room temperature slowly overnight. The reaction mixture was quenched with saturated NaHCO₃ (200 mL) and extracted with EtOAc (3 x 200 mL). Combined organics were washed with saturated NH₄Cl (150 mL), brine (150 mL), dried (MgSO₄), and concentrated *in vacuo*. Flash chromatography (hexanes-EtOAc 6:1 → 3:1) provided a mixture of alkylation products (13.6 g) which were used for the next reaction without further purification (diastereomers were not separable at this stage). A solution of the alkylation products in HOAc-water-THF (3:1:1 = V:V:V, 200 mL) was stirred at room temperature for 4 h. The reaction mixture was concentrated *in vacuo* to remove HOAc, quenched with saturated NaHCO₃ (400 mL), and extracted with EtOAc (3 x 200 mL). Combined organics were dried (MgSO₄), and concentrated *in vacuo*. Flash chromatography (hexanes:EtOAc 3:1 → 2:1) provided the α-hydroxyoxazolidinone (7.55 g, 81% yield for two steps) as clear oil: [α]_D²⁰ (25 °C) -48.2° (*c* 1.08, CHCl₃); IR (film) 3486 (br s), 3030 (m), 2983 (s), 2925 (m), 1790 (s), 1682 (s), 1481 (m), 1393 (m), 1360 (m), 1217 (m), 1171 (m), 1113 (m), 992 (m), 919 (m), 847 (w) cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.32 (m, 3 H), 7.17 (m, 2 H), 6.33 (td, *J* = 7.2, 1.5 Hz, 1 H), 5.77 (ddt, *J* = 16.6, 10.1, 6.2 Hz, 1 H), 5.08 (m, 3 H), 4.74 (ddt, *J* = 4.8, 3.7, 4.4 Hz, 1 H), 4.33 (dd, *J* = 8.6, 8.6 Hz, 1 H), 4.26 (dd, *J* = 9.2, 3.4 Hz, 1 H), 3.42 (br d, *J* = 6.4 Hz, 1 H), 3.24 (dd, *J* = 13.5, 3.4 Hz, 1 H), 2.99 (m, 2 H), 2.79 (dd, *J* = 13.5, 9.4 Hz, 1 H), 2.70 (m, 1 H), 2.50 (m, 1 H); ¹³C NMR (125 MHz, CDCl₃) δ 173.93, 153.05, 134.43, 133.64, 129.98 (q, *J* = 6 Hz), 129.82 (q, *J* = 28 Hz), 129.29, 120.01, 127.58, 124.00 (q, *J* = 272 Hz), 116.34, 69.60, 67.31, 54.95, 37.78, 32.29, 29.84; high resolution mass spectrum *m/z* 384.1421 [(M+H)⁺; calcd for C₁₉H₂₁NO₄F₃: 384.1423].



α-Hydroxyamide. A suspension of (MeO)NHMe•HCl (10.1 g, 5.25 equiv) in THF (100 mL) at 0 °C was treated with a solution of AlMe₃ (50 mL, 5.1 equiv) in toluene

(2.0 M) dropwise and the resulting clear solution was stirred at room temperature for 34 min, then added slowly to a cooled (0 °C) solution of the α -hydroxyoxazolidinone (7.55 g, 19.7 mmol) in THF (70 mL) (cloudy $\rightarrow$ clear, pale yellow). The resulting mixture was warmed to room temperature and stirred for 12 h. The reaction mixture was cooled to 0 °C, quenched by slow addition of 1N aq. tartaric acid (100 mL), stirred at room temperature for 25 min, and extracted with EtOAc (3 x 200 mL). Combined organics were dried (MgSO₄), and concentrated *in vacuo*. Flash chromatography (hexanes:EtOAc 2:1 $\rightarrow$ 1:1) gave the α -hydroxyamide (5.12 g, 97% yield) as clear oil: $[\alpha]_D^{20}$ (24 °C) -57.2° (*c* 1.03, CHCl₃); IR (film) 3432 (br s), 3084 (w), 2980 (m), 2943 (m), 1652 (s), 1464 (m), 1373 (m), 1318 (m), 1214 (m), 1171 (m), 1112 (m), 991 (m), 919 (m), 818 (w) cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 6.32 (td, *J* = 7.3, 1.5 Hz, 1 H), 5.74 (ddt, *J* = 16.9, 10.3, 6.1 Hz, 1 H), 5.05 (m, 2 H), 4.43 (dd, *J* = 7.6, 3.5 Hz, 1 H), 3.70 (s, 3 H), 3.35 (br s, 1 H), 3.24 (s, 3 H), 2.94 (d, *J* = 6.1 Hz, 2 H), 2.59 (m, 1 H), 2.36 (m, 1 H); ¹³C NMR (100 MHz, CDCl₃) δ 173.43, 133.68, 130.59 (q, *J* = 6 Hz), 129.25 (q, *J* = 28 Hz), 124.05 (q, *J* = 271 Hz), 116.17, 67.57, 61.44, 32.56, 32.38, 29.75; high resolution mass spectrum *m/z* 268.1161 [(M+H)⁺; calcd for C₁₁H₁₇NO₃F₃: 268.1161].



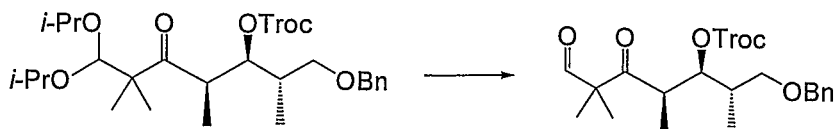
α -Hydroxyketone. To a cooled (0 °C) solution of α -hydroxyamide (4.87 g, 18.2 mmol) in THF (150 mL) was added a solution of MeMgBr (75 mL, 12 equiv) in ether (3.0 M). After 5 min, the reaction mixture was quenched with saturated NH₄Cl (250 mL), and extracted with EtOAc (5 x 200 mL). Combined organics were dried (MgSO₄), and concentrated *in vacuo*. Flash chromatography (hexanes:EtOAc 4:1 → 2:1 → 1:2) provided the α -hydroxyketone (2.16 g, 53% yield, 73% yield based on the recovered starting material) as clear oil and the starting material α -hydroxyamide (1.30

g, 27% yield): $[\alpha]_D^{20}$ (23 °C) +58.5° (*c* 1.30, CHCl₃); IR (film) 3460 (br s), 3085 (w), 2984 (m), 2926 (m), 1716 (s), 1679 (m), 1641 (m), 1417 (m), 1361 (m), 1319 (s), 1247 (m), 1216 (s), 1172 (s), 1113 (s), 1020 (m), 994 (m), 968 (w), 919 (m) cm⁻¹; ¹H NMR (500 MHz, CDCl₃) δ 6.21 (t, *J* = 7.0 Hz, 1 H), 5.75 (ddt, *J* = 16.7, 10.4, 6.2 Hz, 1 H), 5.07 (m, 2 H), 4.26 (dt, *J* = 7.1, 4.5 Hz, 1 H), 3.51 (d, *J* = 4.7 Hz, 1 H), 2.96 (d, *J* = 6.1 Hz, 2 H), 2.66 (m, 1 H), 2.42 (m, 1 H), 2.19 (s, 3 H); ¹³C NMR (100 MHz, CDCl₃) δ 208.53, 133.43, 129.80 (q, *J* = 28 Hz), 129.76 (q, *J* = 6 Hz), 123.85 (q, *J* = 271 Hz), 116.32, 75.36, 31.22, 29.81, 25.11; high resolution mass spectrum *m/z* 223.0945 [(*M*+H)⁺; calcd for C₁₀H₁₄NO₂F₃: 223.0946].

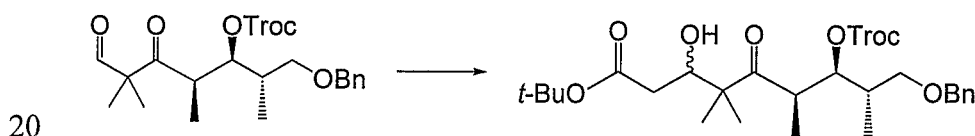
10 This reaction was not complete despite excess amount of MeMgBr.



Carbonic acid 1-(2-benzyloxy-1-methylethyl)-5,5-diisopropoxy-2,4,4-trimethyl-3-oxopentyl ester 2,2,2-trichloroethyl ester. To a solution of 7-Benzyloxy-5-hydroxy-1,1-diisopropoxy-2,2,4,6-tetramethyl-heptan-3-one (1.0 g, 2.4 mmol) and pyridine (0.8 mL, 7.3 mmol) in CH₂Cl₂ (10.0 mL) at 0 °C was added 2,2,2-trichloroethyl chloroformate (668.0 μL, 4.9 mmol) and the mixture was then allowed to warm to rt. After 1 h, the reaction mixture was quenched with brine and then extracted with CH₂Cl₂. The combined organic layers were dried over MgSO₄ and concentrated under reduced pressure. The crude product was purified by flash chromatography (gradient hexane to hexane/EtOAc 93:7) to give the desired product (1.285 g, 92%) as a clear oil: ¹H NMR (400 MHz, CDCl₃) δ 1.03-1.09 (m, 12H), 1.15 (d, *J* = 1.8 Hz, 3H), 1.17 (d, *J* = 1.9 Hz, 3H), 1.19-1.21 (m, 6H), 1.97-2.11 (m, 1H), 3.2 (dd, *J* = 6.2 and 9.0 Hz, 1H), 3.54 (dd, *J* = 4.8 and 9.1 Hz, 1H), 3.57-3.60 (m, 1H), 3.82 (qd, *J* = 3.6 and 5.9 Hz, 2H), 4.47 (s, 2H), 4.57 (s, 1H), 4.72 (d, *J* = 11.9 Hz, 1H), 4.81 (d, *J* = 11.9 Hz, 1H), 5.08 (t, *J* = 6.0 Hz, 1H), 7.29-7.35 (m, 5H); ¹³C NMR (100 MHz, CDCl₃) δ 11.9, 15.0, 18.8, 21.4, 21.7, 22.3, 23.2, 23.4, 35.7, 42.5, 53.4, 53.9, 69.4, 70.9, 71.4, 73.3, 81.3, 94.7, 103.4, 127.5, 127.6, 128.2, 138.2, 154.0, 215.6; IR (film, NaCl, cm⁻¹) 2966, 1760, 1698, 1247; LRMS (ESI) calcd for C₂₇H₄₁O₇Cl₃Na [*M*+Na⁺] 605.2, found 605.2; $[\alpha]_D^{23}$ = -20.4 (*c* = 1.0, CHCl₃).

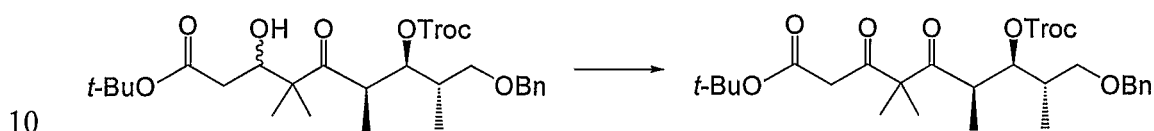


Carbonic acid 1-(2-benzyloxy-1-methylethyl)-2,4,4-trimethyl-3,5-dioxopentyl ester 2,2,2-trichloroethyl ester. To solution of the starting material (1.28 g, 2.25 mmol) in 4:1 THF/H₂O (25 mL) was added *p*-TsOH (111.0 mg, 0.6 mmol). After heating at 70 °C for 5 h, the reaction mixture was poured into a cold (0 °C) sat. NaHCO₃ aq solution (12 mL) and then extracted with EtOAc. The combined organic layers were washed with brine, dried over MgSO₄ and concentrated under reduced pressure. The crude product was purified by flash chromatography (gradient hexane to hexane/EtOAc 84:16) to give product (793.2 mg, 76%) as a clear oil: ¹H NMR (400 MHz, CDCl₃) δ 0.90 (d, *J* = 5.8 Hz, 3H), 1.0 (d, *J* = 6.9 Hz, 3H), 1.24 (s, 6H), 1.97-2.04 (m, 1H), 3.24 (dd, *J* = 4.8 and 9.2 Hz, 1H), 3.34 (m, 1H), 3.42 (dd, *J* = 5.8 and 9.2 Hz, 1H), 4.35 (d, *J* = 11.9 Hz, 1H), 4.39 (d, *J* = 11.9 Hz, 1H), 4.64 (d, *J* = 11.9 Hz, 1H), 4.69 (d, *J* = 11.9 Hz, 1H), 4.96 (t, *J* = 6.0 Hz, 1H), 7.19-7.28 (m, 5H), 9.49 (s, 1H); ¹³C NMR (100 MHz, CDCl₃) – 12.0, 14.8, 19.5, 19.6, 35.4, 43.3, 60.9, 71.1, 73.3, 80.37, 94.5, 127.7, 127.8, 128.3, 137.9, 154.1, 201.0, 210.1; IR (film, NaCl, cm⁻¹) 2973, 2880, 1758, 1701, 1453, 1380, 1248; LRMS (ESI) calcd for C₂₁H₂₇O₆Cl₃Na [M+Na⁺] 503.0, found 503.0; [α]_D²³ = –18.5 (*c* = 0.8, CHCl₃).



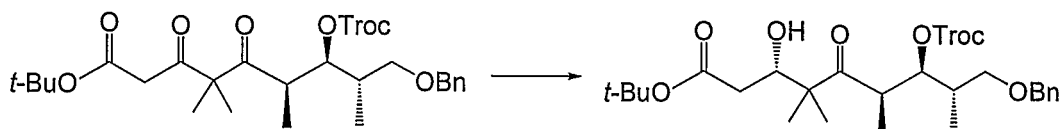
9-Benzyloxy-4,4,6,8-tetramethyl-3,5-dioxo-7-(2,2,2-trichloroethoxycarbonyloxy)-nonanoic acid *tert*-butyl ester. To a solution of LDA (1.17 mmol, 0.3 M in Et₂O) at –78 °C was added *t*-butyl acetate (1.0 mmol, 135.0 μL). After 30 min, a solution of starting material (464.0 mg, 1 mmol) in Et₂O (2 mL) was slowly added over 15 min. After stirring for 1 h, the reaction was quenched with a sat. NH₄Cl aq solution and then extracted with EtOAc. The combined organic layers were washed with brine, dried over MgSO₄ and concentrated under reduced pressure. The crude product was purified by flash chromatography (gradient hexane to hexane/EtOAc 86:14) to give product (1:1 epimeric mixture, 461.4 mg, 80%) as a clear oil: ¹H NMR (400 MHz, CDCl₃) δ 0.87

(d, $J = 5.3$ Hz, 3H), 0.89 (d, $J = 5.5$ Hz, 3H), 1.02-1.10 (m, 18H), 1.38 (s, 18H), 1.97-2.2 (m, 2H), 2.27-2.31 (m, 2H), 3.22-3.27 (m, 3H), 3.39-3.48 (m, 5H), 4.03-4.06 (m, 1H), 4.11-4.14 (m, 1H), 4.38-4.45 (m, 4H), 4.58-4.73 (m, 4H), 4.97 (t, $J = 5.8$ Hz, 1H), 5.02 (t, $J = 5.8$ Hz, 1H), 7.18-7.27 (m, 10H); ^{13}C NMR (100 MHz, CDCl_3) δ 11.9, 12.7, 14.9, 15.2, 18.7, 19.3, 21.4, 21.6, 28.0, 35.6, 37.4, 41.7, 42.0, 51.8, 51.9, 71.3, 71.3, 72.5, 73.0, 73.3, 73.3, 80.6, 81.2, 81.3, 94.6, 127.5, 127.7, 127.8, 128.3, 138.0, 138.1, 154.0, 154.1, 172.3, 172.4, 216.0, 216.3; IR (film, NaCl, cm^{-1}) 3509, 2975, 1759, 1707, 1368, 1248, 1152; LRMS (ESI) calcd for $\text{C}_{27}\text{H}_{39}\text{O}_8\text{Cl}_3\text{Na}$ $[\text{M}+\text{Na}^+]$ 619.1, found 619.2.

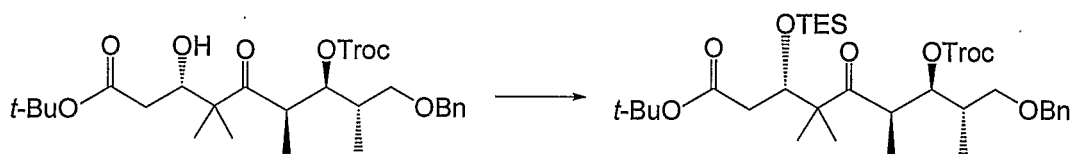


To a 0 °C solution of starting material (350.0 mg, 0.6 mmol) in CH_2Cl_2 (10 mL) was added Dess-Martin periodinane (398.0 mg, 0.9 mmol). The mixture was stirred at rt for 1 h and then poured into a well-stirred mixture of 1:1 sat. $\text{Na}_2\text{S}_2\text{O}_3$ /sat. NaHCO_3 . The layers were separated after 30 min. The aqueous layer was extracted three times with Et_2O . The combined organic extracts were washed with sat. NaHCO_3 , brine, dried over MgSO_4 and concentrated under vacuum. The crude product was purified by flash chromatography (gradient hexane to hexane/ EtOAc 91:9) to give product (258.4 mg, 74%) as a clear oil: ^1H NMR (400 MHz, CDCl_3) δ 0.80 (d, $J = 6.9$ Hz, 3H), 0.87 (d, $J = 6.9$ Hz, 3H), 1.13 (s, 3H), 1.19 (s, 3H), 1.23 (s, 9H), 2.04-2.12 (m, 1H), 3.09-3.28 (m, 5H), 4.23 (s, 2H), 4.48 (d, $J = 11.9$ Hz, 1H), 4.55 (d, $J = 11.9$ Hz, 1H), 4.79 (dd, $J = 4.6$ and 7.3 Hz, 1H), 7.04-7.13 (m, 5H); ^{13}C NMR (100 MHz, CDCl_3) δ 11.7, 14.6, 20.7, 21.5, 27.9, 35.5, 42.2, 43.4, 63.3, 71.3, 73.3, 79.9, 81.5, 90.5, 94.5, 127.6, 127.7, 128.2, 138.0, 154.0, 166.2, 202.9, 210.0; IR (film, NaCl, cm^{-1}) 2977, 1758, 1697, 1368, 1248, 1154; LRMS (ESI) calcd for $\text{C}_{27}\text{H}_{37}\text{O}_8\text{Cl}_3\text{Na}$ $[\text{M}+\text{Na}^+]$ 617.1, found 617.1; $[\alpha]_D^{23} = -49.1$ ($c = 0.9$, CHCl_3).

25

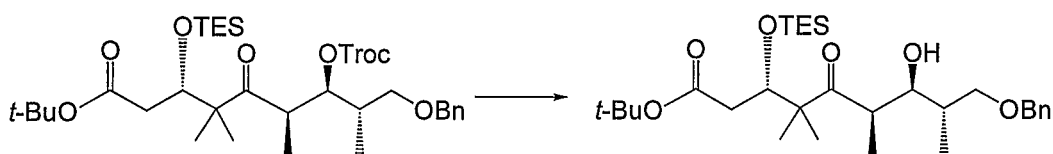


9-Benzyloxy-3-hydroxy-4,4,6,8-tetramethyl-5-oxo-7-(2,2,2-trichloroethoxycarbonyloxy)-nonanoic acid *tert*-butyl ester. A bomb liner was charged with (*R*)-RuBINAP catalyst (16.8 mg, 10.0 μ mol). HCl (555 μ L, 0.2N in MeOH) was added and the mixture was then sonicated for 15 sec. Then a solution of starting material (59.4 mg, 0.1 mmol) in MeOH (555 μ L) was added and the mixture transferred to a Parr apparatus. The vessel was purged with H₂ for 5 min and then pressurized to 1200 psi. After 17 h, the reaction was returned to atmospheric pressure and poured into a sat NaHCO₃ aq solution. The aqueous layer was extracted three times with EtOAc. The combined organic extracts were dried over MgSO₄ and concentrated under reduced pressure. The crude product was purified by flash chromatography (gradient hexane to hexane/EtOAc 88:12) to give product (47.6 mg, 80%) as a clear oil: ¹H NMR (400 MHz, CDCl₃) δ 1.06 (d, *J* = 6.9 Hz, 3H), 1.11 (d, *J* = 6.8 Hz, 3H), 1.14 (s, 3H), 1.18 (s, 3H), 1.47 (s, 9H), 2.05-2.12 (m, 1H), 2.35-2.40 (m, 1H), 3.31-3.37 (m, 2H), 3.51-3.54 (m, 2H), 4.11-4.14 (m, 1H), 4.46 (s, 2H), 4.72 (d, *J* = 11.9 Hz, 1H), 4.80 (d, *J* = 11.9 Hz, 1H), 5.05 (dd, *J* = 5.0 and 6.7 Hz, 1H), 7.27-7.35 (m, 5H); ¹³C NMR (100 MHz, CDCl₃) δ 12.0, 15.0, 19.3, 21.7, 28.0, 35.6, 37.5, 41.7, 51.8, 71.3, 73.0, 73.3, 80.6, 81.3, 94.7, 127.5, 127.7, 128.3, 138.2, 154.1, 172.4, 216; IR (film, NaCl, cm⁻¹) 3849, 2974, 2879, 1758, 1701, 1454, 1368, 1248, 1152, 926, 734; LRMS (ESI) calcd for C₂₇H₃₉O₈Cl₃Na [M+Na⁺] 619.1, found 619.2; [α]_D²³ = -13.0 (*c* = 0.4, CHCl₃).



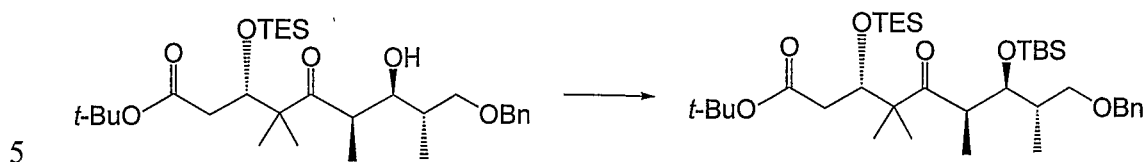
9-Benzyloxy-4,4,6,8-tetramethyl-5-oxo-7-(2,2,2-trichloroethoxycarbonyloxy)-3-(triethylsilanyloxy)-nonanoic acid *tert*-butyl ester. To a solution of starting material (37.6 mg, 6.3 μ mol) and imidazole (9.4 mg, 13.8 μ mol) in DMF (0.4 mL) at 0 °C was added TESCl (11.6 μ L, 69.3 μ mol). After 3 h, the mixture was diluted with sat aq NaHCO₃. The aqueous layer was extracted three times with hexanes. The combined organic extracts were washed with brine, dried over MgSO₄ and concentrated under reduced pressure. The crude product was purified by flash chromatography (gradient

hexane to hexane/EtOAc 93:7) to yield, in order of elution, product (22.9 mg, 51%), and recovered starting material (12.9 mg, 34%) as clear oils: ^1H NMR (400 MHz, CDCl_3) δ 0.66 (q, $J = 7.9$ Hz, 6H), 0.96 (t, $J = 7.9$ Hz, 9H), 1.01 (s, 3H), 1.05 (d, $J = 5.2$ Hz, 3H), 1.07 (d, $J = 5.3$ Hz, 3H), 1.35 (s, 3H), 1.44 (s, 9H), 2.05-2.11 (m, 2H),
 5 2.50 (dd, $J = 3.5$ and 17.2 Hz, 1H), 3.35 (dd, $J = 5.9$ and 9.0 Hz, 1H), 3.49 (dd, $J = 4.0$ and 9.0 Hz, 1H), 3.53 (dd, $J = 3.8$ and 6.7 Hz, 1H), 4.18 (dd, $J = 3.5$ and 6.5 Hz, 1H), 4.45 (s, 2H), 4.65 (d, $J = 11.9$ Hz, 1H), 4.79 (d, $J = 11.9$ Hz, 1H), 4.97 (dd, $J = 3.7$ and 8.1 Hz, 1H), 7.29-7.52 (m, 5H); ^{13}C NMR (125 MHz, CDCl_3) δ 5.3, 7.3, 10.9, 14.9, 21.3, 22.6, 28.4, 35.9, 41.1, 42.7, 53.7, 71.9, 73.7, 75.7, 80.1, 80.9, 95.1, 127.9, 128.0,
 10 128.7, 138.6, 154.3, 171.7, 215.7; IR (film, NaCl, cm^{-1}) 2956, 2876, 1732, 1694, 1456, 1366, 1257, 1154, 1098, 988, 835, 774, 741; LRMS (ESI) calcd for $\text{C}_{33}\text{H}_{53}\text{O}_8\text{SiCl}_3\text{Na}$ $[\text{M}+\text{Na}^+]$ 733.2, found 733.3. $[\alpha]_D^{23} = -16.1$ ($c = 0.1$, CHCl_3).



15 **9-Benzyloxy-3-(diethylmethylsilyloxy)-7-hydroxy-4,4,6,8-tetramethyl-5-oxo-nonanoic acid *tert*-butyl ester.** To a solution of starting material (22.9 mg, 3.2 μmol) in 1:1 THF/AcOH (1.4 mL) was added Zn (5.0 mg, 7.8 μmol , nanosize). The mixture was sonicated for 15 min. More Zn (5.0 mg, 7.8 μmol , nanosize) was added, followed by sonication for a further 15 min. The suspension was filtered through a celite pad,
 20 washing with EtOAc several times. The filtrates were washed with sat. NaHCO_3 , brine, dried over MgSO_4 and concentrated under vacuum. The crude residue was passed through a short plug of silica gel eluting with hexane/EtOAc 4:1 to give 17.1 mg (99% yield) of product as colorless oil: ^1H NMR (400 MHz, CDCl_3) δ (m, 6H), 0.96 (t, $J = 7.9$ Hz, 9H), 0.97 (d, $J = 6.8$ Hz, 3H), 1.05 (d, $J = 6.8$ Hz, 3H), 1.11 (s, 3H), 1.26 (s, 3H), 1.44 (s, 9H), 1.84-1.90 (m, 1H), 2.21 (dd, $J = 6.7$ and 17.0 Hz, 1H), 2.36 (dd, $J = 6.7$ and 17.0 Hz, 1H), 3.24-3.29 (m, 1H), 3.44-3.52 (m, 2H), 3.67 (dd, $J = 3.9$ and 8.9 Hz, 1H), 4.36 (dd, $J = 3.5$ and 6.5 Hz, 1H), 4.50 (d, $J = 12.0$ Hz, 1H), 4.54 (d, $J = 12.0$ Hz, 1H), 7.32-7.36 (m, 5H); ^{13}C NMR (100 MHz, CDCl_3) δ 5.0, 6.9, 9.7, 13.9, 20.2, 21.8, 28.0, 36.3, 40.8, 41.5, 53.7, 72.5, 72.9, 73.2, 73.6, 80.7, 127.4, 127.5, 128.2,

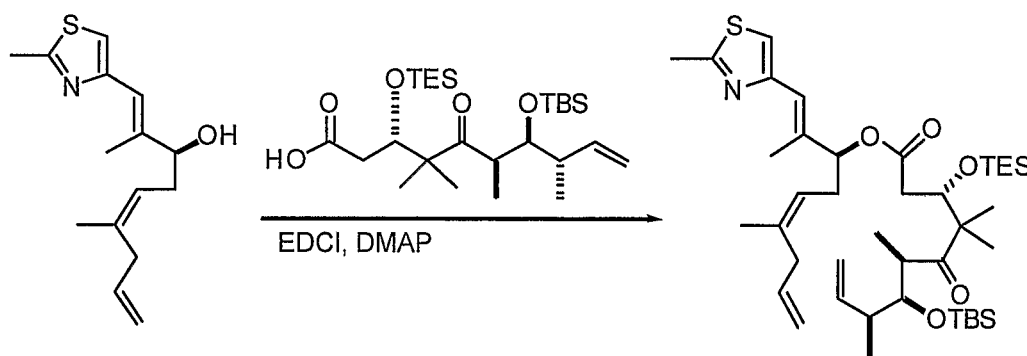
138.6, 171.0, 221.4; IR (film, NaCl, cm^{-1}) 3502, 2959, 2875, 1731, 1683, 1456, 1366, 1154, 1098, 996, 739; LRMS (ESI) calcd for $\text{C}_{30}\text{H}_{52}\text{O}_6\text{SiCl}_3\text{Na}$ $[\text{M}+\text{Na}^+]$ 559.3, found 559.3; $[\alpha]_{\text{D}}^{23} = -41.0$ ($c = 0.4$, CHCl_3).



9-Benzyloxy-7-(tert-butyldimethylsilyloxy)-3-(diethylmethoxysilyloxy)-4,4,6,8-tetramethyl-5-oxo-nonanoic acid tert-butyl ester. To a solution of starting material (4.1 mg, 7.6 μmol) and 2,6-lutidine (10.0 μL , 43.5 mmol) in CH_2Cl_2 (0.2 mL) at -78°C was added TBSOTf (10.0 μL , 85.8 mmol). After 2 h, more 2,6-lutidine (10.0 μL , 43.5 mmol) and TBSOTf (10.0 μL , 85.8 mmol) were added. After 6 h, the mixture was diluted with sat aq NaHCO_3 . The aqueous layer was extracted three times with EtOAc. The combined organic extracts were washed with brine, dried over MgSO_4 and concentrated under reduced pressure. The crude product was purified by flash chromatography (gradient hexane to hexane/EtOAc 91:9) to give the product (5.4 mg, 82%) as a clear oil. Spectroscopic data agreed well with the reported values.

10

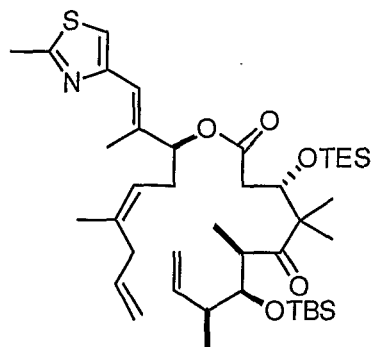
15



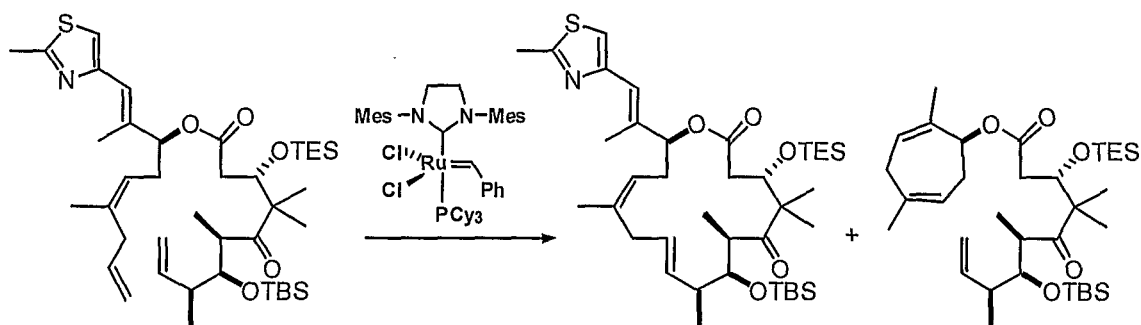
Acid and alcohol were azeotroped with dry benzene (5 mL x 2) and dried under high vacuum prior to the reaction. To a solution of alcohol (639 mg, 2.63 mmol) in CH_2Cl_2 (13 mL) were added EDCI (576 mg, 3.09 mmol) and DMAP (366 mg, 3.09 mmol) at 0°C . To the mixture was added a solution of acid (1.11 g, as 1.88 mmol) in CH_2Cl_2 (5 mL + 2 mL rinse) dropwise over 16 min at 0°C . After stirred at 0°C for 1.5 h, the mixture was stirred at rt for 3.5 h. Following concentration of the reaction mixture, the

20

residue was purified by flash column chromatography (SiO₂, hexane/EtOAc = 30:1 to 20:1) gave ester (1.20 g, 1.61 mmol, 86% from *t*-butyl ester) as a colorless oil.



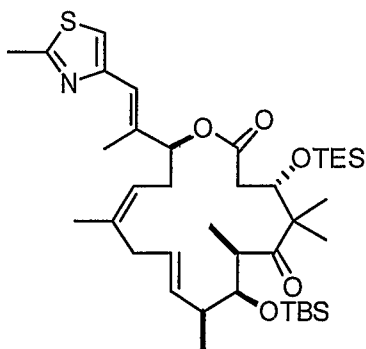
$[\alpha]_D^{24} -25.1$ (c 1.30, CHCl₃); IR (film) ν 2955, 2925, 2872, 1732, 1696, 1461, 1378, 1290, 1243, 1173, 1091, 985, 873, 773 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 0.06 (3H, s), 0.06 (3H, s), 0.58-0.66 (6H, m), 0.92 (9H, s), 0.95 (9H, t, J = 8.0 Hz), 1.02 (3H, d, J = 6.5 Hz), 1.03 (3H, d, J = 6.5 Hz), 1.07 (3H, s), 1.21 (3H, s), 1.67 (3H, s), 2.07 (3H, s), 2.05-2.12 (1H, m), 2.30 (1H, dd, J = 16.9, 7.5 Hz), 2.39 (1H, dt, J = 14.8, 6.7 Hz), 2.49 (1H, dd, J = 17.0, 3.0 Hz), 2.50 (1H, dt, J = 14.8, 6.7 Hz), 2.70 (3H, s), 2.74-2.30 (2H, m), 3.07 (1H, dd, J = 7.0 Hz), 3.83 (1H, dd, J = 7.1, 2.0 Hz), 4.35 (1H, dd, J = 7.4, 2.8 Hz), 4.98-5.07 (4H, m), 5.16 (1H, brt, J = 7.0 Hz), 5.23 (1H, t, J = 6.9 Hz), 5.74 (1H, ddt, J = 16.7, 10.2, 6.5 Hz), 5.91 (1H, ddd, J = 17.8, 10.5, 7.8 Hz), 6.50 (1H, s), 6.95 (1H, s); ¹³C NMR (100 MHz, CDCl₃) δ -3.7, -3.3, 5.3 (3C), 7.2 (3C), 14.8, 15.2, 18.7, 18.9, 19.4, 20.3, 23.6, 23.7, 26.4 (3C), 31.7, 36.7, 40.1, 43.8, 46.4, 53.3, 74.2, 76.5, 79.6, 115.5, 115.6, 116.5, 120.5, 121.3, 135.8, 136.1, 137.4, 140.2, 152.9, 164.7, 171.5, 218.4; LRMS (ESI) calcd for C₄₁H₇₁NO₅SSi₂Na [M+Na⁺] 768.5, found 768.5; HRMS calcd. for C₄₁H₇₂NO₅SSi₂ [M+H⁺] 746.4670, found 746.4680.



A solution of diene (26.9 mg, 36.1 μ mol) in toluene (70 mL) was heated to reflux and treated with a solution of Grubbs' catalyst (3.1 mg, 3.61 μ mol) in toluene (2 mL). The

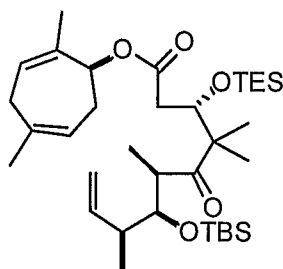
mixture was stirred for 25 min, cooled to 0 °C, filtered through a pad of silica gel, which was rinsed with hexane/EtOAc = 2/1. The combined filtrate was concentrated and purified by flash column chromatography (SiO₂, hexane/Et₂O = 40:1 to 5:1) to give the desired product (9.9 mg, 13.8 μmol, 38%) and cycloheptadiene (14.4 mg, 22.3

5 μmol, 62%) both as a colorless oil.

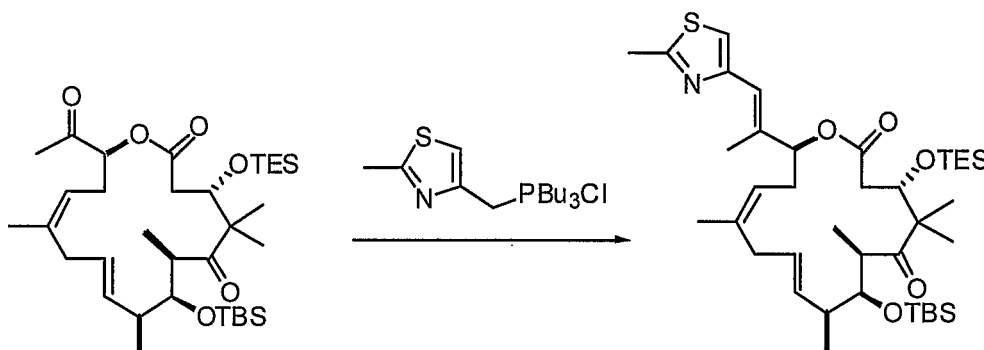


[α]_D²⁵ -41.5 (c 0.715, CHCl₃); IR (film) ν 2955, 2884, 1737, 1690, 1467, 1378, 1249, 1179, 1102, 1014, 979, 879, 826, 773 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 0.08 (3H, s), 0.12 (3H, s), 0.57 (6H, q, *J* = 7.8 Hz), 0.89 (9H, t, *J* = 8.0 Hz), 0.93 (9H, s), 1.04 (3H, s), 1.06 (3H, d, *J* = 7.1 Hz), 1.12 (3H, s), 1.17 (3H, d, *J* = 7.1 Hz), 1.68 (3H, s), 2.15 (3H, d, *J* = 0.8 Hz), 2.14-2.27 (2H, m), 2.45 (1H, dd, *J* = 14.0, 4.8 Hz), 2.50 (1H, dd, *J* = 14.9, 3.2 Hz), 2.64-2.74 (2H, m), 2.72 (3H, s), 3.02 (1H, quintet, *J* = 7.0 Hz), 3.10 (1H, dd, *J* = 14.4, 7.3 Hz), 3.96 (1H, d, *J* = 8.7 Hz), 4.43 (1H, dd, *J* = 8.3, 2.9 Hz), 5.22 (1H, dd, *J* = 9.8, 5.7 Hz), 5.33-5.42 (2H, m), 5.69 (1H, dd, *J* = 15.8, 8.2 Hz), 6.57 (1H, s), 6.96 (1H, s); ¹³C NMR (100 MHz, CDCl₃) δ -3.3, -3.2, 5.6 (3C), 7.1 (3C), 15.0, 17.2, 18.8, 19.4, 21.4, 21.7, 23.8, 24.3, 26.5 (3C), 33.2, 35.6, 41.3, 41.8, 48.2, 54.0, 74.4, 77.4, 79.3, 116.4, 120.5, 121.0, 129.3, 132.1, 137.8, 138.0, 152.7, 164.8, 170.7, 216.8; LRMS (ESI) calcd for C₃₉H₆₈NO₅SSi₂ [M+H⁺] 718.4, found 718.3; HRMS calcd. for C₃₉H₆₈NO₅SSi₂ [M+H⁺] 718.4357, found 718.4355.

20

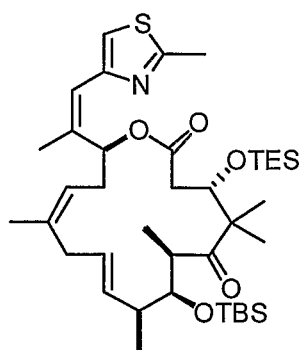


$[\alpha]_D^{26} -38.5$ (c 0.400, CHCl_3); IR (film) ν 2955, 2878, 1741, 1693, 1472, 1458, 1385, 1295, 1253, 1169, 1098, 988, 871, 837, 775 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 0.07 (6H, s), 0.61-0.68 (6H, m), 0.93 (9H, s), 0.97 (9H, t, $J = 8.0$ Hz), 1.03 (3H, d, $J = 7.0$ Hz), 1.04 (3H, d, $J = 7.0$ Hz), 1.10 (3H, s), 1.21 (3H, s), 1.65 (3H, s), 1.75 (3H, s),
 5 2.06-2.14 (1H, m), 2.31 (1H, dd, $J = 17.2, 7.2$ Hz), 2.34-2.51 (2H, m), 2.49 (1H, dd, $J = 17.1, 2.8$ Hz), 2.65-2.81 (2H, m), 3.07 (1H, quintet, $J = 7.0$ Hz), 3.84 (1H, dd, $J = 7.2, 2.1$ Hz), 4.40 (1H, dd, $J = 7.2, 2.8$ Hz), 4.98-5.09 (2H, m), 5.38-5.42 (1H, m), 5.65 (1H, t, $J = 5.9$ Hz), 5.93 (1H, ddd, $J = 17.9, 10.1, 7.8$ Hz); ^{13}C NMR (100MHz, CDCl_3) δ -3.6, -3.3, 5.4 (3C), 7.3 (3C), 15.3, 18.7, 19.0, 20.0, 22.1, 23.8, 25.8, 26.4 (3C), 31.3,
 10 32.3, 40.0, 43.8, 46.3, 54.0, 72.5, 73.8, 76.5, 115.6, 119.8, 125.6, 136.5, 140.1, 140.6, 171.9, 218.5; LRMS (ESI) calcd for $\text{C}_{35}\text{H}_{64}\text{O}_5\text{Si}_2\text{Na}$ $[\text{M}+\text{Na}^+]$ 643.4, found 643.3; HRMS calcd. for $\text{C}_{35}\text{H}_{64}\text{O}_5\text{Si}_2\text{Na}$ $[\text{M}+\text{Na}^+]$ 643.4190, found 643.4219.

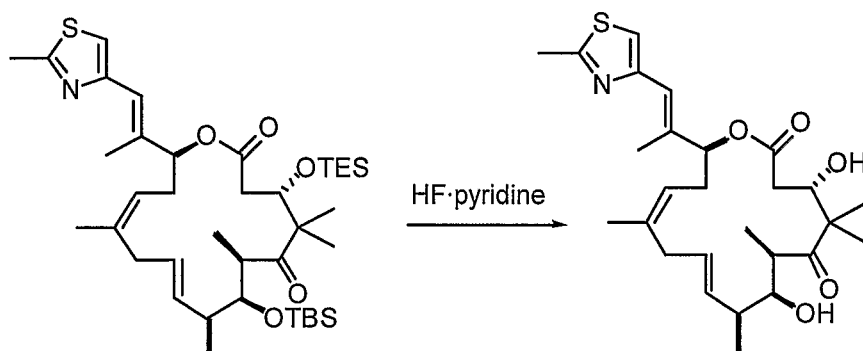


Via Wittig reaction: To a solution of Wittig reagent (19.1 mg, 54.7 μmol) in THF (0.4 mL) was added KHMDS (109 μL of a 0.5 M solution in toluene, 54.7 μmol) at 0 $^\circ\text{C}$.
 15 The mixture was stirred at 0 $^\circ\text{C}$ for 0.5 h and then cooled to -78 $^\circ\text{C}$. To the mixture was added dropwise a solution of ketone (5.7 mg, 9.12 μmol) in THF (0.3 mL), and the resulting mixture was allowed to warm to -20 $^\circ\text{C}$ over 1.5 h. The reaction was quenched with sat. aq. NH_4Cl (2 mL) and extracted with EtOAc (7 mL x 3). The
 20 combined organic layers were dried over Na_2SO_4 and concentrated. The residue was purified by flash column chromatography (SiO_2 , hexane/ Et_2O = 10:1) providing the 5.6 mg of inseparable olefin mixture (E/Z = 9:1). The mixture was purified by preparative TLC (hexane/ Et_2O = 4:1) providing the pure desired isomer (5.0 mg, 6.96 μmol , 76%) as a colorless oil.

25



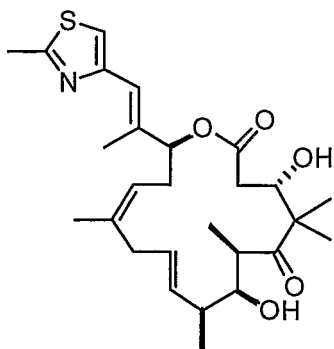
¹H NMR (400 MHz, CDCl₃) δ 0.08 (3H, s), 0.12 (3H, s), 0.51 (6H, q, *J* = 7.9 Hz), 0.86 (9H, t, *J* = 7.9 Hz), 0.97 (9H, s), 1.01 (3H, s), 1.06 (3H, d, *J* = 7.1 Hz), 1.12 (3H, s), 1.18 (3H, d, *J* = 7.1 Hz), 1.69 (3H, s), 1.97 (3H, s), 2.10-2.18 (1H, m), 2.24-2.31 (1H, m), 2.38-2.59 (3H, m), 2.68-2.78 (1H, m), 2.72 (3H, s), 2.98-3.14 (2H, m), 3.97 (1H, d, *J* = 9.0 Hz), 4.45-4.48 (1H, m), 5.29-5.41 (2H, m), 5.73 (1H, dd, *J* = 15.6, 8.3 Hz), 6.30 (1H, s), 6.73 (1H, d, *J* = 8.7 Hz), 6.56 (1H, s); LRMS (ESI) calcd for C₃₉H₆₈NO₅SSi₂ [M+H⁺] 718.4, found 718.1.



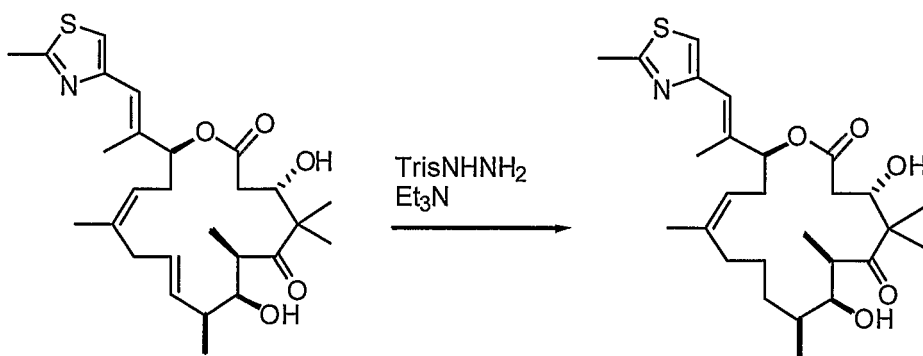
10

To a solution of silylether (298.8 mg, 0.416 mmol) in THF (6.5 mL) in a plastic tube was added HF·pyridine (3.2 mL) at 0 °C, and the mixture was stirred at rt for 3 h. The reaction was quenched with dropwise addition of TMSOMe (30 mL) at 0 °C and the mixture was stirred at rt for 3 h. After concentration and drying under high vacuum, the residue was purified by flash column chromatography (SiO₂, hexane/EtOAc = 1:1) to give alcohol (196.6 mg, 0.402 mmol, 97%) as a colorless solid.

15



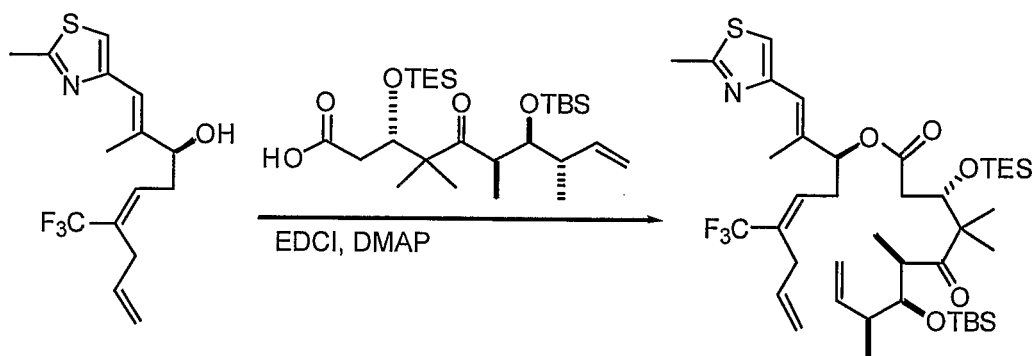
$[\alpha]_D^{25} -96.6$ (c 0.235, CHCl_3); IR (film) ν 3502, 2970, 2927, 1733, 1685, 1506, 1456, 1375, 1251, 1152, 1040, 977 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 1.06 (3H, s), 1.11 (3H, d, $J = 7.0$ Hz), 1.22 (3H, d, $J = 6.8$ Hz), 1.28 (3H, s), 1.72 (3H, s), 2.10 (3H, s),
 5 2.31-2.40 (2H, m), 2.43 (1H, dd, $J = 16.0, 3.7$ Hz), 2.49 (1H, dd, $J = 16.0, 9.2$ Hz), 2.55-2.68 (2H, m), 2.71 (3H, s), 2.98 (1H, dd, $J = 14.4, 6.4$ Hz), 3.16 (1H, quintet, $J = 6.2$ Hz), 3.76 (1H, dd, $J = 5.9, 3.2$ Hz), 4.30 (1H, dd, $J = 9.2, 3.7$ Hz), 5.18 (1H, brt, $J = 7.3$ Hz), 5.32 (1H, dd, $J = 8.4, 2.5$ Hz), 5.63 (1H, dd, $J = 15.7, 6.4$ Hz), 5.60 (1H, ddd, $J = 15.7, 6.9, 5.1$ Hz), 6.60 (1H, s), 6.98 (1H, s); ^{13}C NMR (100 MHz, CDCl_3) δ 15.1,
 10 16.0, 17.7, 19.2, 19.5, 22.5, 23.6, 32.0, 35.0, 39.6, 40.3, 44.8, 53.3, 71.8, 75.6, 78.3, 116.1, 119.6, 120.5, 129.9, 131.3, 137.5, 138.2, 152.2, 165.0, 170.7, 218.8; LRMS (ESI) calcd for $\text{C}_{27}\text{H}_{40}\text{NO}_5\text{S}$ $[\text{M}+\text{H}^+]$ 490.3, found 490.2; HRMS calcd. for $\text{C}_{27}\text{H}_{40}\text{NO}_5\text{S}$ $[\text{M}+\text{H}^+]$ 490.2627, found 490.2602.



15

To a solution of olefin (1.2 mg, 2.5 μmol) and TrisNHNH_2 (29.3 mg, 98 μmol) in $\text{ClCH}_2\text{CH}_2\text{Cl}$ (0.7 mL) at 50 $^\circ\text{C}$ was added Et_3N (13.7 μL , 98 μmol). The reaction was monitored by HPTLC (hexane/ $\text{EtOAc}/\text{CH}_2\text{Cl}_2 = 1/1/2$). After stirring for 7 h, the mixture was cooled to rt, diluted with EtOAc and filtered through a pad of silica gel,
 20 which was rinsed with EtOAc . After concentrating, the residue was purified by

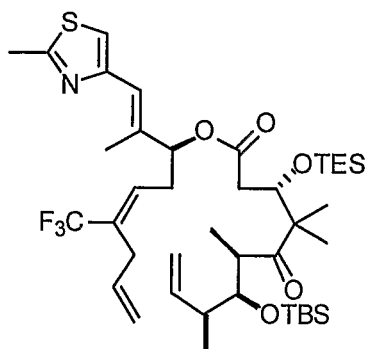
preparative TLC (hexane/EtOAc/CH₂Cl₂ = 1/1/2) providing the reduced product (1.1 mg, 2.2 μ mol, 91%) as a white solid. The spectral data of this compound was identical to those reported of dEpoB.



5

Acid and alcohol were azeotroped with dry benzene (5 mL x 2) and dried under high vacuum prior to the reaction. To a solution of alcohol (10:1 mixture of isomers, 240 mg, 0.756 mmol) in CH₂Cl₂ (5 mL) were added EDCI (192.7 mg, 1.01 mmol) and DMAP (122.8 mg, 1.01 mmol) at 0 °C. To the mixture was added a solution of acid (314.6 mg, 0.628 mmol) in CH₂Cl₂ (2 mL + 1 mL rinse) dropwise over 15 min at 0 °C. After stirring at 0 °C for 2 h, the mixture was stirred at rt for 2 h. After concentrating, the residue was carefully purified by flash column chromatography (SiO₂, hexane/EtOAc = 20:1 to 15:1) to give ester (340.1 mg, 0.425 mmol, 68% based on acid) as a colorless oil.

15

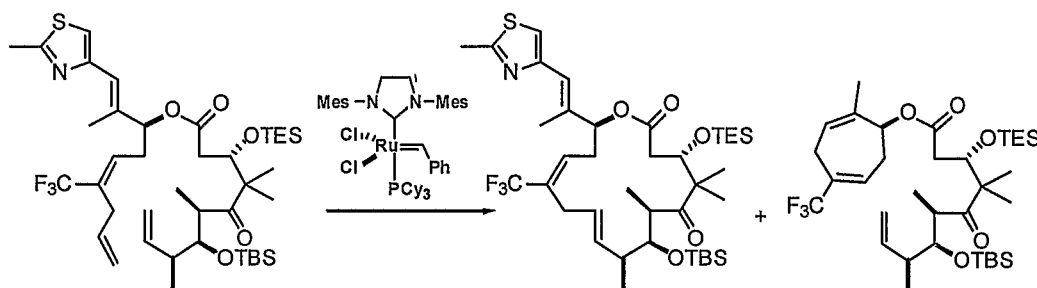


$[\alpha]_D^{24}$ -27.5 (c 0.28, CHCl₃); IR (film) ν 2956, 2878, 1740, 1692, 1472, 1378, 1317, 1253, 1174, 1118, 988, 915, 872, 837, 775 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 0.06 (6H, s), 0.57-0.65 (6H, m), 0.92 (9H, s), 0.94 (9H, t, J = 7.9 Hz), 1.02 (3H, d, J = 6.9 Hz), 1.03 (3H, d, J = 6.8 Hz), 1.07 (3H, s), 1.22 (3H, s), 2.07-2.10 (1H, m), 2.09 (3H, s), 2.31 (1H, dd, J = 16.9, 7.3 Hz), 2.51 (1H, dd, J = 16.8, 3.0 Hz), 2.49-2.65 (2H, m),

20

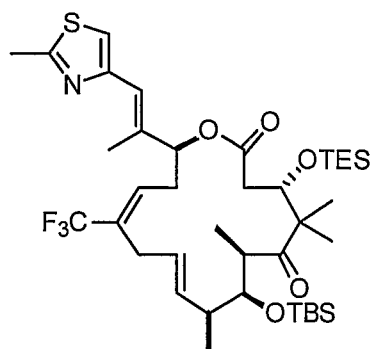
2.71 (3H, s), 2.96-2.99 (2H, m), 3.06 (1H, quintet, $J = 7.1$ Hz), 3.83 (1H, dd, $J = 7.3$,
 2.1 Hz), 4.35 (1H, dd, $J = 7.2$, 3.0 Hz), 4.98-5.12 (4H, m), 5.30 (1H, t, $J = 6.7$ Hz), 5.76
 (1H, ddt, $J = 16.7$, 10.2, 6.2 Hz), 5.92 (1H, ddd, $J = 17.8$, 9.9, 7.8 Hz), 6.19 (1H, t, $J =$
 7.0 Hz), 6.51 (1H, s), 6.97 (1H, s); ^{13}C NMR (100 MHz, CDCl_3) δ -3.7, -3.4, 5.2 (3C),
 7.1 (3C), 14.7, 15.2, 18.6, 18.9, 19.3, 19.9, 23.8, 26.3 (3C), 30.1, 31.2, 40.0, 43.7, 46.3,
 53.3, 73.9, 76.5, 77.9, 115.5, 116.5, 117.0, 121.5, 124.1 [q , $^1J(\text{C},\text{F}) = 273.4$ Hz], 129.6
 [q , $^2J(\text{C},\text{F}) = 28.5$ Hz], 130.5 [q , $^3J(\text{C},\text{F}) = 6.1$ Hz], 133.6, 136.3, 140.1, 152.4, 164.8,
 171.3, 218.3; LRMS (ESI) calcd for $\text{C}_{41}\text{H}_{68}\text{F}_3\text{NO}_5\text{SSi}_2\text{Na}$ [$\text{M} + \text{Na}^+$] 822.4, found 822.4;
 HRMS calcd. for $\text{C}_{41}\text{H}_{69}\text{F}_3\text{NO}_5\text{SSi}_2$ [$\text{M} + \text{H}^+$] 800.4387, found 800.4374.

10



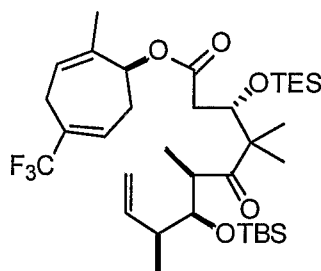
A solution of diene (57.6 mg, 72.0 μmol) in toluene (142 mL) was heated to reflux and
 treated with a solution of Grubbs' catalyst (6.1 mg, 7.20 μmol) in toluene (2 mL). The
 mixture was stirred for 28 min, cooled to 0 $^\circ\text{C}$, and filtered through a pad of silica gel,
 which was rinsed with hexane/EtOAc = 2/1 (300 mL). The combine filtrate was
 concentrated and purified by flash column chromatography (SiO_2 , hexane/ Et_2O = 40:1
 to 15:2) to give the desired product (12.0 mg, 15.5 μmol , 22%) and cycloheptadiene
 (29.2 mg, 43.3 μmol , 60%) both as a colorless oils.

20

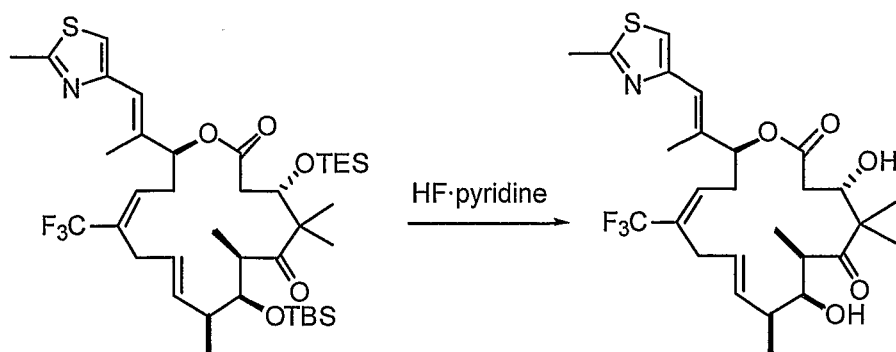


$[\alpha]_{\text{D}}^{26}$ -17.1 (c 0.14, CHCl_3); IR (film) ν 2955, 2884, 1743, 1690, 1472, 1320, 1173,
 1114, 1038, 1008, 873, 832, 773 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 0.09 (3H, s), 0.12

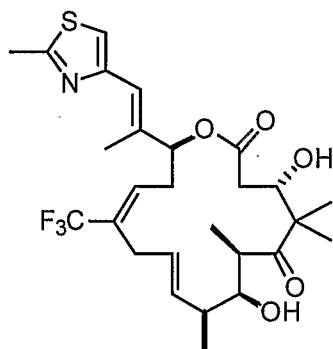
(3H, s), 0.55 (6H, q, $J = 7.7$ Hz), 0.88 (9H, t, $J = 8.0$ Hz), 0.96 (9H, s), 1.01 (3H, s), 1.06 (3H, d, $J = 7.1$ Hz), 1.12 (3H, s), 1.20 (3H, d, $J = 7.1$ Hz), 2.07-2.17 (1H, m), 2.19 (3H, s), 2.38 (1H, dd, $J = 14.3, 3.5$ Hz), 2.39-2.49 (1H, m), 2.50 (1H, dd, $J = 14.3, 7.3$ Hz), 2.73 (3H, s), 2.77-2.91 (2H, m), 2.96-3.09 (2H, m), 3.98 (1H, dd, $J = 8.9$ Hz), 4.54 (1H, dd, $J = 7.3, 3.4$ Hz), 5.28-5.38 (1H, m), 5.63 (1H, dd, $J = 9.6, 2.3$ Hz), 5.77 (1H, dd, $J = 15.9, 8.5$ Hz), 6.21-6.28 (1H, m), 6.60 (1H, s), 6.99 (1H, s); ^{13}C NMR (100 MHz, CDCl_3) δ -3.4, -3.3, 5.5 (3C), 7.0 (3C), 14.6, 17.1, 18.7, 19.4, 19.9, 21.3, 24.8, 26.4 (3C), 29.6, 32.8, 42.0, 42.1, 48.2, 54.1, 73.4, 76.9, 77.8, 117.0, 121.6, 124.3 [q, $^1J(\text{C},\text{F}) = 273.5$ Hz], 127.2, 130.6 [q, $^2J(\text{C},\text{F}) = 28.2$ Hz], 130.8 [q, $^3J(\text{C},\text{F}) = 6.1$ Hz], 133.2, 136.5, 152.3, 165.0, 170.1, 217.1; LRMS (ESI) calcd for $\text{C}_{39}\text{H}_{65}\text{F}_3\text{NO}_5\text{SSi}_2$ [M+H $^+$] 772.4, found 772.4; HRMS calcd. for $\text{C}_{39}\text{H}_{65}\text{F}_3\text{NO}_5\text{SSi}_2$ [M+H $^+$] 772.4074, found 772.4102.



[α] $_{\text{D}}^{26}$ -35.6 (c 0.365, CHCl_3); IR (film) ν 2956, 2878, 1737, 1693, 1472, 1458, 1305, 1279, 1252, 1173, 1116, 988, 871, 775 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 0.08 (6H, s), 0.64 (6H, q, $J = 7.8$ Hz), 0.93 (9H, s), 0.96 (9H, t, $J = 7.8$ Hz), 1.04 (6H, d, $J = 7.0$ Hz), 1.10 (3H, s), 1.22 (3H, s), 1.70 (3H, s), 2.05-2.14 (1H, m), 2.32 (1H, dd, $J = 17.0, 7.1$ Hz), 2.50 (1H, dd, $J = 17.0, 3.0$ Hz), 2.51-2.63 (2H, m), 2.87 (1H, dd, $J = 18.4, 6.7$ Hz), 2.90-3.02 (1H, m), 3.07 (1H, quintet, $J = 7.1$ Hz), 3.85 (1H, dd, $J = 7.2, 2.0$ Hz), 4.39 (1H, dd, $J = 7.1, 2.9$ Hz), 4.98-5.08 (2H, m), 5.51 (1H, dd, $J = 8.1, 3.8$ Hz), 5.67 (1H, t, $J = 5.9$ Hz), 5.93 (1H, ddd, $J = 17.8, 10.5, 7.8$ Hz), 6.29 (1H, t, $J = 5.5$ Hz); ^{13}C NMR (100 MHz, CDCl_3) δ -3.7, -3.4, 5.3 (3C), 7.1 (3C), 15.3, 18.7, 18.9, 19.6, 21.3, 23.9, 24.1, 26.3 (3C), 30.3, 40.0, 43.7, 46.3, 53.4, 70.9, 73.7, 76.5, 115.5, 123.4, 123.8 [q, $^1J(\text{C},\text{F}) = 272.2$ Hz], 129.1 [q, $^3J(\text{C},\text{F}) = 6.1$ Hz], 131.5 [q, $^2J(\text{C},\text{F}) = 28.8$ Hz], 138.1, 140.0, 171.7, 218.5; LRMS (ESI) calcd for $\text{C}_{35}\text{H}_{61}\text{F}_3\text{O}_5\text{Si}_2\text{Na}$ [M+Na $^+$] 697.4, found 697.4; HRMS calcd. for $\text{C}_{35}\text{H}_{61}\text{F}_3\text{O}_5\text{Si}_2\text{Na}$ [M+Na $^+$] 697.3907, found 697.3892.

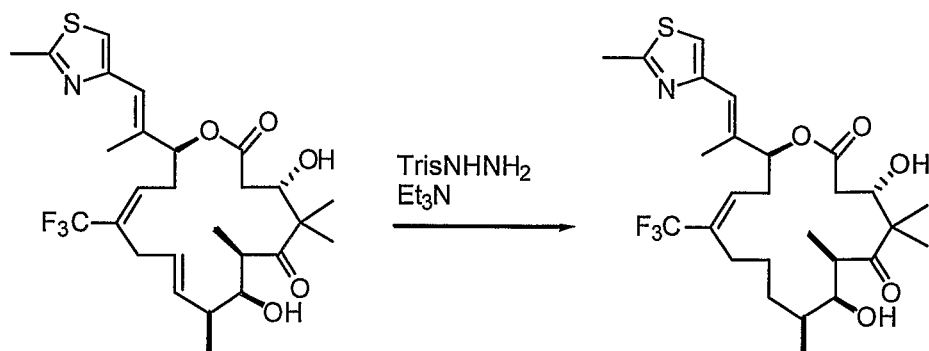


To a solution of silyl ether (1.78 g, 2.31 mmol) in THF (25 mL) in a plastic tube was added slowly HF·pyridine (12.5 mL) at 0 °C, and the mixture was stirred at rt for 4 h. The reaction was quenched with dropwise addition of TMSOMe (80 mL) over 10 min
 5 at 0 °C. The mixture was vigorously stirred at rt for 2.5 h. After concentrating and drying under high vacuum for 2 h, the residue was purified by flash column chromatography (SiO₂~50 g, hexane/EtOAc = 1:1) to give diol (1.20 g, 2.21 mmol, 96%) as a colorless solid.



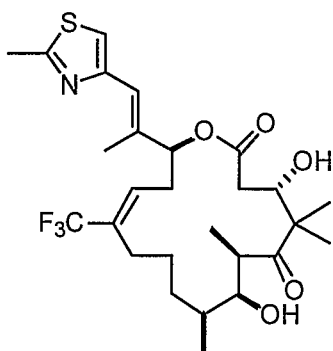
10
 [α]_D²⁵ -54.6 (c 0.28, CHCl₃); IR (film) ν 3478, 2974, 2929, 1736, 1689, 1449, 1381, 1318, 1247, 1169, 1113, 1039, 983, 867, 736 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.05 (3H, s), 1.12 (3H, d, *J* = 7.0 Hz), 1.23 (3H, d, *J* = 6.8 Hz), 1.37 (3H, s), 2.04 (1H, brd, *J* = 3.8 Hz, -OH), 2.12 (3H, s), 2.25-2.33 (1H, m), 2.38 (1H, dd, *J* = 15.3, 3.0 Hz), 2.48 (1H, dd, *J* = 15.4, 9.8 Hz), 2.54-2.61 (1H, m), 2.66-2.76 (1H, m), 2.71 (3H, s), 2.96 (1H, dd, *J* = 16.5, 4.5 Hz), 3.02 (1H, dd, *J* = 16.3, 6.5 Hz), 3.11 (1H, quintet, *J* = 6.7 Hz), 3.19 (1H, brs, =OH), 3.74 (1H, brs), 4.35 (1H, brd, *J* = 9.5 Hz), 5.42 (1H, dd, *J* = 6.2, 4.1 Hz), 5.60 (1H, ddd, *J* = 15.8, 5.6, 4.5 Hz), 5.66 (1H, dd, *J* = 15.8, 5.8 Hz), 6.24 (1H, t, *J* = 7.2 Hz), 6.64 (1H, s), 7.00 (1H, s); ¹³C NMR (100 MHz, CDCl₃) δ 15.1,
 15
 16.1, 17.7, 18.5, 19.3, 22.5, 28.8, 31.1, 39.6, 39.7, 45.0, 53.7, 71.4, 75.3, 76.8, 116.7,
 20 120.2, 124.3 [q, ¹*J*(C,F) = 273.4 Hz], 127.9, 130.2 [q, ³*J*(C,F) = 6.0 Hz], 130.6 [q, ²*J*

(C,F) = 28.4 Hz], 132.5, 136.7, 152.0, 165.4, 170.2, 218.4; LRMS (ESI) calcd for $C_{27}H_{37}F_3NO_5S$ $[M+H]^+$ 544.2, found 544.1; HRMS calcd. for $C_{27}H_{37}F_3NO_5S$ $[M+H]^+$ 544.2345, found 544.2346.



To a solution of diol (1.22 mg, 2.24 μ mol) and TrisNHNH₂ (26.7 mg, 89.6 μ mol) in $ClCH_2CH_2Cl$ (1 mL) at 50 °C was added Et₃N (12.5 μ L, 89.6 μ mol). The reaction was monitored by HPTLC (hexane/EtOAc/ CH_2Cl_2 = 1/1/2). After stirring for 6.5 h, further TrisNHNH₂ (26.7 mg, 89.6 μ mol) and Et₃N (12.5 μ L, 89.6 μ mol) were added to the mixture. After stirring for 14 h, the mixture was cooled to rt, diluted with EtOAc and filtered through a pad of silica gel, which was rinsed with EtOAc. After concentrating, the residue was purified by preparative TLC (hexane/EtOAc/ CH_2Cl_2 = 1/1/2) to give the reduced product (1.16 mg, 2.13 μ mol, 94%) as a white solid.

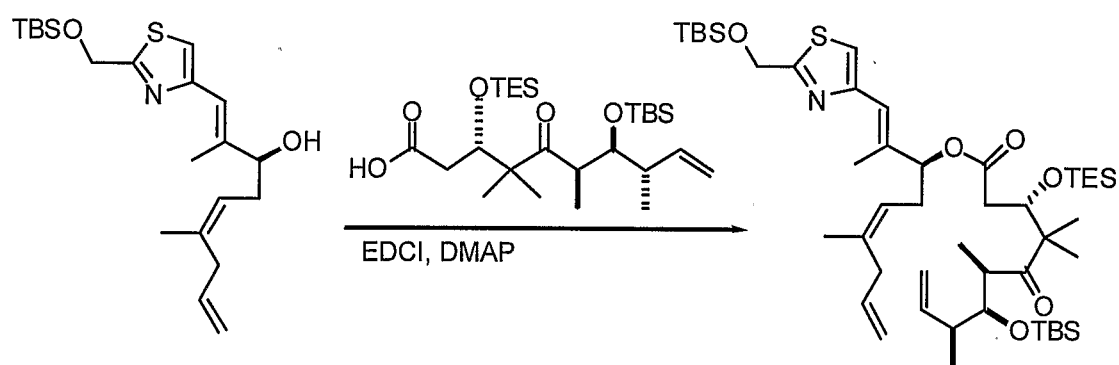
10



$[\alpha]_D^{24}$ -75.1 (c 0.35, $CHCl_3$); IR (film) ν 3483, 2968, 1337, 1685, 1466, 1381, 1322, 1247, 1168, 1113, 1010, 833, 736 cm^{-1} ; ^1H NMR (400 MHz, $CDCl_3$) δ 1.03 (3H, d, J = 7.0 Hz), 1.08 (3H, s), 1.19 (3H, d, J = 6.8 Hz), 1.25-1.35 (2H, m), 1.37 (3H, s), 1.42-1.55 (2H, m), 1.65-1.82 (2H, m), 2.10 (3H, d, J = 0.8 Hz), 2.21-2.47 (2H, m), 2.27 (1H, dd, J = 14.2, 2.6 Hz), 2.48 (1H, dd, J = 14.3, 10.8 Hz), 2.70 (3H, s), 2.70-2.28 (1H, m),

20

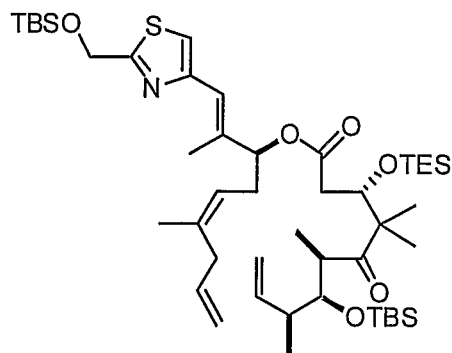
3.02 (1H, d, $J = 2.0$ Hz, -OH), 3.19 (1H, qd, $J = 6.9, 2.2$ Hz), 3.65 (1H, d, $J = 6.2$ Hz, -OH), 3.69-3.72 (1H, m), 4.34 (1H, ddd, $J = 10.8, 6.2, 2.6$ Hz), 5.28 (1H, dd, $J = 10.2, 2.2$ Hz), 6.12 (1H, dd, $J = 10.2, 5.2$ Hz), 6.61 (1H, s), 6.98 (1H, s); ^{13}C NMR (100 MHz, CDCl_3) δ 13.0, 15.9, 16.0, 17.7, 19.1, 23.0, 25.6, 26.2, 31.3, 32.3, 37.4, 39.8, 41.6, 53.9, 72.3, 73.6, 77.7, 116.2, 119.9, 124.3 [$^1J(\text{C},\text{F}) = 274.4$ Hz], 129.8 [$^3J(\text{C},\text{F}) = 6.1$ Hz], 132.6 [$^2J(\text{C},\text{F}) = 27.8$ Hz], 138.3, 151.7, 165.4, 170.2, 220.7; LRMS (ESI) calcd for $\text{C}_{27}\text{H}_{39}\text{F}_3\text{NO}_5\text{S}$ [$\text{M}+\text{H}^+$] 546.3, found 546.2; HRMS calcd. for $\text{C}_{27}\text{H}_{39}\text{F}_3\text{NO}_5\text{S}$ [$\text{M}+\text{H}^+$] 546.2501, found 546.2496.



10

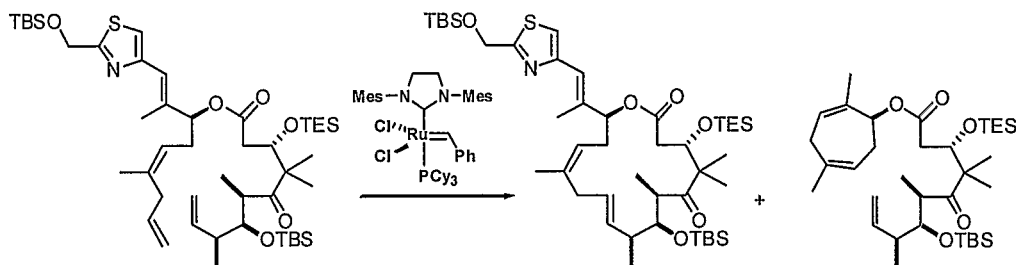
Acid and alcohol were azeotroped with dry benzene (3 mL x 2) and dried under high vacuum prior to the reaction. To a solution of alcohol (68.0 mg, 0.173 mmol) in CH_2Cl_2 (1.3 mL) were added EDCI (37.8 mg, 0.197 mmol) and DMAP (24.1 mg, 0.197 mmol) at 0 °C. To the mixture was added a solution of acid (72.6 mg, as 0.123 mmol) in CH_2Cl_2 (0.7 mL) dropwise over 5 min at 0 °C. After stirring at 0 °C for 1 h, the mixture was stirred at rt for 2.5 h. After concentrating, the residue was purified by flash column chromatography (SiO_2 , hexane/EtOAc = 30:1) to give ester (99.5 mg, 0.114 mmol, 92% from *t*-butyl ester) as a colorless oil.

15



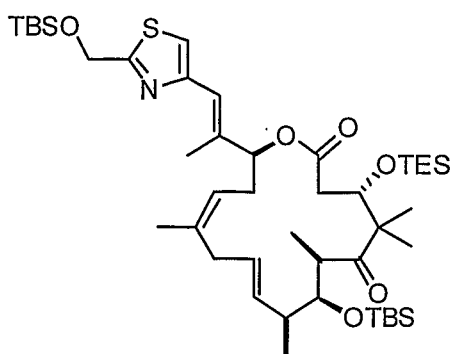
20

$[\alpha]_D^{25} -23.4$ (c 0.56, CHCl_3); IR (film) ν 2955, 2931, 2880, 1735, 1696, 1506, 1472, 1386, 1362, 1294, 1254, 1174, 1104, 988, 878, 776, 742 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 0.06 (3H, s), 0.06 (3H, s), 0.14 (6H, s), 0.63 (6H, q, $J = 8.0$ Hz), 0.92 (9H, s), 0.94 (9H, t, $J = 8.0$ Hz), 0.97 (9H, s), 1.02 (3H, d, $J = 6.6$ Hz), 1.05 (3H, d, $J = 6.5$ Hz),
 5 1.07 (3H, s), 1.21 (3H, s), 1.67 (3H, s), 2.06 (3H, d, $J = 0.8$ Hz), 2.05-2.14 (1H, m), 2.30 (1H, dd, $J = 16.9, 7.5$ Hz), 2.33-2.53 (2H, m), 2.50 (1H, dd, $J = 16.9, 2.7$ Hz), 2.76-2.80 (2H, m), 3.07 (1H, quintet, $J = 7.0$ Hz), 3.83 (1H, dd, $J = 7.0, 2.2$ Hz), 4.35 (1H, dd, $J = 7.4, 2.8$ Hz), 4.97 (2H, s), 4.97-5.07 (4H, m), 5.16 (1H, t, $J = 7.2$ Hz), 5.24 (1H, t, $J = 6.9$ Hz), 5.74 (1H, ddt, $J = 16.6, 10.0, 6.5$ Hz), 5.91 (1H, ddd, $J = 17.6, 9.9,$
 10 7.7 Hz), 6.50 (1H, s), 7.06 (1H, s); ^{13}C NMR (100 MHz, CDCl_3) δ -5.2 (2C), -3.7, -3.3, 5.3 (3C), 7.2 (3C), 14.7, 15.2, 18.5, 18.7, 18.9, 20.3, 23.6, 23.7, 26.0 (3C), 26.4 (3C), 31.7, 36.7, 40.1, 43.8, 46.4, 53.3, 63.4, 74.2, 76.5, 79.6, 115.5, 115.6, 116.6, 120.5, 121.3, 135.8, 136.1, 137.4, 140.1, 153.0, 171.5, 172.2, 218.4; LRMS (ESI) calcd for $\text{C}_{47}\text{H}_{86}\text{NO}_6\text{SSi}_3$ $[\text{M}+\text{H}^+]$ 876.6, found 876.5; HRMS calcd. for $\text{C}_{47}\text{H}_{86}\text{NO}_6\text{SSi}_3$
 15 $[\text{M}+\text{H}^+]$ 876.5484, found 876.5482.



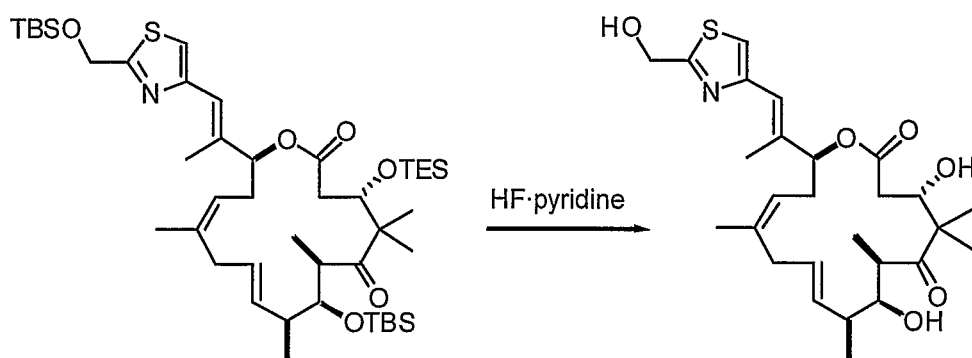
A solution of diene (69.7 mg, 79.5 μmol) in toluene (158 mL) was heated to reflux and treated with a solution of Grubbs' catalyst (6.7 mg, 7.95 μmol) in toluene (2 mL). The
 20 mixture was stirred for 11 min, cooled to 0 $^{\circ}\text{C}$, filtered through a pad of silica gel, which was rinsed with hexane/EtOAc = 3/1 (280 mL). The combined filtrate was concentrated and purified by flash column chromatography (SiO_2 , hexane/ Et_2O = 20:1 to 15:1) to give the desired product (18.4 mg, 21.7 μmol , 27%) and cycloheptadiene (28.3 mg, 45.5 μmol , 57%) both as a colorless oils.

25



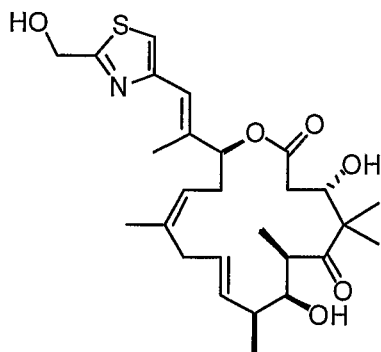
$[\alpha]_D^{24} -40.4$ (c 0.26, CHCl_3); IR (film) ν 2955, 2930, 2879, 1740, 1694, 1472, 1387, 1362, 1253, 1200, 1107, 1007, 838, 776, 742 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 0.08 (3H, s), 0.12 (3H, s), 0.15 (6H, s), 0.57 (6H, q, $J = 7.9$ Hz), 0.88 (9H, t, $J = 8.0$ Hz),
 5 0.95 (9H, s), 0.97 (9H, s), 1.04 (3H, s), 1.06 (3H, d, $J = 7.1$ Hz), 1.12 (3H, s), 1.17 (3H, d, $J = 7.0$ Hz), 1.69 (3H, s), 2.06-2.30 (2H, m), 2.14 (3H, s), 2.45 (1H, dd, $J = 15.6, 3.6$ Hz), 2.50 (1H, dd, $J = 14.9, 3.1$ Hz), 2.63-2.75 (2H, m), 2.97-3.06 (1H, m), 3.10 (1H, dd, $J = 14.6, 7.7$ Hz), 3.97 (1H, d, $J = 8.5$ Hz), 4.44 (1H, dd, $J = 8.4, 2.9$ Hz), 4.97 (2H, s), 5.22 (1H, dd, $J = 8.7, 5.2$ Hz), 5.33-5.44 (2H, m), 5.70 (1H, dd, $J = 15.6, 8.1$ Hz),
 10 6.57 (1H, s), 7.07 (1H, s); ^{13}C NMR (125 MHz, CDCl_3) δ -5.2, -3.3, -3.2, 5.6, 7.2, 7.3, 15.0, 17.2, 18.5, 18.8, 21.4, 23.9, 24.4, 26.0, 26.5, 33.3, 35.6, 41.4, 41.8, 48.2, 54.0, 63.5, 74.4, 78.1, 79.3, 116.6, 120.6, 121.0, 129.3, 132.1, 137.8, 137.9, 153.0, 170.7, 172.3, 216.8; LRMS (ESI) calcd for $\text{C}_{45}\text{H}_{82}\text{NO}_6\text{SSi}_3$ $[\text{M}+\text{H}^+]$ 848.5, found 848.5; HRMS (ESI) calcd. for $\text{C}_{45}\text{H}_{82}\text{NO}_6\text{SSi}_3$ $[\text{M}+\text{H}^+]$ 848.5171, found 848.5161.

15

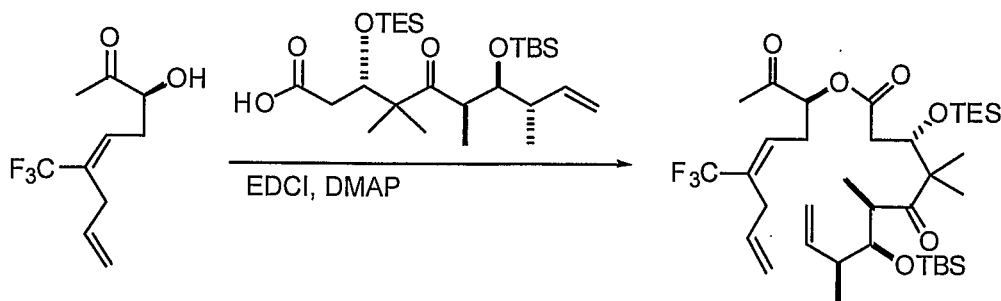


To a solution of silylether (61.8 mg, 72.8 μmol) in THF (2 mL) in a plastic tube was added HF-pyridine (1 mL) at 0 $^\circ\text{C}$, and the mixture was stirred at rt for 3.2 h. The reaction was quenched with dropwise addition of TMSOMe (15 mL) at 0 $^\circ\text{C}$. The
 20 mixture was stirred at rt for 2 h. After concentrating and drying under high vacuum,

the residue was purified by flash column chromatography (SiO₂, hexane/EtOAc = 1:3) to give triol (32.4 mg, 64.1 μmol, 88%) as a white solid.

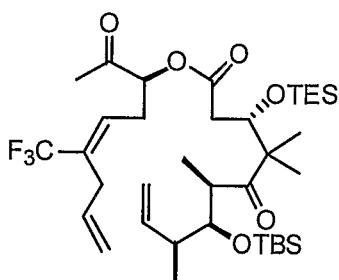


- 5 $[\alpha]_D^{25} -108.4$ (c 0.285, CHCl₃); IR (film) ν 3422, 2968, 2919, 2729, 1689, 1449, 1377, 1252, 1152, 1064, 978 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.05 (3H, s), 1.12 (3H, d, J = 6.9 Hz), 1.22 (3H, d, J = 6.8 Hz), 1.32 (3H, s), 1.72 (3H, s), 2.08 (3H, s), 2.31-2.40 (3H, m), 2.43 (1H, dd, J = 15.5, 3.5 Hz), 2.49 (1H, dd, J = 15.5, 9.5 Hz), 2.55-2.67 (2H, m), 2.95 (1H, dd, J = 14.6, 6.3 Hz), 3.13 (1H, quintet, J = 6.6 Hz), 3.34 (1H, brs, -OH),
 10 3.75 (1H, dd, J = 6.6, 2.4 Hz), 4.06 (1H, brs, -OH), 4.33 (1H, dd, J = 9.4, 3.0 Hz), 4.92 (2H, s), 5.18 (1H, t, J = 6.9 Hz), 5.33 (1H, dd, J = 8.0, 2.5 Hz), 5.52 (1H, dd, J = 15.8, 6.4 Hz), 5.59 (1H, ddd, J = 15.8, 6.6, 5.0 Hz), 6.63 (1H, s), 7.13 (1H, s); ¹³C NMR (100 MHz, CDCl₃) δ 15.3, 16.3, 17.8, 19.2, 22.8, 23.7, 31.9, 35.1, 39.7, 40.2, 45.0, 53.4, 61.8, 71.7, 75.8, 78.1, 116.7, 119.0, 120.5, 130.0, 131.2, 137.6, 138.9, 152.5, 170.0,
 15 170.7, 218.7; LRMS (ESI) calcd for C₂₇H₃₉NO₆SNa [M+Na⁺] 528.2, found 528.0; HRMS calcd. for C₂₇H₄₀NO₆S [M+H⁺] 506.2576, found 506.2552.

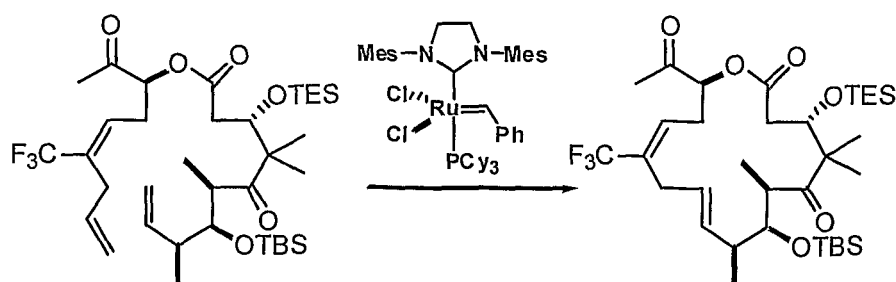


- Crude acid (4.65 g, as 7.27 mmol) and alcohol (2.18 g, 9.84 mmol) were azeotroped
 20 with dry benzene and then dried under high vacuum for 20 min before reaction. To a solution of alcohol (2.18 g, 9.84 mmol) in CH₂Cl₂ (65 mL) were added EDCI (2.09 g,

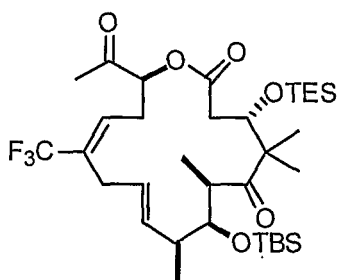
10.9 mmol) and DMAP (1.33 g, 10.9 mmol) at 0 °C. To the mixture was added a solution of crude acid (4.65 g, as 7.27 mmol) in CH₂Cl₂ (20 mL + 5 mL rinse) dropwise over 20 min at 0 °C. After stirring at 0 °C for 40 min, the mixture was stirred at rt for 4 h. After concentrating, the residue was purified by flash column chromatography (SiO₂~160 g, hexane/EtOAc = 20:1) to give ester (4.85 g, 6.87 mmol, 94% from *t*-butyl ester) as a colorless oil.



[α]_D²⁵ -22.7 (c 0.26, CHCl₃); IR (film) ν 2958, 2936, 2800, 1748, 1732, 1693, 1473, 1416, 1360, 1317, 1296, 1254, 1174, 1119, 989, 916, 872, 838, 776 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 0.08 (3H, s), 0.08 (3H, s), 0.60 (6H, q, *J* = 7.8 Hz), 0.93 (9H, s), 0.94 (9H, t, *J* = 8.0 Hz), 1.04 (3H, d, *J* = 7.0 Hz), 1.04 (3H, d, *J* = 7.0 Hz), 1.11 (3H, s), 1.23 (3H, s), 2.05-2.14 (1H, m), 2.17 (3H, s), 2.40 (1H, dd, *J* = 16.9, 7.0 Hz), 2.59 (1H, dd, *J* = 17.0, 3.6 Hz), 2.56-2.64 (2H, m), 2.90-3.01 (2H, m), 3.06 (1H, quintet, *J* = 7.0 Hz), 3.85 (1H, dd, *J* = 7.3, 2.0 Hz), 4.38 (1H, d, *J* = 7.0, 3.4 Hz), 4.97-5.14 (5H, m), 5.75 (1H, ddt, *J* = 16.0, 9.9, 6.2 Hz), 5.92 (1H, ddd, *J* = 17.8, 10.5, 7.8 Hz), 6.21 (1H, td, *J* = 7.2, 1.5 Hz); ¹³C NMR (100 MHz, CDCl₃) δ -3.7, -3.4, 5.2 (3C), 7.1 (3C), 15.4, 18.7, 18.9, 19.5, 23.9, 26.3 (3C), 26.6, 28.5, 30.0, 39.8, 43.7, 46.3, 53.3, 73.6, 76.5, 77.1, 115.6, 117.8, 124.0 [q, ¹*J*(C,F) = 273.5 Hz], 129.2 [q, ³*J*(C,F) = 6.1 Hz], 130.6 [q, ²*J*(C,F) = 28.7 Hz], 133.4, 140.0, 171.8, 204.6, 218.4; LRMS (ESI) calcd for C₃₆H₆₃F₃O₆Si₂Na [M+Na⁺] 727.4, found 727.3; HRMS calcd. for C₃₆H₆₄F₃O₆Si₂ [M+H⁺] 705.4194, found 705.4193.

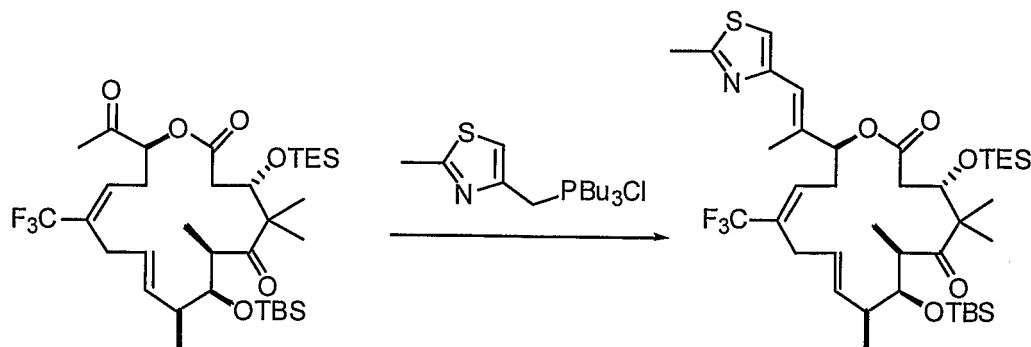


A solution of diene (510.0 mg, 0.723 mmol) in toluene (500 mL) was heated to reflux and treated with a solution of Grubbs' catalyst (92.1 mg, 0.109 mmol) in toluene (10 mL). The mixture was stirred for 17 min under reflux and immediately cooled to 0 °C and kept at 0 °C before filtration through a pad of silica gel. A second batch of diene (510.0 mg, 0.723 mmol) was processed identically and simultaneously. The combined reaction mixture were filtered through a pad of silica gel (100 g), which was rinsed with hexane/EtOAc = 3/1 (1.4 L). The combine filtrate was concentrated and purified by flash column chromatography (SiO₂~65 g, hexane/Et₂O = 10:1 to 5:1) to give
 10 macrolide (742.4 mg, 1.10 mmol, 76%) as a colorless amorphous oil.

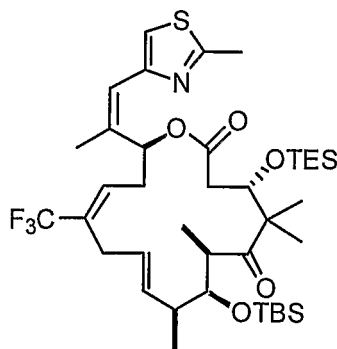


$[\alpha]_D^{25} -7.5$ (c 0.12, CHCl₃); IR (film) ν 2956, 2979, 1748, 1732, 1695, 1472, 1415, 1384, 1252, 1170, 1119, 1018, 986, 876, 835 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 0.08 (3H, s), 0.10 (3H, s), 0.60 (6H, q, J = 7.8 Hz), 0.93 (9H, s), 0.94 (9H, t, J = 7.8 Hz), 1.03 (3H, d, J = 7.1 Hz), 1.08 (3H, s), 1.13 (3H, d, J = 7.0 Hz), 1.17 (3H, s), 2.26 (3H, s), 2.25-2.34 (1H, m), 2.64 (1H, dd, J = 15.5, 5.0 Hz), 2.68-2.75 (2H, m), 2.76 (1H, dd, J = 15.6, 6.4 Hz), 2.85 (1H, dd, J = 15.6, 5.7 Hz), 2.97 (1H, dq, J = 8.3, 6.9 Hz), 3.04 (1H, dd, J = 15.6, 6.3 Hz), 3.92 (1H, dd, J = 8.3, 1.2 Hz), 4.36 (1H, t, J = 5.3 Hz), 5.30-5.39 (2H, m), 5.58 (1H, dd, J = 15.5, 8.0 Hz), 6.13 (1H, brt, J = 7.2 Hz); ¹³C NMR (100 MHz, CDCl₃) δ -3.6, -3.6, 5.4 (3C), 7.0 (3C), 17.5, 18.5, 19.0, 21.6, 23.5, 26.3 (3C), 26.5, 28.6, 29.1, 41.0, 42.3, 47.3, 54.1, 74.2, 76.8, 77.7, 124.0 [q, ¹ J (C,F) = 273.7 Hz], 126.0, 128.7 [q, ³ J (C,F) = 5.9 Hz], 132.2 [q, ² J (C,F) = 28.1 Hz], 133.8, 170.5, 204.1,

216.1; LRMS (ESI) calcd for $C_{34}H_{59}F_3O_6Si_2Na$ $[M+Na^+]$ 699.4, found 699.4; HRMS calcd. for $C_{34}H_{60}F_3O_6Si_2$ $[M+H^+]$ 677.3881, found 677.3892.

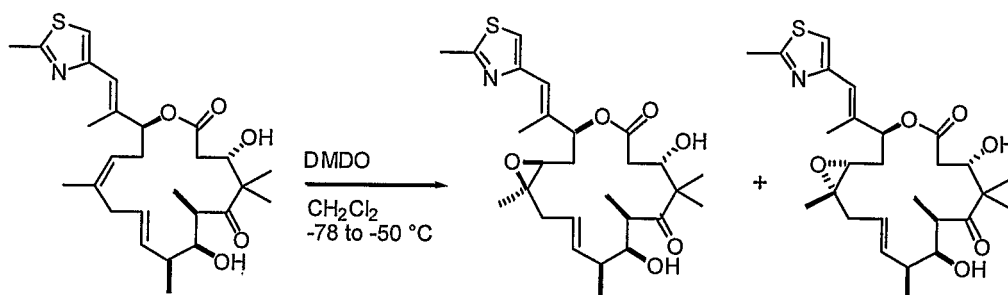


- 5 via Wittig reaction: Ketone was azeotroped with benzene (5 mL x 2) and then dried under high vacuum for 0.5 h. To a solution of Wittig salt (907 mg, 2.59 mmol) in THF (19 mL) was added *t*-BuOK (2.4 mL of a 1.0 M solution in THF, 2.43 mmol) dropwise over 5 min at 0 °C. The mixture was stirred at 0 °C for 0.5 h and then cooled to -78 °C. To the mixture was added dropwise a solution of ketone (1.10 g, 1.62 mmol) in
- 10 THF (13 mL) over 10 min, and the resulting mixture was allowed to warm to -20 °C over 2 h. The reaction was quenched with sat. aqueous NH_4Cl (15 mL) and extracted with EtOAc (50 mL x 3). The combined organic layers were washed with brine (20 mL), dried over Na_2SO_4 and concentrated. The residue was purified by flash column chromatography (SiO_2 , hexane/ Et_2O = 20:1 to 10:1) to give the desired 16(*E*)-isomer
- 15 (940 mg, 1.22 mmol, 75%) and the undesired 16(*Z*)-isomer (140.9 mg, 0.182 mmol, 11%) both as a colorless amorphous oil.

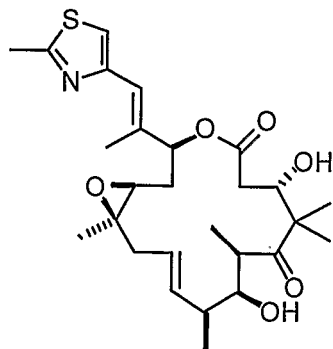


- $[\alpha]_D^{25}$ 62.7 (c 0.33, $CHCl_3$); IR (film) ν 2955, 2878, 1743, 1692, 1472, 1379, 1320, 1253, 1169, 1114, 1007, 956, 877, 835, 775 cm^{-1} ; ^1H NMR (400 MHz, $CDCl_3$) δ 0.09 (3H, s), 0.13 (3H, s), 0.49 (6H, q, J = 7.8 Hz), 0.85 (9H, t, J = 7.8 Hz), 0.97 (9H, s),
- 20 0.99 (3H, s), 1.06 (3H, d, J = 7.1 Hz), 1.11 (3H, s), 1.20 (3H, d, J = 7.1 Hz), 2.00 (3H,

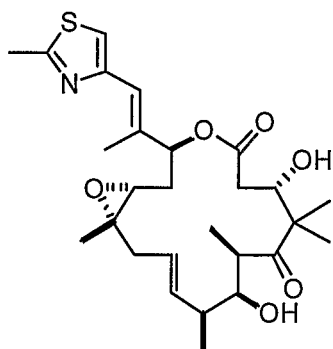
s), 2.03-2.13 (1H, m), 2.35 (1H, dd, $J = 14.3, 3.0$ Hz), 2.46 (1H, dd, $J = 14.3, 7.8$ Hz), 2.41-2.50 (1H, m), 2.73 (3H, s), 2.71-2.90 (2H, m), 2.98-3.12 (2H, m), 3.99 (1H, d, $J = 9.2$ Hz), 4.56 (1H, dd, $J = 7.7, 2.8$ Hz), 5.33 (1H, ddd, $J = 15.6, 8.9, 4.1$ Hz), 5.82 (1H, dd, $J = 15.6, 8.4$ Hz), 6.29 (1H, s), 6.33-6.40 (1H, m), 6.94 (1H, m), 7.09 (1H, brd, $J = 8.4$ Hz); ^{13}C NMR (100 MHz, CDCl_3) δ -3.2, -3.2, 5.5 (3C), 7.0 (3C), 17.2, 18.7, 19.3, 19.6, 20.0, 22.3, 24.9, 26.4 (3C), 29.7, 32.9, 41.9, 42.0, 48.6, 54.0, 72.2, 73.3, 77.0, 116.7, 120.7, 124.5 [q, $^1J(\text{C},\text{F}) = 273.3$ Hz], 127.9, 129.7 [q, $^2J(\text{C},\text{F}) = 28.0$ Hz], 131.9 [q, $^3J(\text{C},\text{F}) = 6.1$ Hz], 132.9, 136.6, 152.1, 165.4, 170.2, 217.4; LRMS (ESI) calcd for $\text{C}_{39}\text{H}_{65}\text{F}_3\text{NO}_5\text{SSi}_2$ $[\text{M}+\text{H}^+]$ 772.4, found 772.4; HRMS calcd. for $\text{C}_{39}\text{H}_{65}\text{F}_3\text{NO}_5\text{SSi}_2$ $[\text{M}+\text{H}^+]$ 772.4074, found 772.4044.



A solution of deH-dEpoB (12.2 mg, 24.9 μmol) in CH_2Cl_2 (1.25 mL) was cooled to -78 °C and treated with a cooled solution of DMDO (-78 °C, 0.06 M in acetone, 914 μL , 54.8 μmol). The mixture was allowed to warm to -50 °C and stirred at -50 °C for 2.7 h. The excess DMDO was quenched at -50 °C by the addition of dimethylsulfide (117 μL) and the mixture was stirred at this temperature for 0.5 h. The solvent was removed in vacuo. Purification by preparative thin layer chromatography (hexane/EtOAc = 1/2) gave β -epoxide (3.0 mg, 5.93 μmol , 24%) and α -epoxide (7.9 mg, 15.6 μmol , 63%) both as a colorless solids.



$[\alpha]_D^{25} -78.5$ (c 0.33, CHCl_3); IR (film) ν 3454, 2974, 2928, 1734, 1689, 1450, 1379, 1250, 1152, 1061, 978, 735 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 1.03 (3H, s), 1.11 (3H, d, $J = 7.0$ Hz), 1.14 (3H, d, $J = 6.9$ Hz), 1.34 (3H, s), 1.36 (3H, s), 2.00 (1H, ddd, $J = 15.1, 7.3, 4.0$ Hz), 2.14 (1H, dt, $J = 15.1, 5.2$ Hz), 2.14 (3H, s), 2.21 (1H, dd, $J = 14.6, 8.0$ Hz), 2.33 (1H, dd, $J = 14.7, 4.8$ Hz), 2.47 (1H, dd, $J = 13.8, 3.3$ Hz), 2.59 (1H, dd, $J = 13.8, 9.4$ Hz), 2.73 (3H, s), 2.77 (1H, brs, OH), 2.93 (1H, dd, $J = 7.3, 4.8$ Hz), 3.34 (1H, qd, $J = 6.9, 3.8$ Hz), 3.75-3.82 (1H, m), 4.12-4.24 (2H, m, including OH), 5.54 (1H, ddd, $J = 15.7, 7.4, 5.0$ Hz), 5.54-5.60 (1H, m), 5.64 (1H, dd, $J = 15.7, 5.6$ Hz), 6.94 (1H, s), 7.01 (1H, s); ^1H NMR (500 MHz, CD_2Cl_2) δ 0.91 (3H, s), 1.01 (3H, d, $J = 6.9$ Hz), 1.03 (3H, d, $J = 6.9$ Hz), 1.22 (3H, s), 1.27 (3H, s), 1.96-2.02 (1H, m), 2.04 (3H, d, $J = 0.7$ Hz), 2.16-2.23 (2H, m), 2.33 (1H, dd, $J = 14.2, 3.1$ Hz), 2.30-2.35 (1H, m), 2.44 (1H, dd, $J = 14.4, 10.3$ Hz), 2.69 (3H, s), 2.77 (1H, t, $J = 5.9$ Hz), 3.24 (1H, qd, $J = 6.9, 4.5$ Hz), 3.63 (1H, t, $J = 4.1$ Hz), 4.18-4.26 (1H, m), 5.37 (1H, t, $J = 4.5$ Hz), 5.48 (1H, dtd, $J = 15.7, 6.7, 0.5$ Hz), 5.58 (1H, dd, $J = 15.7, 6.2$ Hz), 6.58 (1H, s), 7.00 (1H, s); ^{13}C NMR (100 MHz, CDCl_3) δ 14.4, 16.3 (2C), 19.3, 19.7, 21.6, 22.6, 31.8, 35.9, 38.7, 39.6, 44.1, 52.8, 60.8, 61.8, 74.0, 75.7, 75.9, 116.5, 119.6, 124.3, 135.8, 136.2, 152.1, 165.2, 170.8, 221.5; LRMS calcd for $\text{C}_{27}\text{H}_{40}\text{NO}_6\text{S}$ $[\text{M}+\text{H}^+]$ 506.3, found 506.3; HRMS (ESI) calcd. for $\text{C}_{27}\text{H}_{40}\text{NO}_6\text{S}$ $[\text{M}+\text{H}^+]$ 506.2576, found 506.2566.

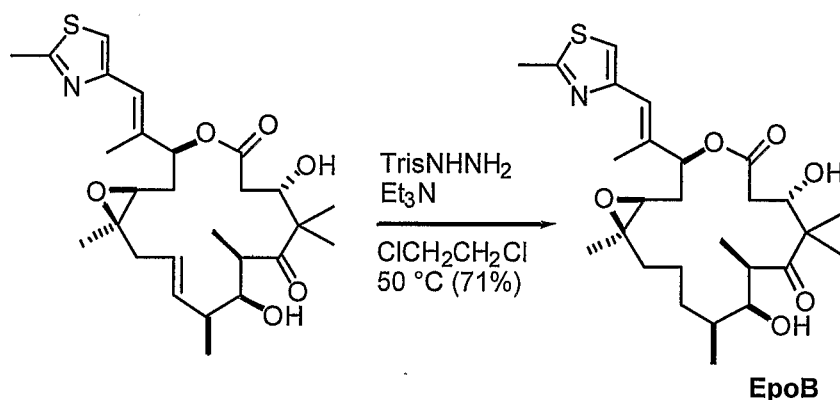


20

$[\alpha]_D^{25} -53.9$ (c 0.700, CHCl_3); IR (film) ν 3460, 2976, 2928, 1735, 1688, 1506, 1451, 1378, 1252, 1186, 1151, 1087, 1042, 976, 879, 735 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 1.00 (3H, s), 1.04 (3H, d, $J = 6.9$ Hz), 1.12 (3H, d, $J = 7.0$ Hz), 1.35 (3H, s), 1.35 (3H, s), 1.87 (1H, dt, $J = 15.0, 9.2$ Hz), 2.03 (1H, dd, $J = 13.9, 9.2$ Hz), 2.13 (3H, s), 2.13-2.19 (1H, m), 2.36 (1H, dd, $J = 13.9, 3.4$ Hz), 2.39 (1H, dd, $J = 12.2, 2.1$ Hz), 2.42-2.51 (1H, m), 2.49 (1H, dd, $J = 12.4, 10.9$ Hz), 2.69 (1H, d, $J = 2.7$ Hz), 2.72 (3H,

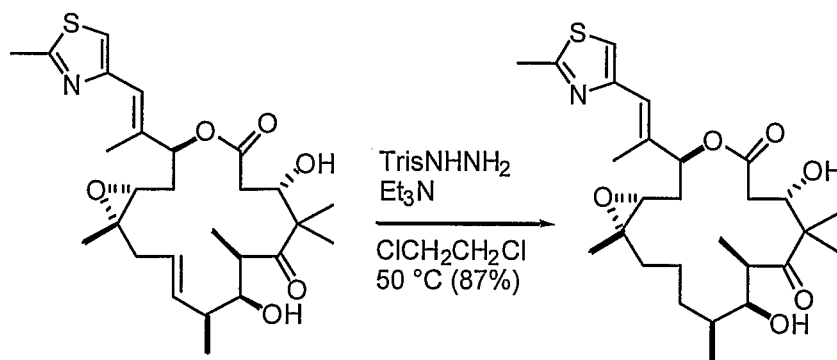
25

s), 3.06 (1H, dd, $J = 9.7, 3.1$ Hz), 3.54 (1H, qd, $J = 7.0, 1.8$ Hz), 3.76-3.80 (1H, m), 4.07-4.14 (1H, m), 4.31 (1H, d, $J = 4.1$ Hz), 5.52 (1H, dd, $J = 15.5, 8.7$ Hz), 5.60 (1H, ddd, $J = 15.1, 9.4, 3.4$ Hz), 5.71 (1H, d, $J = 8.4$ Hz), 6.63 (1H, s), 6.99 (1H, s); ^{13}C NMR (100 MHz, CDCl_3) δ 13.7, 15.3, 15.7, 18.5, 19.4, 21.2, 22.4, 32.5, 35.5, 39.1, 43.4, 43.8, 51.9, 61.3, 64.8, 73.5, 75.9, 76.4, 116.7, 120.1, 124.3, 137.5, 137.7, 152.3, 165.2, 171.0, 222.3; LRMS (ESI) calcd for $\text{C}_{27}\text{H}_{39}\text{NO}_6\text{SNa}$ [$\text{M}+\text{Na}^+$] 528.2, found 528.2; HRMS calcd. for $\text{C}_{27}\text{H}_{40}\text{NO}_6\text{S}$ [$\text{M}+\text{H}^+$] 506.2576, found 506.2583.

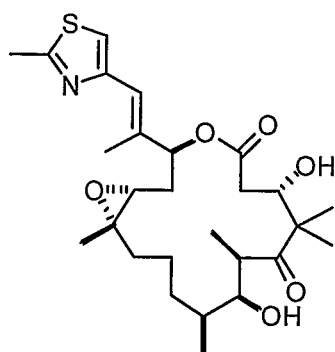


To a solution of epoxide (0.7 mg, 1.38 μmol) and TrisNHNH_2 (20.6 mg, 69 μmol) in $\text{ClCH}_2\text{CH}_2\text{Cl}$ (0.4 mL) at 50 °C was added Et_3N (9.6 μL , 69 μmol). The reaction was monitored by HPTLC (hexane/ $\text{EtOAc} = 1/2$). After stirring for 6 h, the mixture was cooled to rt, diluted with EtOAc and filtered through a pad of silica gel, which was rinsed with EtOAc . After concentrating, the residue was purified by preparative TLC (hexane/ $\text{EtOAc} = 1/2$) to give reduced product (0.5 mg, 0.985 μmol , 71%) as a white solid.

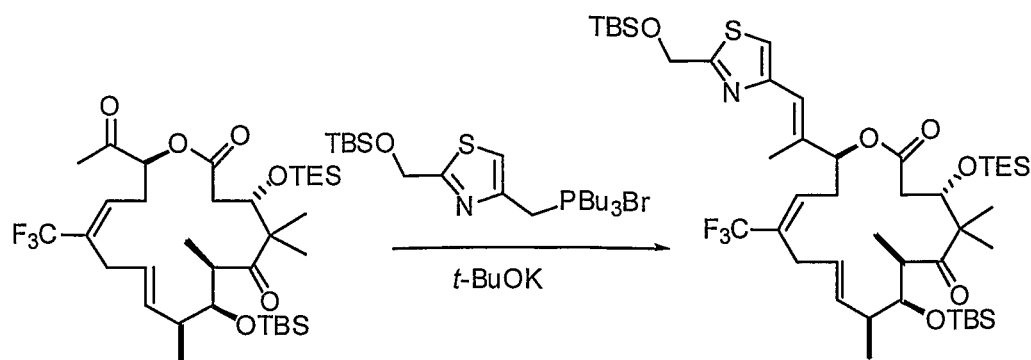
The spectral data of this compound was identical to those reported for EpoB.



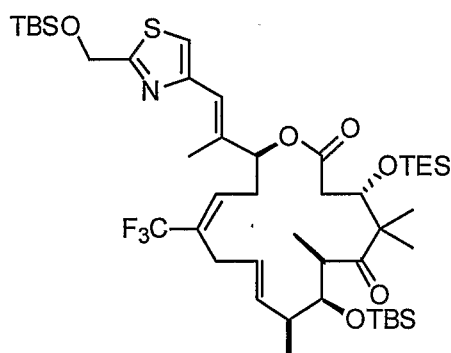
To a solution of epoxide (14.0 mg, 27.7 μmol) and TrisNHNH_2 (165 mg, 0.554 mmol) in $\text{ClCH}_2\text{CH}_2\text{Cl}$ (3.3 mL) at 50 $^\circ\text{C}$ was added Et_3N (77.0 μL , 0.554 mmol). The reaction was monitored by HPTLC (hexane/ EtOAc = 1/2). After stirring for 6 h, the mixture was cooled to rt, diluted with EtOAc and filtered through a pad of silica gel, which was rinsed with EtOAc . After concentrating, the residue was purified by preparative TLC (hexane/ EtOAc = 1/2) to give reduced product (12.3 mg, 24.2 μmol , 87%) as a colorless solid.



$[\alpha]_{\text{D}}^{24} -13.8$ (c 0.61, CHCl_3); IR (film) ν 3475, 2971, 2875, 1735, 1689, 1456, 1382, 1253, 1181, 1151, 1056, 980, 884, 735 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 0.95 (3H, d, J = 7.1 Hz), 1.04 (3H, s), 1.11 (3H, d, J = 7.0 Hz), 1.28 (3H, s), 1.37 (3H, s), 1.25-1.44 (2H, m), 1.45-1.59 (2H, m), 1.71-1.82 (3H, m), 1.86 (1H, dt, J = 15.3, 9.5 Hz), 2.10 (1H, dd, J = 15.3, 3.6 Hz), 2.13 (3H, s), 2.40 (1H, dd, J = 12.5, 2.5 Hz), 2.49 (1H, dd, J = 12.5, 11.0 Hz), 2.74 (3H, s), 2.80 (1H, brs, OH), 3.07 (1H, dd, J = 10.3, 3.3 Hz), 3.34 (1H, qd, J = 7.0, 0.5 Hz), 3.89 (1H, brs, OH), 4.03-4.09 (1H, m), 4.12-4.17 (1H, m), 5.69 (1H, d, J = 9.1 Hz), 6.63 (1H, s), 7.00 (1H, s); ^{13}C NMR (100 MHz, CDCl_3) δ 12.9, 15.4, 16.3, 18.8, 19.3, 21.6, 22.0, 23.0, 31.5, 32.1, 33.6, 38.6, 38.9, 42.6, 51.7, 62.6, 65.5, 71.2, 74.5, 76.3, 116.6, 119.9, 138.0, 152.2, 165.2, 170.6, 222.7; LRMS (ESI) calcd for $\text{C}_{27}\text{H}_{41}\text{NO}_6\text{SNa}$ $[\text{M}+\text{Na}^+]$ 530.3, found 530.2; HRMS calcd. for $\text{C}_{27}\text{H}_{42}\text{NO}_6\text{S}$ $[\text{M}+\text{H}^+]$ 508.2733, found 508.2754.

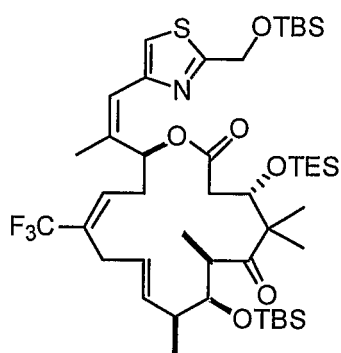


- Ketone was azeotroped with benzene (5 mL x 2) and then dried under high vacuum for 0.5 h. To a solution of Wittig salt (1.19 g, 2.27 mmol) in THF (18 mL) was added *t*-BuOK (2.2 mL of a 1.0 M solution in THF, 2.20 mmol) dropwise over 5 min at 0 °C.
- 5 The mixture was stirred at 0 °C for 20 min and then cooled to -78 °C. To the mixture was added dropwise a solution of ketone (1.06 g, 1.51 mmol) in THF (10 mL + 2 mL rinse) over 10 min, and the resulting mixture was allowed to warm to -20 °C over 2 h. The reaction was quenched with sat. aqueous NH₄Cl (15 mL) and extracted with EtOAc (50 mL x 3). The combined organic layers were washed with brine (20 mL),
- 10 dried over Na₂SO₄ and concentrated. The residue was purified by flash column chromatography (SiO₂ ~ 65 g, hexane/Et₂O = 30:1 to 20:1) to give the desired 16(*E*)-isomer (1.01 g, 1.11 mmol, 74%) and undesired 16(*Z*)-isomer (154.5 mg, 0.182 mmol, 11%) both as a colorless amorphous oil.



- 15 $[\alpha]_D^{24} -19.0$ (c 0.10, CHCl₃); IR (film) ν 2954, 2930, 2880, 1744, 1692, 1472, 1381, 1321, 1252, 1171, 1114, 1038, 1006, 837, 776 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 0.09 (3H, s), 0.12 (3H, s), 0.15 (6H, s), 0.55 (6H, q, *J* = 7.8 Hz), 0.87 (9H, t, *J* = 8.0 Hz), 0.96 (9H, s), 0.97 (9H, s), 1.01 (3H, s), 1.06 (3H, d, *J* = 7.1 Hz), 1.12 (3H, s), 1.20 (3H, d, *J* = 7.1 Hz), 2.07-2.16 (1H, m), 2.18 (3H, d, *J* = 1.0 Hz), 2.38 (1H, dd, *J* = 14.4,
- 20 3.3 Hz), 2.34-2.46 (1H, m), 2.49 (1H, dd, *J* = 14.4, 7.4 Hz), 2.78-2.90 (2H, m), 2.97-3.09 (2H, m), 3.98 (1H, d, *J* = 8.9 Hz), 4.54 (1H, dd, *J* = 7.3, 3.3 Hz), 4.97 (2H, s), 5.33

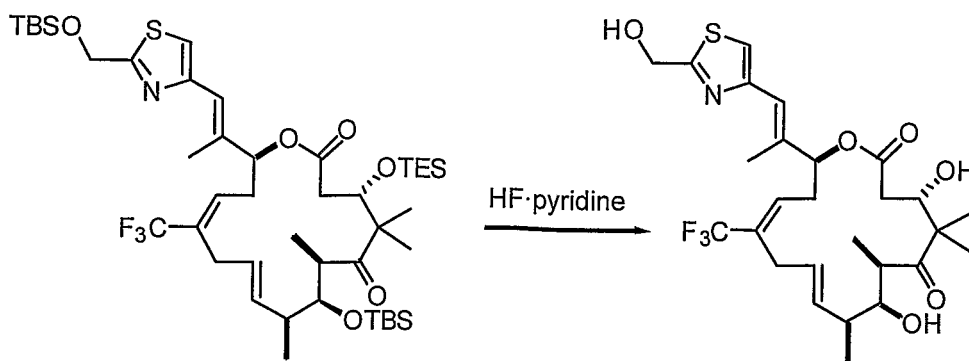
(1H, ddd, $J = 15.8, 8.6, 4.9$ Hz), 5.63 (1H, dd, $J = 9.6, 2.4$ Hz), 5.78 (1H, dd, $J = 15.8, 8.2$ Hz), 6.22-6.27 (1H, m), 6.60 (1H, s), 7.09 (1H, s); ^{13}C NMR (100 MHz, CDCl_3) δ -5.3 (2C), -3.4, -3.3, 5.5 (3C), 7.0 (3C), 14.6, 17.1, 18.4, 18.7, 19.8, 21.3, 24.8, 25.9 (3C), 26.4 (3C), 29.6, 32.9, 42.0, 42.1, 48.2, 54.1, 63.4, 73.4, 76.9, 77.8, 117.2, 121.7, 124.3 [q, $^1J(\text{C},\text{F}) = 273.6$ Hz], 127.2, 130.7 [q, $^2J(\text{C},\text{F}) = 27.5$ Hz], 130.8 [q, $^3J(\text{C},\text{F}) = 6.2$ Hz], 133.2, 136.4, 152.6, 170.1, 172.4, 217.1; LRMS (ESI) calcd. for $\text{C}_{45}\text{H}_{78}\text{F}_3\text{NO}_6\text{SSi}_3\text{Na}$ [$\text{M}+\text{Na}^+$] 924.5, found 924.5; HRMS calcd. for $\text{C}_{45}\text{H}_{79}\text{F}_3\text{NO}_6\text{SSi}_3$ [$\text{M}+\text{H}^+$] 902.4888, found 902.4887.



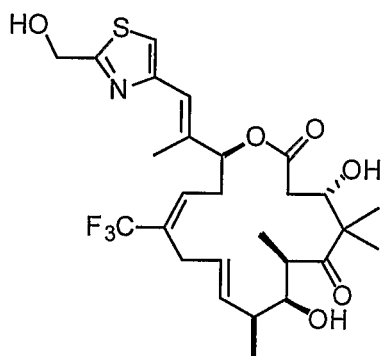
10

$[\alpha]_{\text{D}}^{26}$ 65.7 (c 1.76, CHCl_3); IR (film) ν 2955, 2931, 2879, 1743, 1692, 1472, 1380, 1321, 1253, 1170, 1113, 1007, 836, 776 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 0.07 (3H, s), 0.13 (3H, s), 0.16 (6H, s), 0.48 (6H, q, $J = 7.8$ Hz), 0.84 (9H, t, $J = 7.9$ Hz), 0.97 (18H, s), 0.98 (3H, s), 1.06 (3H, d, $J = 7.1$ Hz), 1.11 (3H, s), 1.20 (3H, d, $J = 7.2$ Hz), 2.00 (3H, s), 2.03-2.11 (1H, m), 2.33 (1H, dd, $J = 14.1, 2.8$ Hz), 2.43 (1H, dd, $J = 14.0, 7.8$ Hz), 2.40-2.48 (1H, m), 2.76-2.89 (2H, m), 2.97-3.10 (2H, m), 3.99 (1H, d, $J = 9.3$ Hz), 4.57 (1H, dd, $J = 7.8, 2.6$ Hz), 4.95 (1H, d, $J = 14.6$ Hz), 5.00 (1H, d, $J = 14.6$ Hz), 5.33 (1H, ddd, $J = 15.6, 9.1, 3.8$ Hz), 5.82 (1H, dd, $J = 15.6, 8.3$ Hz), 6.30 (1H, s), 6.32-6.38 (1H, m), 7.04 (1H, s), 7.11 (1H, dd, $J = 11.0, 2.3$ Hz); ^{13}C NMR (100 MHz, CDCl_3) δ -5.3 (2C), -3.2, -3.2, 5.5 (3C), 7.0 (3C), 17.2, 18.4, 18.8, 19.3, 19.8, 22.4, 25.1, 25.9 (3C), 26.5 (3C), 29.7, 33.0, 41.9, 42.1, 48.6, 54.0, 63.5, 72.1, 73.3, 76.9, 117.0, 120.8, 124.5 [q, $^1J(\text{C},\text{F}) = 273.5$ Hz], 127.9, 129.7 [q, $^2J(\text{C},\text{F}) = 27.6$ Hz], 131.9 [q, $^3J(\text{C},\text{F}) = 6.1$ Hz], 132.9, 136.4, 152.4, 170.1, 172.9, 217.5; LRMS (ESI) calcd for $\text{C}_{45}\text{H}_{78}\text{F}_3\text{NO}_6\text{SNa}$ [$\text{M}+\text{Na}^+$] 924.5, found 924.5.

25

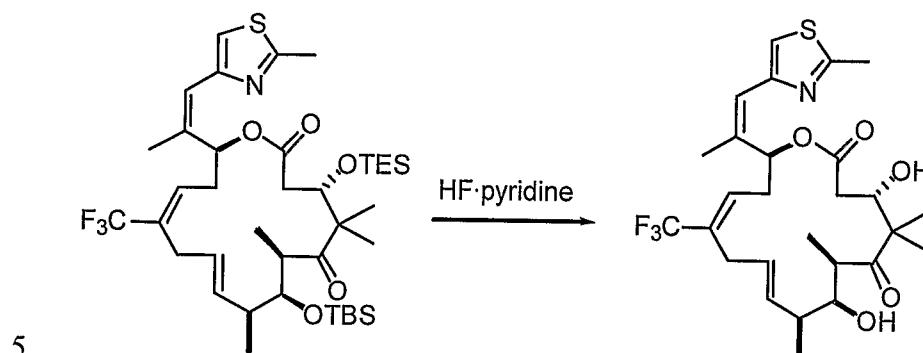


To a solution of silyl ether (1.04 g, 2.25 mmol) in THF (22 mL) in a plastic tube was added slowly HF·pyridine (11 mL) at 0 °C, and the mixture was stirred at rt for 4.3 h. The reaction was quenched with dropwise addition of TMSOMe (75 mL) over 10 min
 5 at 0 °C. The mixture was vigorously stirred at rt for 4.2 h. After concentrating and drying under high vacuum for 1 h, the residue was purified by flash column chromatography (SiO₂~25 g, hexane/EtOAc = 3:4 to 1:2) to give triol (615.7 mg, 1.00 mmol, 96%) as a colorless powder.



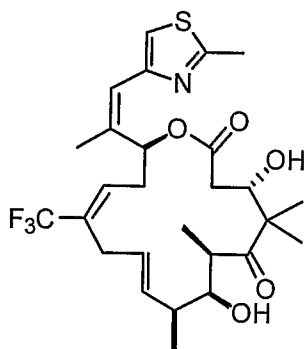
- 10 $[\alpha]_D^{25} -57.7$ (c 1.20, CHCl₃); IR (film) ν 3441, 2974, 2932, 1734, 1685, 1507, 1456, 1374, 1318, 1248, 1169, 1112, 1054, 982, 888, 737 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.04 (3H, s), 1.12 (3H, d, J = 6.9 Hz), 1.25 (3H, d, J = 6.8 Hz), 1.36 (3H, s), 1.90 (1H, d, J = 6.6 Hz, OH), 2.08 (3H, s), 2.23-2.32 (1H, m), 2.34 (1H, dd, J = 15.7, 2.4 Hz), 2.49 (1H, dd, J = 15.7, 10.1 Hz), 2.59-2.69 (2H, m), 2.95-3.01 (2H, m), 3.04 (1H, quintet, J = 6.8 Hz), 3.72 (1H, td, J = 7.0, 3.0 Hz), 3.78 (1H, d, J = 5.7 Hz, OH), 4.38 (1H, ddd, J = 10.1, 5.7, 2.4 Hz), 4.90 (2H, d, J = 6.1 Hz), 5.10 (1H, t, J = 6.1 Hz, OH), 5.44 (1H, t, J = 4.7 Hz), 5.60 (1H, dd, J = 15.9, 4.4 Hz), 5.66 (1H, dd, J = 15.9, 5.0 Hz), 6.28 (1H, t, J = 6.7 Hz), 6.73 (1H, s), 7.16 (1H, s); ¹³C NMR (100 MHz, CDCl₃) δ 16.0, 16.5, 17.4, 17.5, 22.9, 28.5, 30.3, 39.0, 39.6, 45.6, 54.0, 60.9, 70.6, 75.6, 75.7, 116.8, 119.2, 124.2 [q, ¹ J (C,F) = 273.6 Hz], 127.9, 129.8 [q, ² J (C,F) = 28.4 Hz], 130.3 [q, ³ J
- 15
- 20

(C,F) = 5.9 Hz], 131.2, 137.0, 152.2, 169.8, 170.0, 218.3; LRMS (ESI) calcd for $C_{27}H_{37}F_3NO_6SNa$ $[M+H]^+$ 560.2, found 560.1; HRMS calcd. for $C_{27}H_{37}F_3NO_6S$ $[M+H]^+$ 560.2294, found 560.2299.



To a solution of silyl ether (42.8 mg, 55.4 μ mol) in THF (1 mL) in a plastic tube was added slowly HF-pyridine (0.5 mL) at 0 °C, and the mixture was stirred at rt for 4.3 h. The reaction was quenched with dropwise addition of TMSOMe (3.2 mL) over 10 min at 0 °C. The mixture was vigorously stirred at rt for 1.5 h. After concentrating and drying under high vacuum for 1 h, the residue was purified by flash column chromatography (SiO₂, hexane/EtOAc = 1:1) gave diol (23.6 mg, 43.4 μ mol, 78%) as a colorless oil.

10



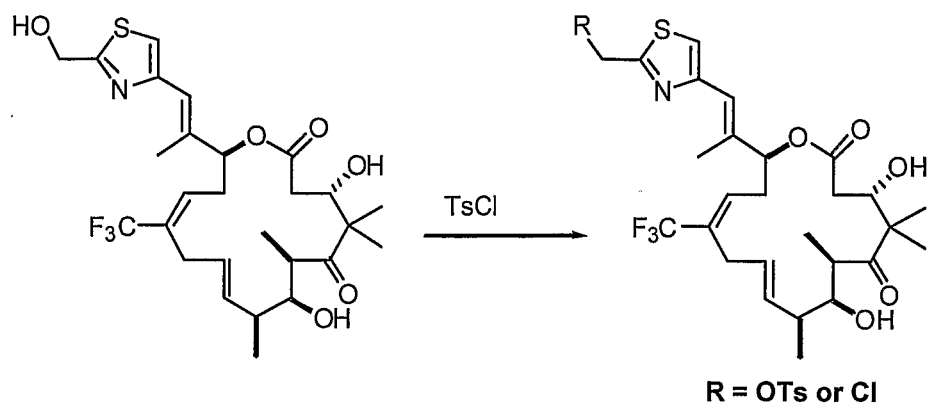
$[\alpha]_D^{25}$ 31.6 (c 1.00, CHCl₃); IR (film) ν 2955, 2878, 1743, 1692, 1471, 1379, 1320, 1253, 1169, 1114, 1007, 877, 835, 741 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 1.04 (3H, s), 1.11 (3H, d, J = 6.9 Hz), 1.20 (3H, d, J = 6.9 Hz), 1.30 (3H, s), 1.93 (3H, brs), 2.22 (1H, d, J = 4.3 Hz, OH), 2.25-2.33 (1H, m), 2.38-2.41 (2H, m), 2.51-2.59 (2H, m), 2.70 (3H, s), 2.80-2.90 (1H, m), 2.94 (1H, dd, J = 15.6, 4.7 Hz), 3.06 (1H, dd, J = 15.6, 7.4 Hz), 3.19 (1H, quintet, J = 6.6 Hz), 3.71-3.76 (1H, m), 4.26-4.32 (1H, m), 5.57 (1H, ddd, J = 15.8, 7.2, 5.0 Hz), 5.67 (1H, dd, J = 15.8, 6.8 Hz), 6.27 (1H, s), 6.33 (1H, dd, J = 7.6, 6.3 Hz), 6.76 (1H, dd, J = 8.3, 2.9 Hz), 6.94 (1H, s); ¹³C NMR (100 MHz,

15

20

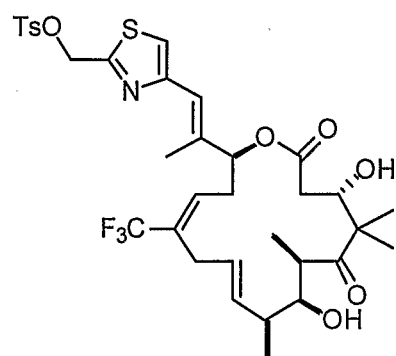
CDCl₃) δ 14.5, 17.9, 19.2, 19.5, 19.8, 22.2, 28.7, 32.4, 39.8 (2C), 44.7, 53.3, 71.9, 74.1, 75.1, 117.0, 120.4, 124.4 [q, ^1J (C,F) = 272.7 Hz], 128.4, 130.1 [q, ^2J (C,F) = 28.9 Hz], 131.5 [q, ^3J (C,F) = 5.9 Hz], 133.0, 136.9, 152.2, 165.5, 170.7, 218.5; LRMS (ESI) calcd for C₂₇H₃₆F₃NO₅SNa [M+Na⁺] 566.2, found 566.3.

5



To a solution of alcohol (18.9 mg, 33.8 μ mol) and Et₃N (18.8 μ L, 0.135 mmol) in CH₂Cl₂ (1 mL) was added TsCl (12.9 mg, 67.5 μ mol) followed by DMAP (2.1 mg, 16.9 μ mol) at 0 °C. After stirring at rt for 1.5 h, the mixture was filtered through a pad of silica gel (EtOAc rinse). After concentrated, the residue was purified by preparative TLC (hexane/EtOAc= 1/1) to give tosylate (8.5 mg, 11.9 μ mol, 35%) and chloride (4.3 mg, 7.44 μ mol, 22%) both as a colorless powders.

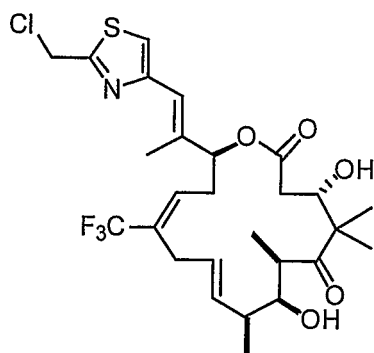
10



¹H NMR (400 MHz, CDCl₃) δ 1.06 (3H, s), 1.12 (3H, d, J = 7.0 Hz), 1.23 (3H, d, J = 6.7 Hz), 1.33 (3H, s), 1.99 (1H, d, J = 5.5 Hz), 2.10 (3H, s), 2.25-2.34 (1H, m), 2.41 (1H, dd, J = 15.5, 3.3 Hz), 2.47 (3H, s), 2.48 (1H, dd, J = 15.7, 9.4 Hz), 2.51-2.63 (1H, m), 2.63 (1H, d, J = 6.1 Hz, OH), 2.64-2.75 (1H, m), 2.91-3.05 (2H, m), 3.10 (1H, quintet, J = 6.8 Hz), 3.70-3.75 (1H, m), 4.30 (1H, ddd, J = 9.3, 6.1, 3.2 Hz), 5.32 (2H, s), 5.41 (1H, dd, J = 5.8, 4.5 Hz), 5.57 (1H, ddd, J = 15.8, 6.4, 4.6 Hz), 5.65 (1H, dd, J

15

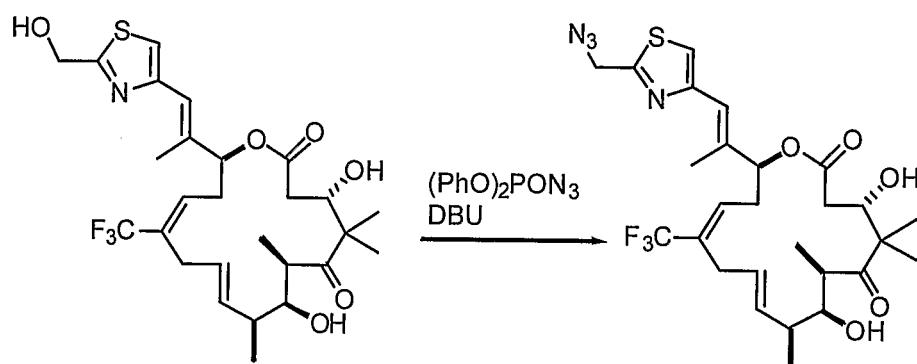
= 15.8, 6.0 Hz), 6.21 (1H, t, $J = 7.1$ Hz), 6.59 (1H, s), 7.18 (1H, s), 7.37 (2H, d, $J = 8.1$ Hz), 7.84 (2H, d, $J = 8.3$ Hz); LRMS (ESI) calcd for $C_{34}H_{42}F_3NO_8S_2Na$ $[M+Na^+]$ 736.2, found 736.3.



5

IR (film) ν 3494, 2975, 2935, 1734, 1689, 1319, 1248, 1170, 1113, 1040, 979, 738 cm^{-1} ; ^1H NMR (400 MHz, $CDCl_3$) δ 1.06 (3H, s), 1.12 (3H, d, $J = 6.9$ Hz), 1.23 (3H, d, $J = 6.7$ Hz), 1.34 (3H, s), 2.00 (1H, d, $J = 5.6$ Hz, OH), 2.15 (3H, s), 2.25-2.35 (1H, m), 2.41 (1H, dd, $J = 15.5, 3.2$ Hz), 2.49 (1H, dd, $J = 15.5, 9.4$ Hz), 2.53-2.62 (1H, m), 2.69 (1H, d, $J = 6.1$ Hz, OH), 2.66-2.76 (1H, m), 2.92-3.05 (2H, m), 3.11 (1H, quintet, $J = 6.4$ Hz), 3.70-3.76 (1H, m), 4.32 (1H, ddd, $J = 9.2, 5.9, 3.1$ Hz), 4.85 (2H, s), 5.43 (1H, dd, $J = 6.0, 4.4$ Hz), 5.59 (1H, ddd, $J = 15.9, 6.4, 4.5$ Hz), 5.66 (1H, dd, $J = 15.9, 6.1$ Hz), 6.23 (1H, t, $J = 6.8$ Hz), 6.63 (1H, s), 7.20 (1H, s); LRMS (ESI) calcd for $C_{27}H_{35}ClF_3NO_5SNa$ $[M+Na^+]$ 600.2, found 600.2.

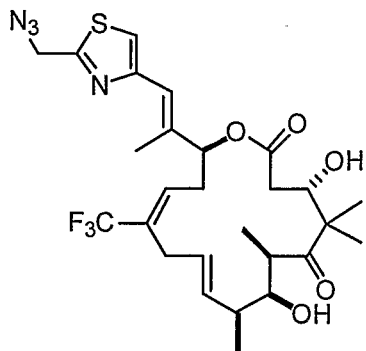
15



To a solution of triol (50.4 mg, 90.1 μ mol) in THF (1 mL) was added $(PhO)_2PON_3$ (27.2 μ L, 0.126 mmol) at 0 $^{\circ}C$. After 5 min, DBU (16.2 μ L, 0.108 mmol) was added, and the mixture was stirred at 0 $^{\circ}C$ for 2 h then rt for 20.5 h. The mixture was diluted with EtOAc and quenched with water (2 mL), extracted with EtOAc (three times), and

20

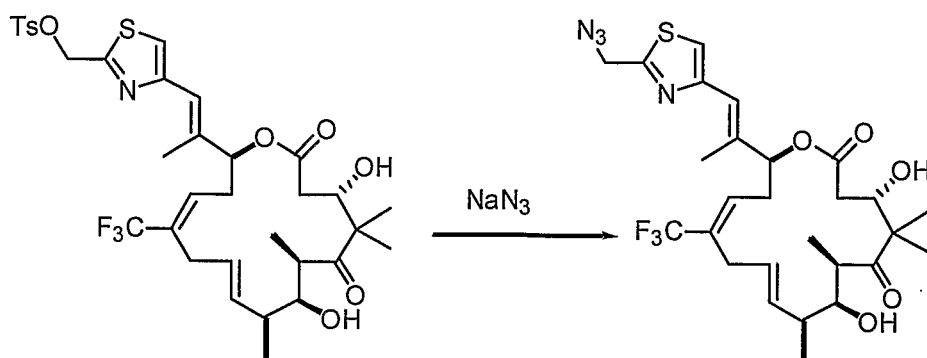
the combined organic layers were dried over Na_2SO_4 . After concentrating, the residue was purified by flash column chromatography (SiO_2 , hexane/EtOAc = 3:2) to give azide (45.6 mg, 78.0 μmol , 87%) as a colorless powder.



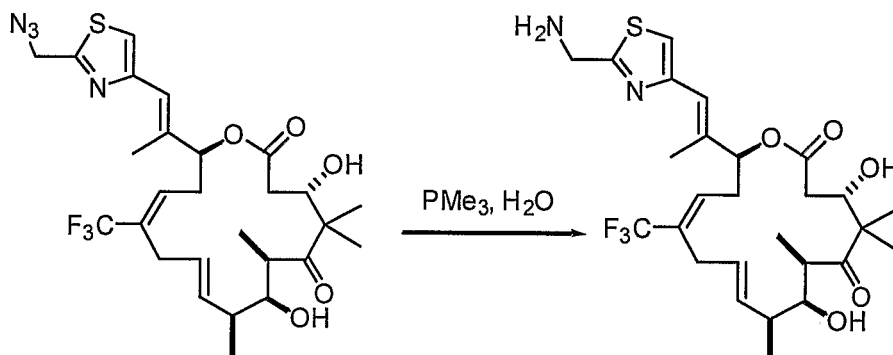
5

$[\alpha]_D^{24} -60.3$ (c 0.345, CHCl_3); IR (film) ν 3492, 2975, 2931, 2105, 1732, 1688, 1319, 1248, 1169, 1113, 982, 733 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 1.05 (3H, s), 1.12 (3H, d, $J = 7.0$ Hz), 1.23 (3H, d, $J = 6.8$ Hz), 1.33 (3H, s), 2.01 (1H, d, $J = 5.5$ Hz, OH), 2.17 (3H, s), 2.25-2.35 (1H, m), 2.41 (1H, dd, $J = 15.5, 3.2$ Hz), 2.49 (1H, dd, $J = 15.5, 9.5$ Hz), 2.54-2.60 (1H, m), 2.66 (1H, d, $J = 6.0$ Hz), 2.65-2.76 (1H, m), 2.96 (1H, dd, $J = 16.0, 4.2$ Hz), 3.03 (1H, dd, $J = 16.1, 6.7$ Hz), 3.11 (1H, quintet, $J = 6.8$ Hz), 3.71-3.76 (1H, m), 4.31 (1H, ddd, $J = 9.2, 5.9, 3.2$ Hz), 4.65 (2H, s), 5.43 (1H, dd, $J = 6.0, 4.3$ Hz), 5.58 (1H, ddd, $J = 15.8, 6.4, 4.6$ Hz), 5.66 (1H, dd, $J = 15.8, 6.1$ Hz), 6.23 (1H, t, $J = 7.3$ Hz), 6.63 (1H, s), 7.18 (1H, s); ^{13}C NMR (100 MHz, CDCl_3) δ 15.4, 15.9, 17.8, 18.6, 22.8, 28.7, 30.9, 39.5, 39.7, 45.1, 51.3, 53.5, 71.5, 75.4, 76.8, 118.2, 119.6, 122.7 [q, $^1J(\text{C},\text{F}) = 273.6$ Hz], 127.9, 130.0 [q, $^3J(\text{C},\text{F}) = 6.1$ Hz], 130.6 [q, $^2J(\text{C},\text{F}) = 27.9$ Hz], 132.3, 137.2, 153.1, 163.9, 170.0, 218.3; LRMS (ESI) calcd for $\text{C}_{27}\text{H}_{35}\text{F}_3\text{N}_4\text{O}_5\text{SNa}$ $[\text{M}+\text{Na}^+]$ 607.2, found 607.2; HRMS calcd for $\text{C}_{27}\text{H}_{36}\text{F}_3\text{N}_4\text{O}_5\text{S}$ $[\text{M}+\text{H}^+]$ 585.2359 found 585.2344.

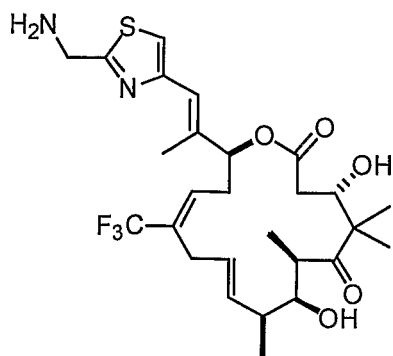
20



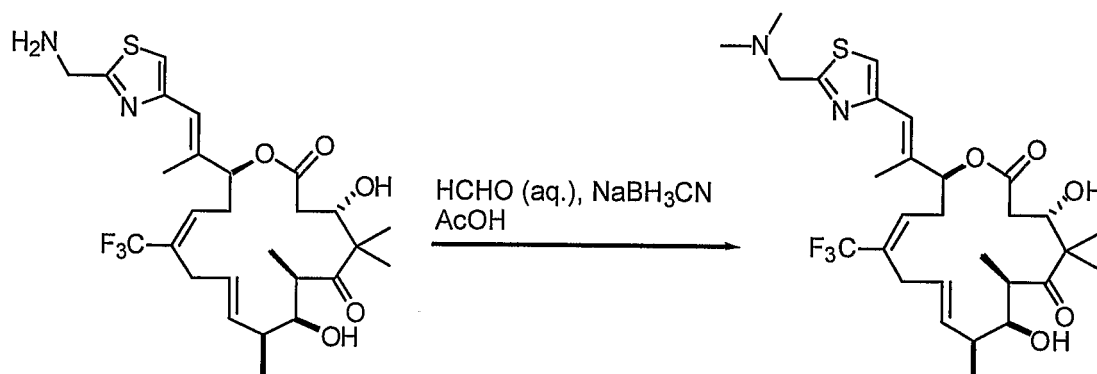
To a solution of tosylate (8.9 mg, 12.5 μ mol) in DMF (0.4 mL) was added NaN₃ (12.2 mg, 0.188 mmol). After stirring at rt for 21 h, the mixture was quenched with sat. NH₄Cl (aq.) and extracted with EtOAc (three times). After concentrating, the residue
 5 was purified by preparative TLC (hexane/EtOAc = 1:1) to give azide (6.9 mg, 11.8 μ mol, 94%) as a colorless powder.



To a solution of azide (21.0 mg, 35.9 μ mol) in THF (0.6 mL) was added PMe₃ (1.0 M in THF, 43.1 μ L, 43.1 μ mol). After 2 min, water (0.1 mL) was added and the mixture
 10 was stirred at rt for 3 h. Further PMe₃ (1.0 M in THF, 7.2 μ L, 7.2 μ mol) was added, and the mixture was stirred at rt for 1.5 h. 28% aqueous NH₄OH (54.5 μ L) was added. After stirring at rt for 1 h, the mixture was directly purified by preparative TLC (CH₂Cl₂/MeOH = 100:7.5) to give amine (15.9 mg, 28.5 μ mol, 79%) as a colorless
 15 powder.

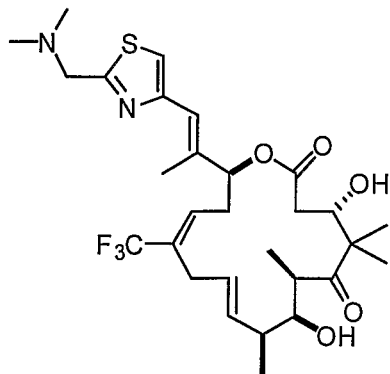


$[\alpha]_D^{26} -64.2$ (c 0.815, CHCl_3); IR (film) ν 3504, 3363, 2975, 2931, 1733, 1688, 1450, 1383, 1318, 1248, 1169, 1113, 1054, 984, 736 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 1.05 (3H, s), 1.12 (3H, d, $J = 7.0$ Hz), 1.23 (3H, d, $J = 6.8$ Hz), 1.34 (3H, s), 2.12 (3H, d, $J = 0.7$ Hz), 2.24-2.35 (1H, m), 2.39 (1H, dd, $J = 15.4, 3.0$ Hz), 2.49 (1H, dd, $J = 15.4, 9.8$ Hz), 2.54-2.63 (1H, m), 2.66-2.76 (1H, m), 2.97 (1H, dd, $J = 16.2, 4.2$ Hz), 3.03 (1H, dd, $J = 16.3, 6.5$ Hz), 3.10 (1H, quintet, $J = 6.8$ Hz), 3.74 (1H, dd, $J = 6.7, 3.5$ Hz), 4.18 (2H, s), 4.34 (1H, dd, $J = 9.8, 2.9$ Hz), 5.43 (1H, dd, $J = 6.0, 4.3$ Hz), 5.55-5.64 (1H, m), 5.67 (1H, dd, $J = 15.9, 5.8$ Hz), 6.24 (1H, brt, $J = 7.3$ Hz), 6.66 (1H, s), 7.10 (1H, s); ^{13}C NMR (100 MHz, CDCl_3) δ 15.3, 16.1, 17.7, 18.2, 22.6, 28.7, 30.9, 39.4, 39.7, 43.9, 45.1, 53.8, 71.2, 75.3, 76.6, 116.8, 120.1, 124.2 [$^1J(\text{C},\text{F}) = 273.5$ Hz], 127.8, 130.2 [$^3J(\text{C},\text{F}) = 6.1$ Hz], 130.4 [$^2J(\text{C},\text{F}) = 28.6$ Hz], 132.2, 136.6, 152.3, 170.1, 172.7, 218.4; LRMS (ESI) calcd for $\text{C}_{27}\text{H}_{38}\text{F}_3\text{N}_2\text{O}_5\text{S}$ [$\text{M}+\text{H}^+$] 559.2, found 559.2; HRMS calcd for $\text{C}_{27}\text{H}_{38}\text{F}_3\text{N}_2\text{O}_5\text{S}$ [$\text{M}+\text{H}^+$] 559.2454 found 559.2440.



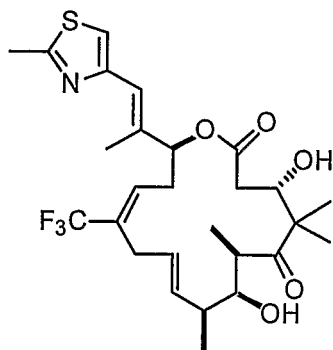
To a stirred solution of amine (15.9 mg, 28.5 μmol) in CH_3CN (0.78 mL) was added HCHO (37% aqueous solution, 31.4 μL , 0.143 mmol) followed by NaBH_3CN (1.0 M in THF, 85.5 μL , 85.5 μmol). The mixture was stirred at rt for 20 min. AcOH (1 drop) was added, and the mixture was stirred at rt for 40 min. The mixture was directly

purified by preparative TLC ($\text{CH}_2\text{Cl}_2/\text{MeOH} = 100:8$) to give dimethylamine (15.6 mg, 26.6 μmol , 93%) as a colorless powder.

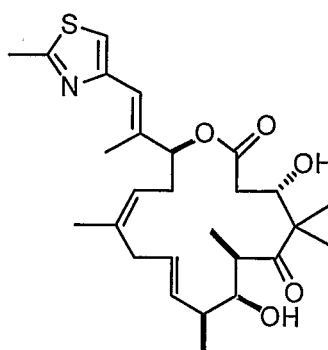


- 5 $[\alpha]_D^{24} -49.9$ (c 0.74, CHCl_3); IR (film) ν 3424, 2974, 1729, 1689, 1468, 1318, 1247, 1169, 1112, 754 cm^{-1} ; ^1H NMR (400 MHz, CDCl_3) δ 1.05 (3H, s), 1.12 (3H, d, $J = 6.9$ Hz), 1.23 (3H, d, $J = 6.8$ Hz), 1.33 (3H, s), 2.17 (3H, s), 2.24-2.35 (1H, m), 2.43 (1H, dd, $J = 15.7, 3.6$ Hz), 2.49 (1H, dd, $J = 15.6, 9.1$ Hz), 2.55-2.64 (2H, m, including OH), 2.68-2.77 (1H, m), 2.80 (3H, s), 2.81 (3H, s), 2.92-3.06 (2H, m), 3.10 (1H, quintet, $J =$
- 10 6.8 Hz), 3.69-3.76 (1H, m), 4.25-4.34 (1H, m), 4.33 (2H, s), 5.42 (1H, t, $J = 5.5$ Hz), 5.57 (1H, dt, $J = 15.8, 6.3$ Hz), 5.66 (1H, dd, $J = 15.7, 6.4$ Hz), 6.22 (1H, brt, $J = 7.2$ Hz), 6.64 (1H, s), 7.30 (1H, s); ^{13}C NMR (100 MHz, CDCl_3) δ 15.3, 15.8, 17.8, 18.8, 22.3, 28.8, 30.9, 39.6, 39.6, 45.2, 49.7, 49.7, 53.4, 61.5, 71.7, 75.4, 77.4, 119.2, 120.2, 124.2 [q, $^1J(\text{C},\text{F}) = 273.5$ Hz], 127.8, 129.9 [q, $^3J(\text{C},\text{F}) = 6.2$ Hz], 130.7 [q, $^2J(\text{C},\text{F}) =$
- 15 28.4 Hz], 132.4, 137.6, 154.2, 157.2, 170.0, 218.3; LRMS (ESI) calcd for $\text{C}_{29}\text{H}_{42}\text{F}_3\text{N}_2\text{O}_5\text{S}$ $[\text{M}+\text{H}^+]$ 580.2, found 580.2.

Melting points; Both samples were not recrystallized, but purified by SiO_2 .



mp 90-94 $^\circ\text{C}$

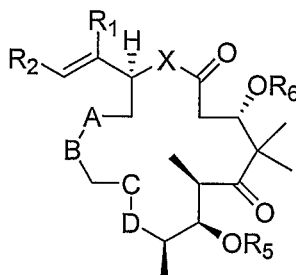


mp 78-81 $^\circ\text{C}$

Claims

What is claimed is:

1. A compound of the formula:



wherein R_1 is hydrogen or lower alkyl;

- R_2 is a substituted or unsubstituted aryl, heteroaryl, arylalkyl, or heteroarylalkyl moiety;

R_5 and R_6 are each independently hydrogen or a protecting group;

X is O, S, $C(R_7)_2$, or NR_7 , wherein each occurrence of R_7 is independently hydrogen or lower alkyl;

- A-B represents $CR_A=CR_B-$, $C(R_A)_2-C(R_B)_2-$, or $-C\equiv C-$;

C-D represents $-CR_C=CR_D-$, $-C(R_C)_2-C(R_D)_2-$, or $-C\equiv C-$;

wherein each occurrence of R_A is independently hydrogen; halogen; -

$OR_{A'}$; $-SR_{A'}$; $-N(R_{A'})_2$; $-C(O)OR_{A'}$; $-C(O)R_{A'}$; $-CONHR_{A'}$; $-O(C=O)R_{A'}$; $-O(C=O)OR_{A'}$; $-NR_{A'}(C=O)R_{A'}$; N_3 ; $N_2R_{A'}$; cyclic acetal; or cyclic or acyclic, linear or branched aliphatic, heteroaliphatic, aryl, or heteroaryl, optionally substituted with one or more of hydrogen; halogen; $-OR_{A'}$; $-SR_{A'}$; $-N(R_{A'})_2$; $-C(O)OR_{A'}$; $-C(O)R_{A'}$; $-CONHR_{A'}$; $-O(C=O)R_{A'}$; $-O(C=O)OR_{A'}$; $-NR_{A'}(C=O)R_{A'}$; N_3 ; $N_2R_{A'}$; cyclic acetal; or cyclic or acyclic, linear or branched substituted or unsubstituted aliphatic, heteroaliphatic, aryl, or heteroaryl moiety;

- R_B is, independently for each occurrence, hydrogen; halogen; $-OR_{B'}$; $-SR_{B'}$; $-N(R_{B'})_2$; $-C(O)OR_{B'}$; $-C(O)R_{B'}$; $-CONHR_{B'}$; $-O(C=O)R_{B'}$; $-O(C=O)OR_{B'}$; $-NR_{B'}(C=O)R_{B'}$; N_3 ; $N_2R_{B'}$; cyclic acetal; or cyclic or acyclic, linear or branched aliphatic, heteroaliphatic, aryl, or heteroaryl, optionally substituted with one or more of hydrogen; halogen; $-OR_{B'}$; $-SR_{B'}$; $-N(R_{B'})_2$; $-C(O)OR_{B'}$; $-C(O)R_{B'}$; $-CONHR_{B'}$; $-O(C=O)R_{B'}$; $-O(C=O)OR_{B'}$; $-NR_{B'}(C=O)R_{B'}$; N_3 ; $N_2R_{B'}$; cyclic acetal; or cyclic or acyclic, linear or branched substituted or unsubstituted aliphatic, heteroaliphatic, aryl, or heteroaryl moiety;

$\text{NR}_B(\text{C}=\text{O})\text{R}_B$; N_3 ; N_2R_B ; cyclic acetal; or cyclic or acyclic, linear or branched substituted or unsubstituted aliphatic, heteroaliphatic, aryl, or heteroaryl moiety;

R_C is, independently for each occurrence, hydrogen; halogen; $-\text{OR}_C$; $-\text{SR}_C$;

5 $-\text{N}(\text{R}_C)_2$; $-\text{C}(\text{O})\text{OR}_C$; $-\text{C}(\text{O})\text{R}_C$; $-\text{CONHR}_C$; $-\text{O}(\text{C}=\text{O})\text{R}_C$; $-\text{O}(\text{C}=\text{O})\text{OR}_C$;
 $-\text{NR}_C(\text{C}=\text{O})\text{R}_C$; N_3 ; N_2R_C ; cyclic acetal; or cyclic or acyclic, linear or branched aliphatic, heteroaliphatic, aryl, or heteroaryl, optionally substituted with one or more of hydrogen; halogen; $-\text{OR}_C$; $-\text{SR}_C$; $-\text{N}(\text{R}_C)_2$; $-\text{C}(\text{O})\text{OR}_C$; $-\text{C}(\text{O})\text{R}_C$; $-\text{CONHR}_C$; $-\text{O}(\text{C}=\text{O})\text{R}_C$; $-\text{O}(\text{C}=\text{O})\text{OR}_C$; $-\text{NR}_C(\text{C}=\text{O})\text{R}_C$; N_3 ; N_2R_C ;
 10 cyclic acetal; or cyclic or acyclic, linear or branched substituted or unsubstituted aliphatic, heteroaliphatic, aryl, or heteroaryl moiety;

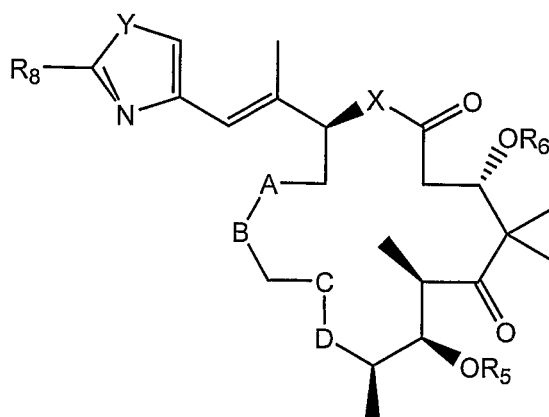
R_D is, independently for each occurrence, hydrogen; halogen; $-\text{OR}_D$; $-\text{SR}_D$;

15 $-\text{N}(\text{R}_D)_2$; $-\text{C}(\text{O})\text{OR}_D$; $-\text{C}(\text{O})\text{R}_D$; $-\text{CONHR}_D$; $-\text{O}(\text{C}=\text{O})\text{R}_D$; $-\text{O}(\text{C}=\text{O})\text{OR}_D$; $-\text{NR}_D(\text{C}=\text{O})\text{R}_D$; N_3 ; N_2R_D ; cyclic acetal; or cyclic or acyclic, linear or branched aliphatic, heteroaliphatic, aryl, or heteroaryl, optionally substituted with one or more of hydrogen; halogen; $-\text{OR}_D$; $-\text{SR}_D$; $-\text{N}(\text{R}_D)_2$; $-\text{C}(\text{O})\text{OR}_D$; $-\text{C}(\text{O})\text{R}_D$; $-\text{CONHR}_D$; $-\text{O}(\text{C}=\text{O})\text{R}_D$; $-\text{O}(\text{C}=\text{O})\text{OR}_D$; $-\text{NR}_D(\text{C}=\text{O})\text{R}_D$; N_3 ; N_2R_D ; cyclic acetal; or cyclic or acyclic, linear or branched substituted or unsubstituted
 20 aliphatic, heteroaliphatic, aryl, or heteroaryl moiety; or

wherein any two of R_A , R_B , R_C or R_D taken together may form a cyclic moiety and may be linked through an oxygen, sulfur, carbon or nitrogen atom, or any two adjacent groups R_A , R_B , R_C , or R_D , taken together, may form a 3-6-membered substituted or unsubstituted aliphatic, heteroaliphatic, aryl or
 25 heteroaryl ring;

wherein each occurrence of R_A , R_B , R_C and R_D is independently hydrogen; a protecting group; a linear or branched, substituted or unsubstituted, cyclic or acyclic, aliphatic, heteroaliphatic, aryl, heteroaryl, arylalkyl, arylalkenyl, arylalkynyl, arylalkenyl, arylalkynyl, or heteroarylalkyl, heteroarylalkenyl, or heteroarylalkynyl moiety; and pharmaceutically
 30 acceptable derivatives thereof.

2. A compound of the formula:



wherein

- 5 X is O, S, C(R₇)₂, or NR₇, wherein each occurrence of R₇ is independently hydrogen or lower alkyl;
 Y is O or S;
 R₅ and R₆ are each independently hydrogen or a protecting group;
 R₈ is independently hydrogen, halogen, -OR₉, -SR₉, -N(R₉)₂, -CZ₃, -CHZ₂, -
 10 CH₂Z, where Z is F, Br, Cl, I, OR_{B'}, NHR_{B'}, N(R_{B'})₂, or SR_{B'}; -(CV₂)_nOR₉, -
 (CV₂)_nN(R₉)₂, -(CV₂)_nSR₉, -(C=O)R₉, -O(C=O)R₉, -(C=O)OR₉, -O(C=O)OR₉; -
 NH(C=O)R₉, -NH(C=O)OR₉, -(C=O)NHR₉, or a cyclic or acyclic, linear or branched
 aliphatic, heteroaliphatic, aryl, heteroaryl, arylalkyl, or heteroarylalkyl moiety
 optionally substituted with one or more occurrences of halogen, -OR₉, -SR₉, -N(R₉)₂, -
 15 (CV₂)_nOR₉, -(CV₂)_nN(R₉)₂, -(CV₂)_nSR₉, -(C=O)R₉, -O(C=O)R₉, -(C=O)OR₉, -
 O(C=O)OR₉; -NH(C=O)R₉, -NH(C=O)OR₉, -(C=O)NHR₉, or a cyclic or acyclic, linear
 or branched, substituted or unsubstituted aliphatic, heteroaliphatic, aryl, heteroaryl,
 arylalkyl, or heteroarylalkyl moiety,
 wherein each occurrence of R₉ is independently hydrogen; a protecting group; a
 20 cyclic or acyclic, linear or branched, substituted or unsubstituted aliphatic,
 heteroaliphatic, aryl, or heteroaryl moiety; or is an epothilone, desoxyepothilone or
 analogues thereof; a polymer; carbohydrate; photoaffinity label; or radiolabel;
 wherein each occurrence of V is independently hydrogen, halogen, hydroxyl,
 thio, amino, alkylamino, or protected hydroxyl, thio or amino; each occurrence of t is
 25 independently 0, 1 or 2; and each occurrence of n is independently 0-10;

A-B represents $\text{CR}_A=\text{CR}_B$ -, $\text{C}(\text{R}_A)_2\text{-C}(\text{R}_B)_2$ -, or $\text{-C}\equiv\text{C-}$;

C-D represents $\text{-CR}_C=\text{CR}_D$ -, $\text{-C}(\text{R}_C)_2\text{-C}(\text{R}_D)_2$ -, or $\text{-C}\equiv\text{C-}$;

wherein each occurrence of R_A is independently hydrogen; halogen; -
 OR_A ;; - SR_A ;; - $\text{N}(\text{R}_A)_2$;; - $\text{C}(\text{O})\text{OR}_A$;; - $\text{C}(\text{O})\text{R}_A$;; - CONHR_A ;; - $\text{O}(\text{C}=\text{O})\text{R}_A$;; -
 5 $\text{O}(\text{C}=\text{O})\text{OR}_A$;; - $\text{NR}_A(\text{C}=\text{O})\text{R}_A$;; N_3 ; N_2R_A ;; cyclic acetal; or cyclic or acyclic,
 linear or branched aliphatic, heteroaliphatic, aryl, or heteroaryl, optionally
 substituted with one or more of hydrogen; halogen; - OR_A ;; - SR_A ;; - $\text{N}(\text{R}_A)_2$;; -
 $\text{C}(\text{O})\text{OR}_A$;; - $\text{C}(\text{O})\text{R}_A$;; - CONHR_A ;; - $\text{O}(\text{C}=\text{O})\text{R}_A$;; - $\text{O}(\text{C}=\text{O})\text{OR}_A$;; -
 $\text{NR}_A(\text{C}=\text{O})\text{R}_A$;; N_3 ; N_2R_A ;; cyclic acetal; or cyclic or acyclic, linear or branched
 10 substituted or unsubstituted aliphatic, heteroaliphatic, aryl, or heteroaryl moiety;

R_B is, independently for each occurrence, hydrogen; halogen; - OR_B ;; -
 SR_B ;; - $\text{N}(\text{R}_B)_2$;; - $\text{C}(\text{O})\text{OR}_B$;; - $\text{C}(\text{O})\text{R}_B$;; - CONHR_B ;; - $\text{O}(\text{C}=\text{O})\text{R}_B$;; -
 $\text{O}(\text{C}=\text{O})\text{OR}_B$;; - $\text{NR}_B(\text{C}=\text{O})\text{R}_B$;; N_3 ; N_2R_B ;; cyclic acetal; or cyclic or acyclic,
 linear or branched aliphatic, heteroaliphatic, aryl, or heteroaryl, optionally
 15 substituted with one or more of hydrogen; halogen; - OR_B ;; - SR_B ;; - $\text{N}(\text{R}_B)_2$;; -
 $\text{C}(\text{O})\text{OR}_B$;; - $\text{C}(\text{O})\text{R}_B$;; - CONHR_B ;; - $\text{O}(\text{C}=\text{O})\text{R}_B$;; - $\text{O}(\text{C}=\text{O})\text{OR}_B$;; -
 $\text{NR}_B(\text{C}=\text{O})\text{R}_B$;; N_3 ; N_2R_B ;; cyclic acetal; or cyclic or acyclic, linear or branched
 substituted or unsubstituted aliphatic, heteroaliphatic, aryl, or heteroaryl moiety;

R_C is, independently for each occurrence, hydrogen; halogen; - OR_C ;; -
 20 SR_C ;;
 $\text{-N}(\text{R}_C)_2$;; - $\text{C}(\text{O})\text{OR}_C$;; - $\text{C}(\text{O})\text{R}_C$;; - CONHR_C ;; - $\text{O}(\text{C}=\text{O})\text{R}_C$;; - $\text{O}(\text{C}=\text{O})\text{OR}_C$;;
 $\text{-NR}_C(\text{C}=\text{O})\text{R}_C$;; N_3 ; N_2R_C ;; cyclic acetal; or cyclic or acyclic, linear or
 branched aliphatic, heteroaliphatic, aryl, or heteroaryl, optionally substituted
 with one or more of hydrogen; halogen; - OR_C ;; - SR_C ;; - $\text{N}(\text{R}_C)_2$;; - $\text{C}(\text{O})\text{OR}_C$;; -
 25 $\text{C}(\text{O})\text{R}_C$;; - CONHR_C ;; - $\text{O}(\text{C}=\text{O})\text{R}_C$;; - $\text{O}(\text{C}=\text{O})\text{OR}_C$;; - $\text{NR}_C(\text{C}=\text{O})\text{R}_C$;; N_3 ; N_2R_C ;;
 cyclic acetal; or cyclic or acyclic, linear or branched substituted or unsubstituted
 aliphatic, heteroaliphatic, aryl, or heteroaryl moiety;

R_D is, independently for each occurrence, hydrogen; halogen; - OR_D ;; -
 SR_D ;;
 30 $\text{-N}(\text{R}_D)_2$;; - $\text{C}(\text{O})\text{OR}_D$;; - $\text{C}(\text{O})\text{R}_D$;; - CONHR_D ;; - $\text{O}(\text{C}=\text{O})\text{R}_D$;; - $\text{O}(\text{C}=\text{O})\text{OR}_D$;; -
 $\text{NR}_D(\text{C}=\text{O})\text{R}_D$;; N_3 ; N_2R_D ;; cyclic acetal; or cyclic or acyclic, linear or branched
 aliphatic, heteroaliphatic, aryl, or heteroaryl, optionally substituted with one or

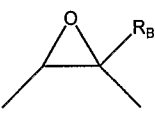
more of hydrogen; halogen; $-OR_D$; $-SR_D$; $-N(R_D)_2$; $-C(O)OR_D$; $-C(O)R_D$; $-CONHR_D$; $-O(C=O)R_D$; $-O(C=O)OR_D$; $-NR_D(C=O)R_D$; N_3 ; N_2R_D ; cyclic acetal; or cyclic or acyclic, linear or branched substituted or unsubstituted aliphatic, heteroaliphatic, aryl, or heteroaryl moiety; or

5 wherein any two of R_A , R_B , R_C or R_D taken together may form a cyclic moiety and may be linked through an oxygen, sulfur, carbon or nitrogen atom, or any two adjacent groups R_A , R_B , R_C , or R_D , taken together, may form a 3-6-membered substituted or unsubstituted aliphatic, heteroaliphatic, aryl or heteroaryl ring;

10 wherein each occurrence of R_A , R_B , R_C and R_D is independently hydrogen; a protecting group; a linear or branched, substituted or unsubstituted, cyclic or acyclic, aliphatic, heteroaliphatic, aryl, heteroaryl, arylalkyl, arylalkenyl, arylalkynyl, arylalkenyl, arylalkynyl, or heteroarylalkyl, heteroarylalkenyl, or heteroarylalkynyl moiety; and

15 pharmaceutically acceptable derivatives thereof.

3. The compound of claim 2, wherein A-B is $-\text{CH}=\text{C}(\text{R}_B)-$.

4. The compound of claim 2, wherein A-B is .

20

5. The compound of claim 2 wherein R_B is methyl.

6. The compound of claim 2 wherein R_B is $-\text{CF}_3$.

25 7. The compound of claim 2, 3, or 4, wherein R_8 is methyl.

8. The compound of claim 2, 3, or 4, wherein R_8 is $-\text{CH}_2\text{OH}$.

9. The compound of claim 2, 3, or 4, wherein R_8 is $-\text{CH}_2\text{NH}_2$.

30

10. The compound of claim 2, 3, or 4, wherein C-D is , wherein Z is O, S, NR_Z, or C(R_Z)₂, wherein R_Z is hydrogen, halogen, lower acyl, or lower alkyl.

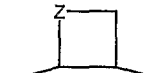
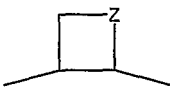
11. The compound of claim 10, wherein Z is NH.

5

12. The compound of claim 10, wherein Z is CH₂.

13. The compound of claim 10, wherein Z is O.

10 14. The compound of claim 10, wherein Z is S.

15. The compound of claim 2, 3, or 4, wherein C-D is  or , wherein Z is O, S, NR_Z, or C(R_Z)₂, wherein R_Z is hydrogen, halogen, lower acyl, or lower alkyl.

15

16. The compound of claim 15, wherein Z is NH.

17. The compound of claim 15, wherein Z is CH₂.

20 18. The compound of claim 2, wherein Y is S.

19. The compound of claim 2, wherein Y is O.

20. The compound of claim 2, 3, or 4, wherein C-D is -C(R_C)₂-C(R_D)₂-.

25

21. The compound of claim 20, wherein each occurrence of R_C and R_D is selected from the group consisting of hydrogen, lower alkyl, hydroxyl, halogen, or lower alkoxy.

22. The compound of claim 20, wherein each occurrence of R_C and R_D is selected from the group consisting of hydrogen and methyl.

30

23. The compound of claim 20, wherein each R_C is methyl and each R_D is hydrogen.

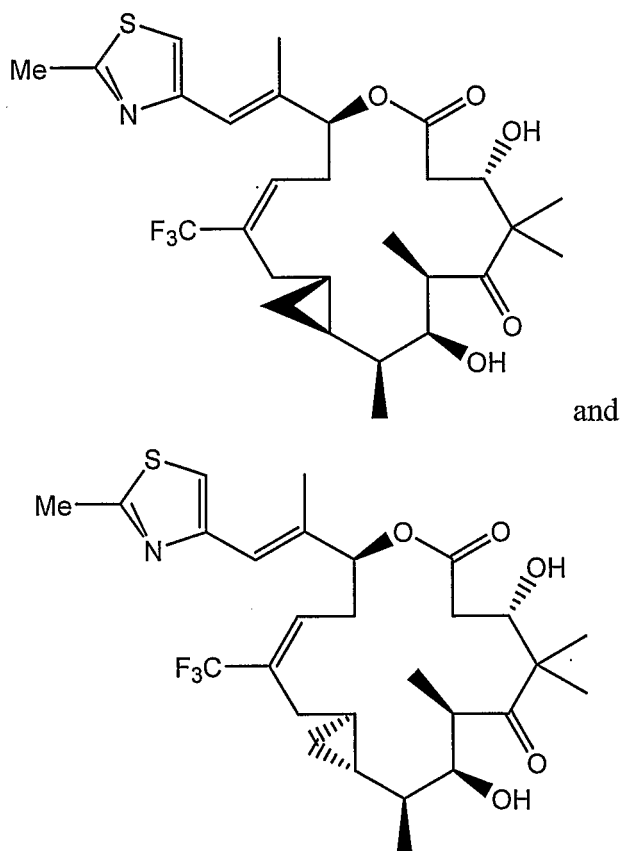
5 24. The compound of claim 20, wherein each R_C is hydrogen and each R_D is methyl.

25. The compound of claim 20, wherein one R_C is hydrogen and the other R_C is methyl.

10

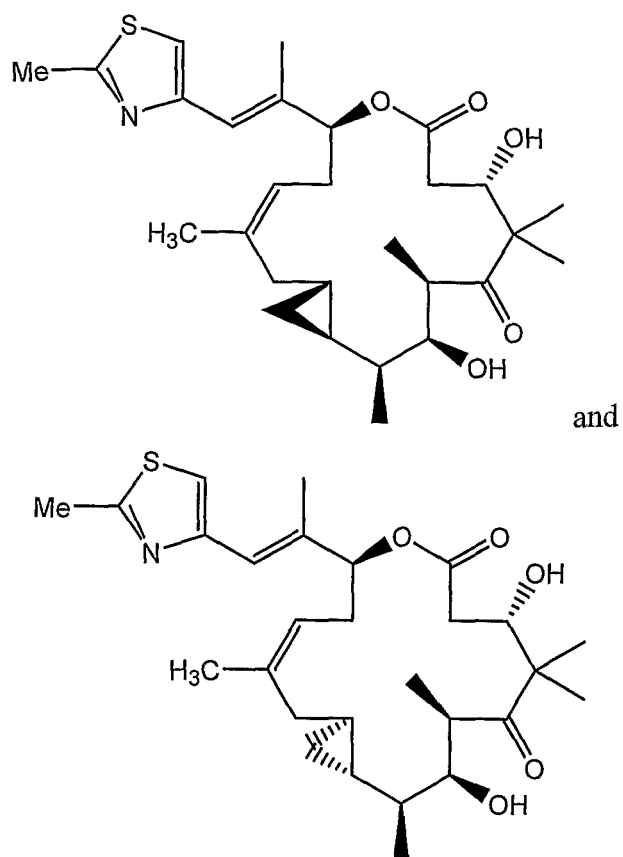
26. The compound of claim 20, wherein one R_D is hydrogen and the other R_D is methyl.

27. The compound of claim 1 selected from the group consisting of formulae:

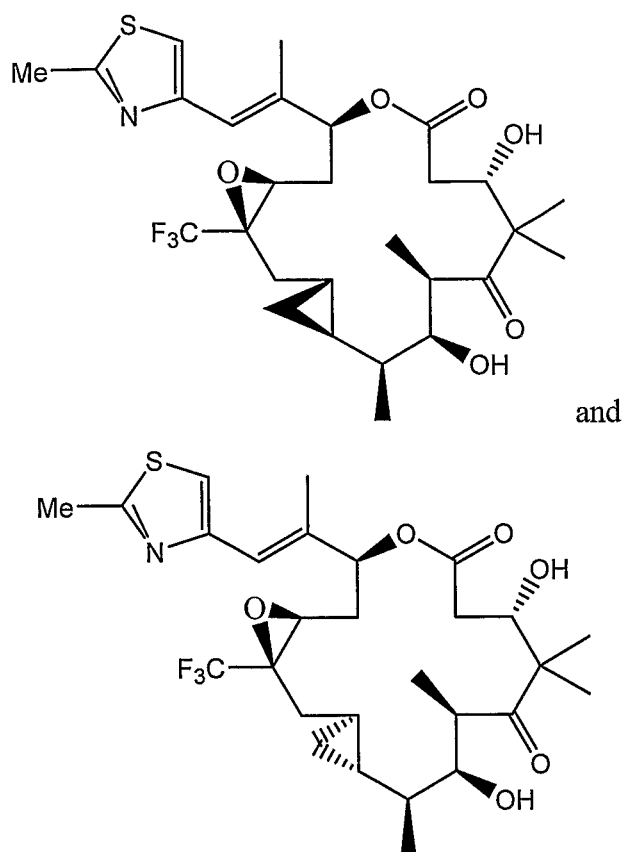


15

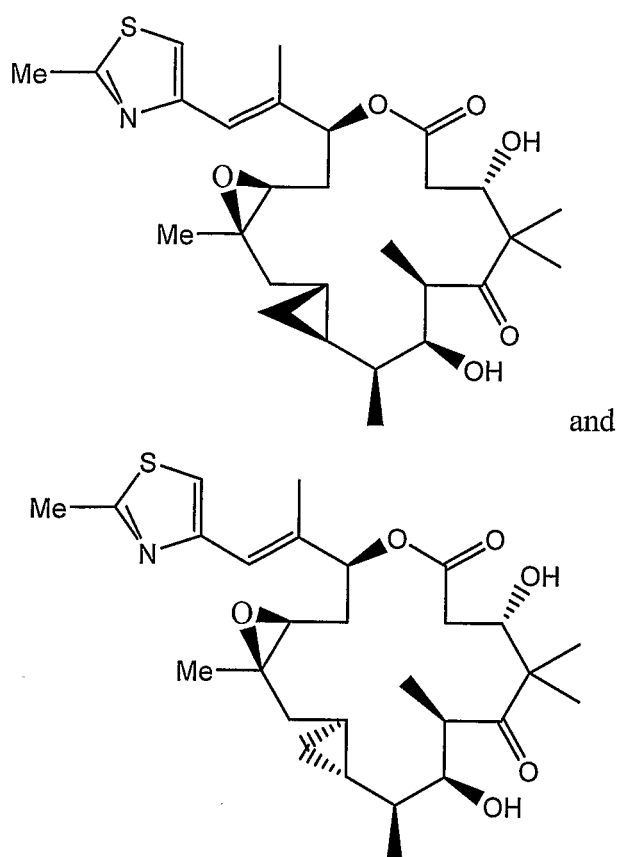
28. The compound of claim 1 selected from the group consisting of formulae:



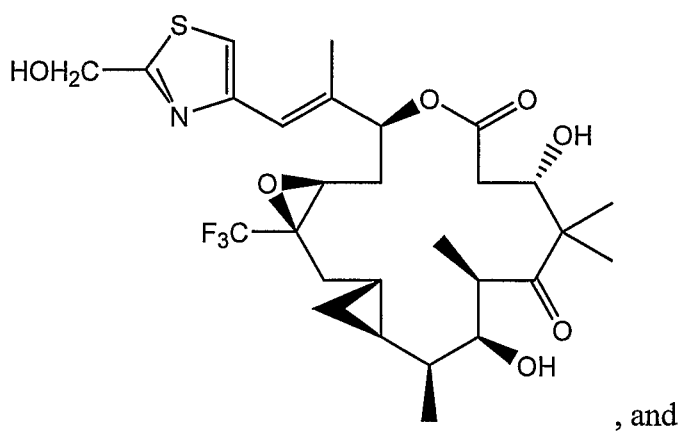
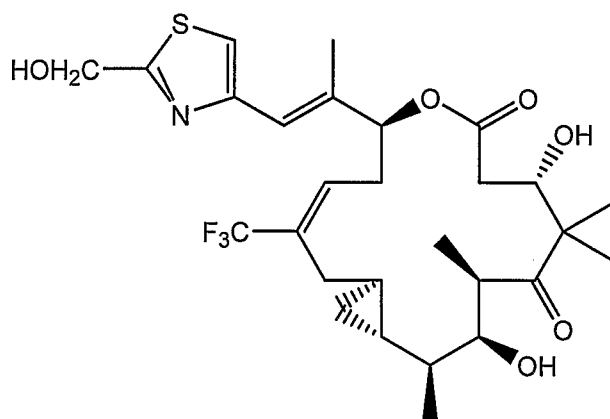
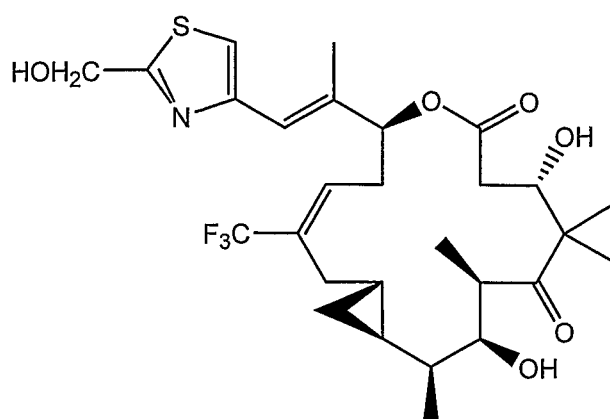
29. The compound of claim 1 selected from the group consisting of formulae:



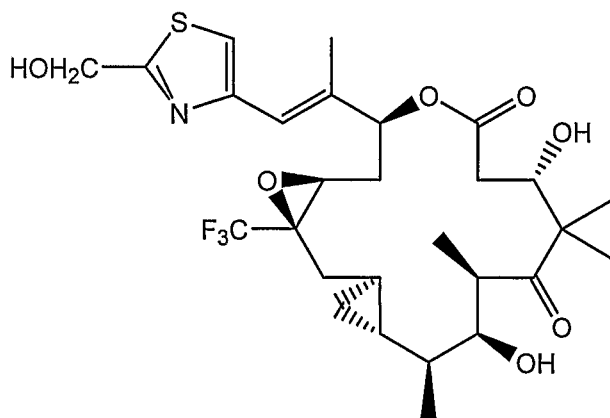
30. The compound of claim 1 selected from the group consisting of formulae:



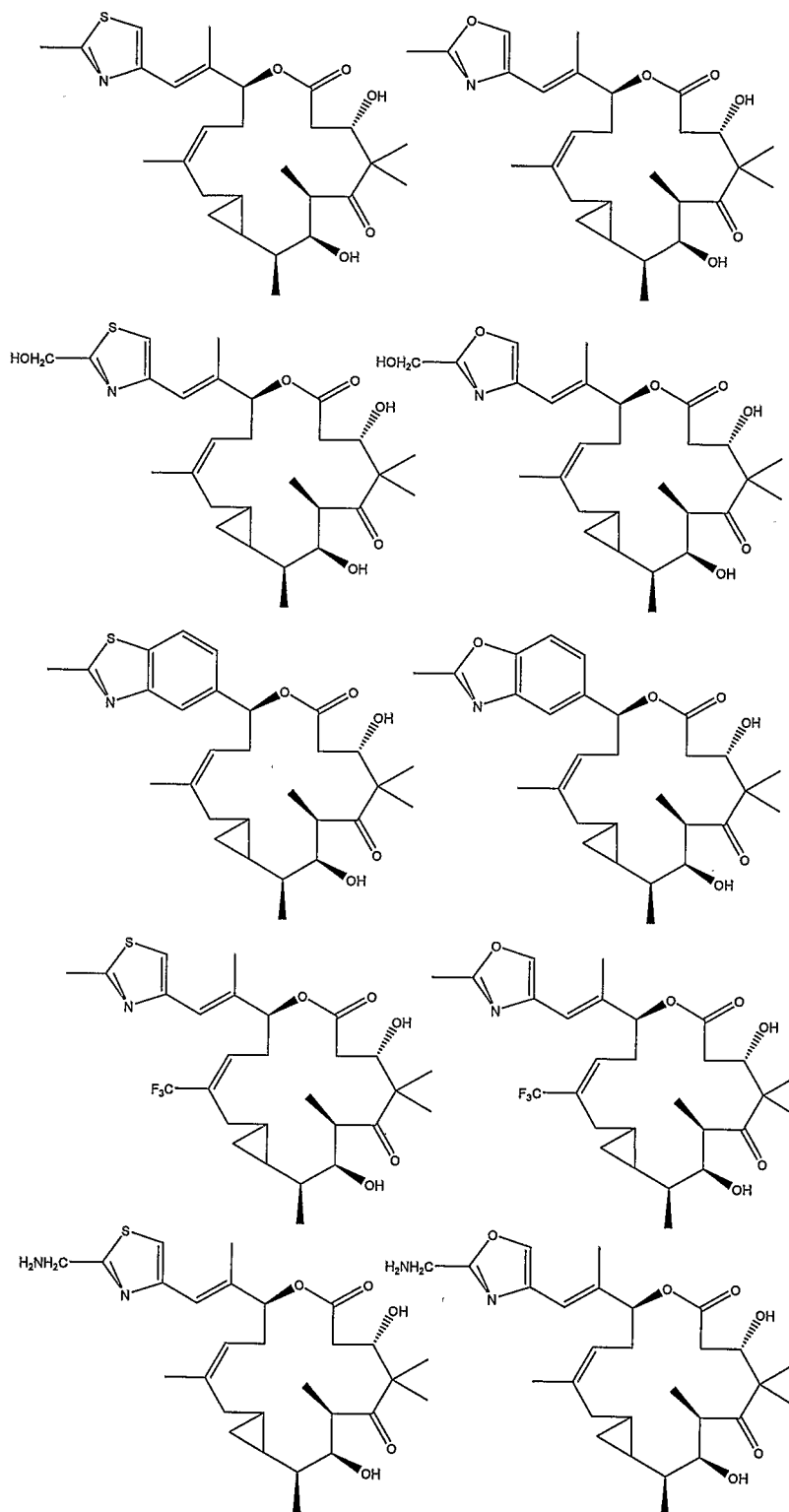
31. The compound of claim 1 selected from the group consisting of formulae:

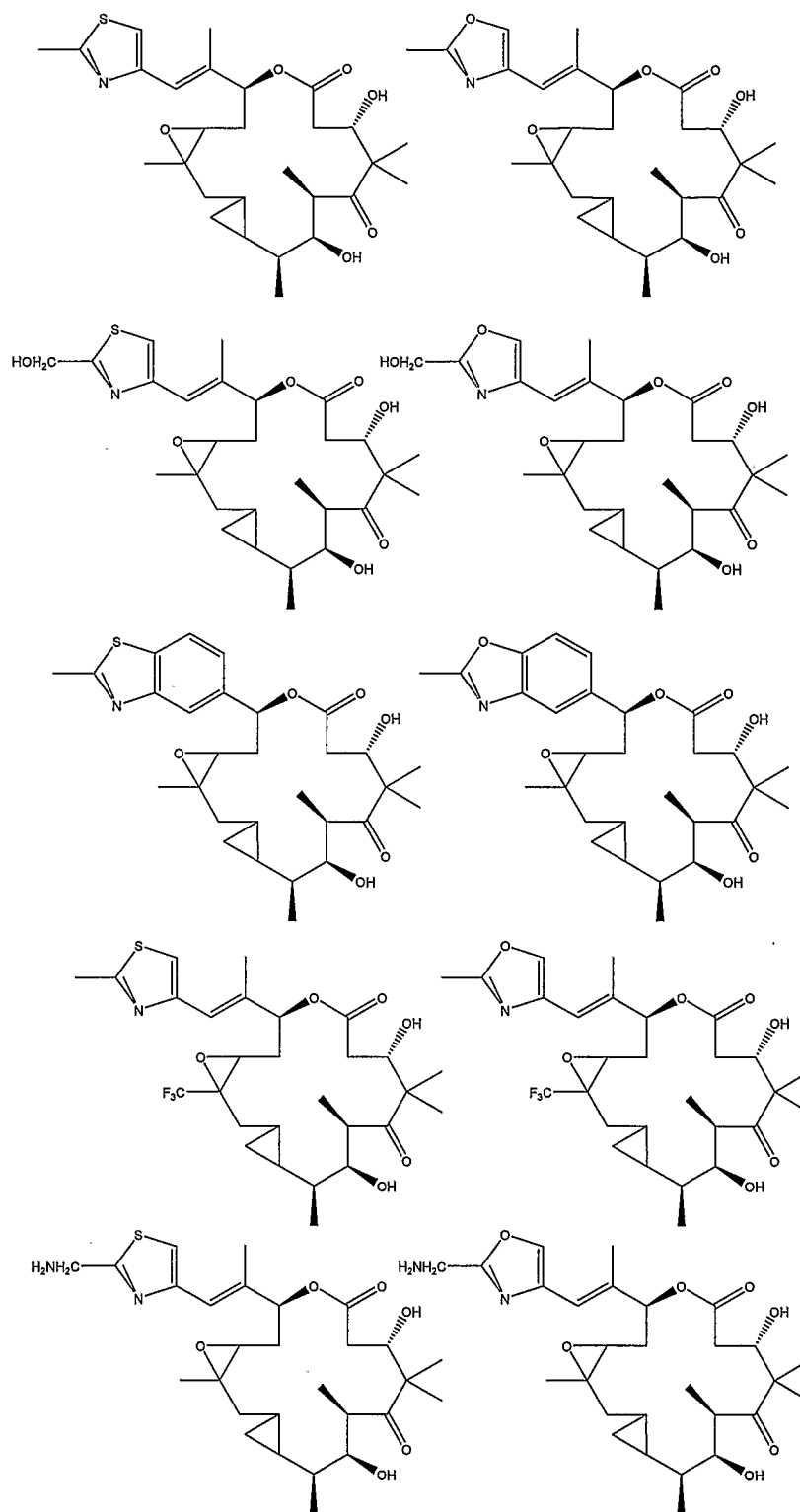


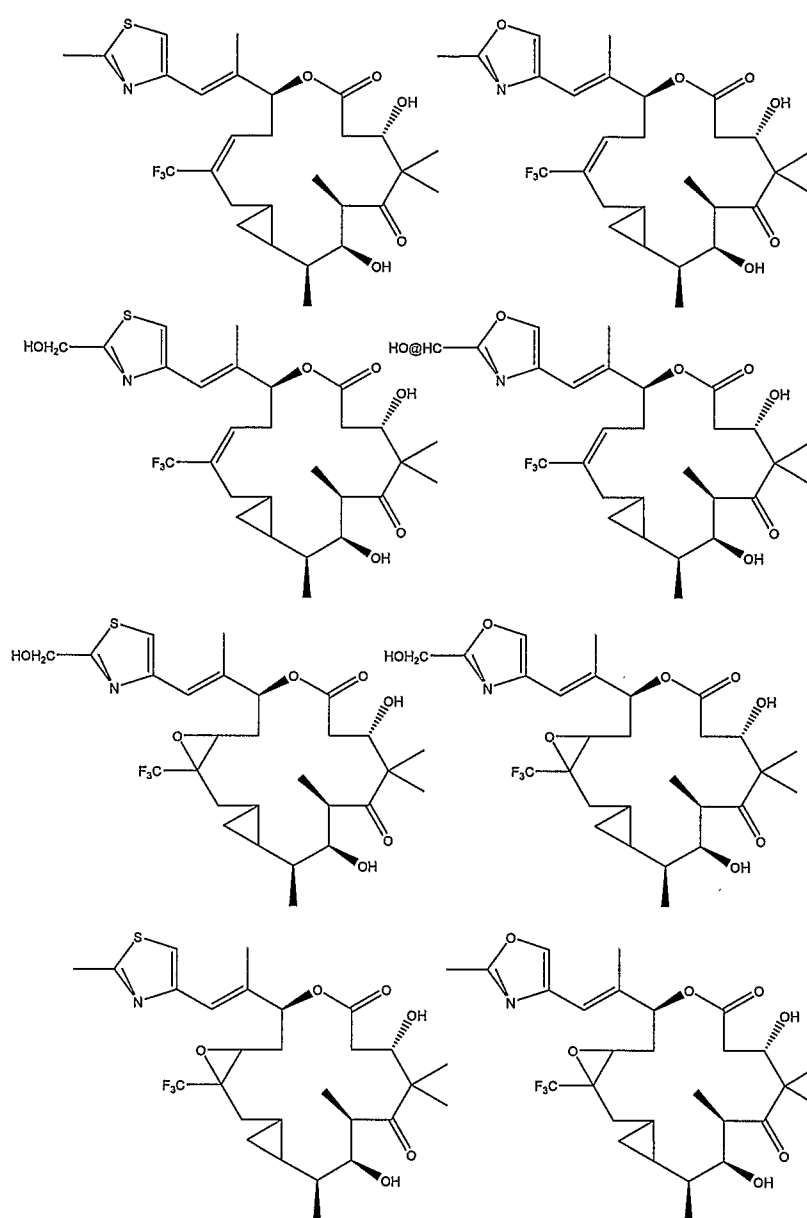
, and

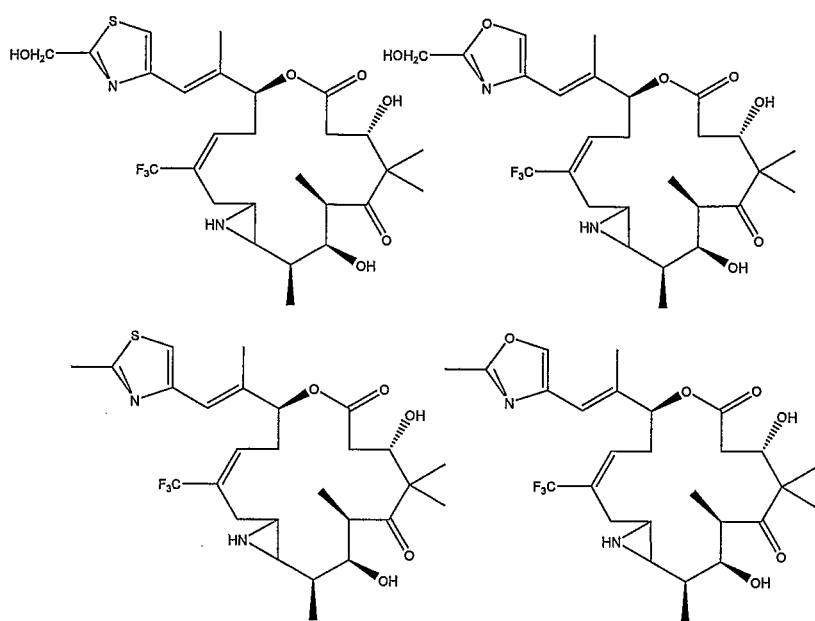


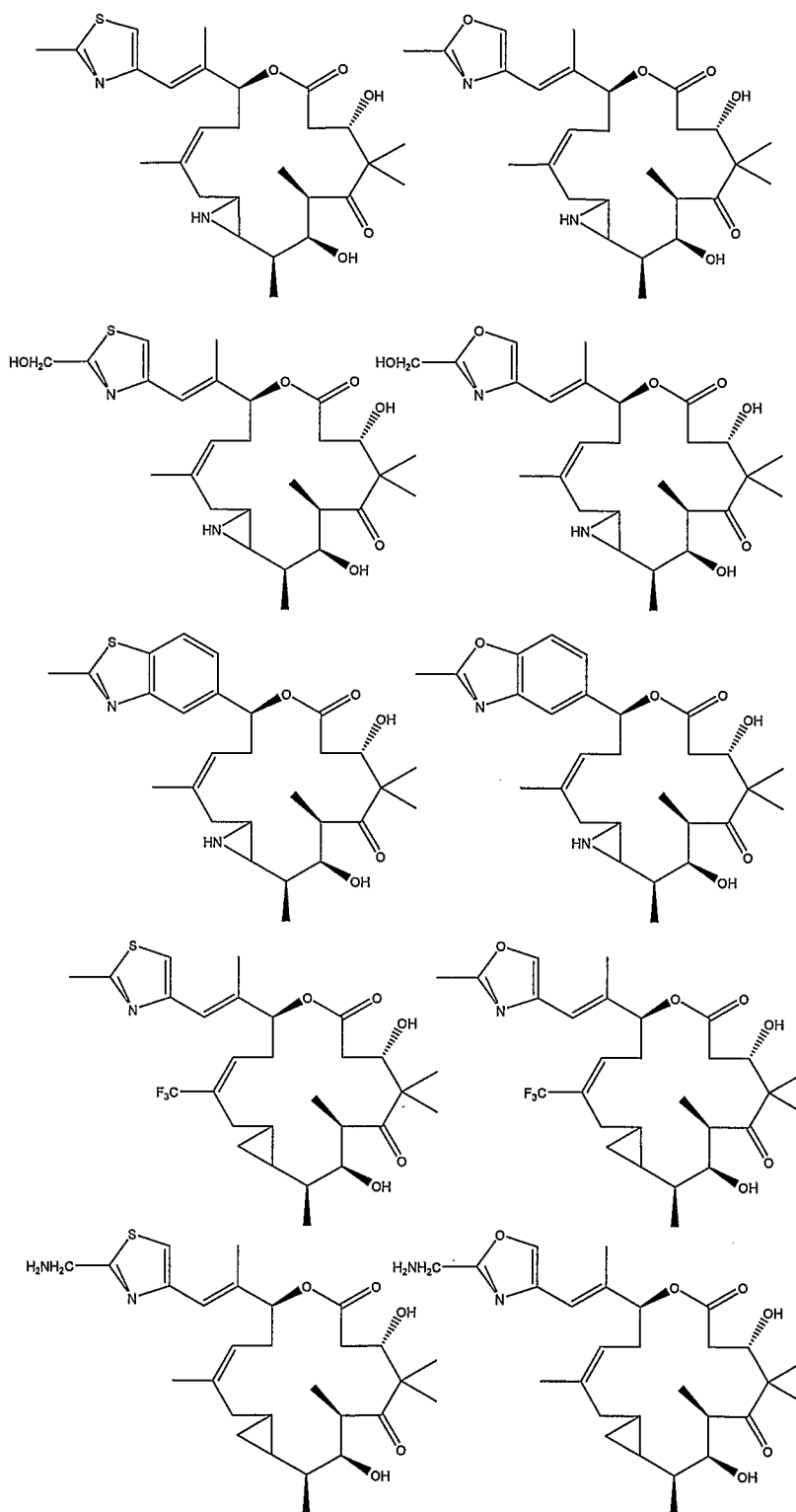
32. The compound of claim 1 selected from the group consisting of formulae:











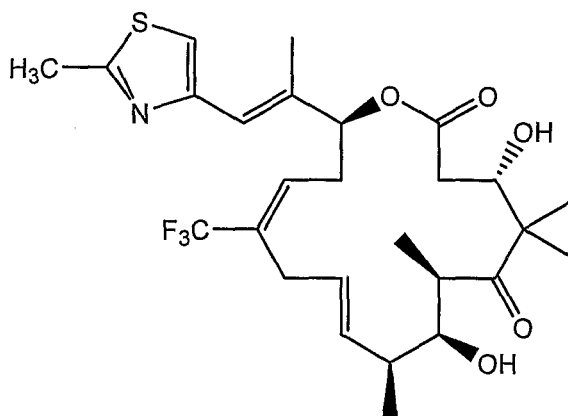
33. A pharmaceutical composition for the treatment of cancer comprising a compound of claim 1 and a pharmaceutically acceptable excipient.

34. The pharmaceutical composition of claim 33 further comprising Cremophor.
35. The pharmaceutical composition of claim 33 further comprising Cremophor and ethanol.
- 5 36. The pharmaceutical composition of claim 33, wherein the compound is suspended in 1:1 Cremophor/EtOH.
- 10 37. The pharmaceutical composition of claim 33 further comprising an additional cytotoxic agent.
38. A pharmaceutical composition for the treatment of cancer comprising:
a therapeutically effective amount of a compound of claim 1, or
pharmaceutically acceptable salts thereof; and
15 a pharmaceutically acceptable carrier or diluent,
wherein the therapeutically effective amount of the compound is an amount sufficient to deliver about 0.001 to about 40 mg compound per kg body weight of a subject.
- 20 39. A pharmaceutical composition for the treatment of cancer comprising a compound of claim 1; and
a pharmaceutically acceptable excipient; wherein the pharmaceutical composition is suitable for oral administration to a subject.
- 25 40. A pharmaceutical composition for the treatment of cancer comprising a compound of claim 2; and
a pharmaceutically acceptable excipient; wherein the pharmaceutical composition is suitable for oral administration to a subject.
- 30 41. The pharmaceutical composition of claim 40, wherein R_8 is $-\text{CH}_2\text{OH}$.

42. The pharmaceutical composition of claim 40, wherein R_B is $-\text{CHF}_2$, $-\text{CH}_2\text{F}$, or $-\text{CF}_3$.

43. The pharmaceutical composition of claim 40, wherein C-D is $-\text{CH}=\text{CH}-$,
5 wherein the double bond is in the trans configuration.

44. The pharmaceutical composition of claim 40, wherein the compound is of the formula:



10

45. The pharmaceutical composition of claim 40, wherein the composition is in solid form.

46. The pharmaceutical composition of claim 40, wherein the composition is in
15 liquid form.

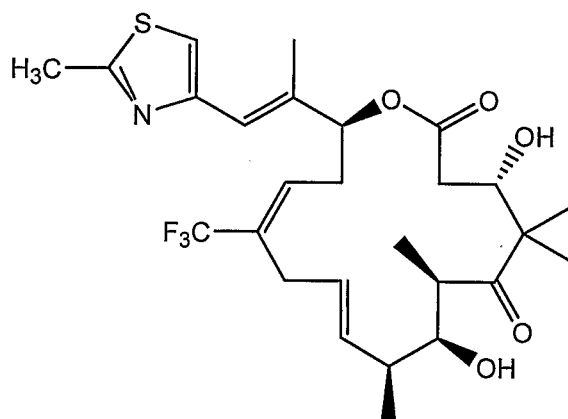
47. A method of treating cancer comprising:
administering a therapeutically effective amount of a compound of claim 1 to a
subject in need thereof.

20

48. The method of claim 47, wherein the therapeutically effective amount of the
compound is an amount sufficient to deliver about 0.001 mg to about 40 mg compound
per kg body weight.

49. The method of claim 47, wherein the therapeutically effective amount of the compound is an amount sufficient to deliver about 0.1 mg to about 25 mg compound per kg body weight.

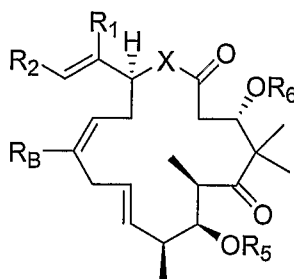
- 5 50. A method of treating cancer comprising:
administering orally a therapeutically effective amount of a compound of formula:



to a subject in need thereof.

10

51. A method of preparing a compound of formula:



wherein R_1 is hydrogen or lower alkyl;

15 R_2 is a substituted or unsubstituted aryl, heteroaryl, arylalkyl, or heteroarylalkyl moiety;

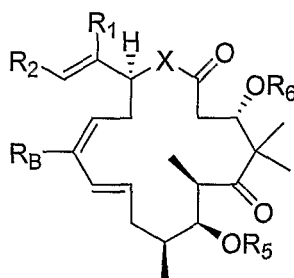
R_5 and R_6 are each independently hydrogen or a protecting group;

X is O, S, $C(R_7)_2$, or NR_7 , wherein each occurrence of R_7 is independently hydrogen or lower alkyl; and

20 R_B is, independently for each occurrence, hydrogen; halogen; $-OR_{B'}$; $-SR_{B'}$; $-N(R_{B'})_2$; $-CY_3$; $-CHY_2$; $-CH_2Y$, where Y is F, Br, Cl, I, $OR_{B'}$, $NHR_{B'}$, $N(R_{B'})_2$, or $SR_{B'}$; $-C(O)OR_{B'}$; $-C(O)R_{B'}$; $-CONHR_{B'}$; $-O(C=O)R_{B'}$; $-O(C=O)OR_{B'}$; -

$\text{NR}_B(\text{C}=\text{O})\text{R}_B$; N_3 ; N_2R_B ; cyclic acetal; or cyclic or acyclic, linear or branched aliphatic, heteroaliphatic, aryl, or heteroaryl, optionally substituted with one or more of hydrogen; halogen; $-\text{OR}_B$; $-\text{SR}_B$; $-\text{N}(\text{R}_B)_2$; $-\text{C}(\text{O})\text{OR}_B$; $-\text{C}(\text{O})\text{R}_B$; $-\text{CONHR}_B$; $-\text{O}(\text{C}=\text{O})\text{R}_B$; $-\text{O}(\text{C}=\text{O})\text{OR}_B$; $-\text{NR}_B(\text{C}=\text{O})\text{R}_B$; N_3 ; N_2R_B ; cyclic acetal; or cyclic or acyclic, linear or branched substituted or unsubstituted aliphatic, heteroaliphatic, aryl, or heteroaryl moiety; or is an epothilone, desoxyepothilone, or analogues thereof; or is a polymer; carbohydrate; photoaffinity label; or radiolabel; wherein each occurrence of R_B is independently hydrogen; a protecting group; a linear or branched, substituted or unsubstituted, cyclic or acyclic, aliphatic, heteroaliphatic, aryl, heteroaryl, arylalkyl, arylalkenyl, arylalkynyl, heteroarylalkyl, heteroarylalkenyl, or heteroarylalkynyl moiety;

isomerizing a compound of the formula:



in the presence of a metal and a reducing agent.

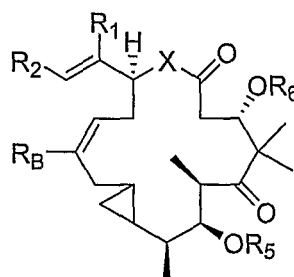
15

52. The method of claim 51, wherein the metal is a transition metal.

53. The method of claim 51, wherein the metal is palladium.

20 54. The method of claim 51, wherein the reducing agent is selected from the group consisting of LiAlH_4 , NaBH_4 , and NaBH_3CN .

55. A method of preparing a compound of formula:



wherein R_1 is hydrogen or lower alkyl;

R_2 is a substituted or unsubstituted aryl, heteroaryl, arylalkyl, or heteroarylalkyl moiety;

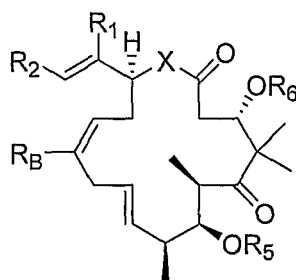
R_5 and R_6 are each independently hydrogen or a protecting group;

5 X is O, S, C(R_7)₂, or NR₇, wherein each occurrence of R_7 is independently hydrogen or lower alkyl; and

R_B is, independently for each occurrence, hydrogen; halogen; -OR_{B'}; -SR_{B'}; -N($R_{B'}$)₂; -CY₃, -CHY₂, -CH₂Y, where Y is F, Br, Cl, I, OR_{B'}, NHR_{B'}, N($R_{B'}$)₂, or SR_{B'}; -C(O)OR_{B'}; -C(O)R_{B'}; -CONHR_{B'}; -O(C=O)R_{B'}; -O(C=O)OR_{B'}; -

10 NR_{B'}(C=O)R_{B'}; N₃; N₂R_{B'}; cyclic acetal; or cyclic or acyclic, linear or branched aliphatic, heteroaliphatic, aryl, or heteroaryl, optionally substituted with one or more of hydrogen; halogen; -OR_{B'}; -SR_{B'}; -N($R_{B'}$)₂; -C(O)OR_{B'}; -C(O)R_{B'}; -CONHR_{B'}; -O(C=O)R_{B'}; -O(C=O)OR_{B'}; -NR_{B'}(C=O)R_{B'}; N₃; N₂R_{B'}; cyclic acetal; or cyclic or acyclic, linear or branched substituted or unsubstituted aliphatic, heteroaliphatic, aryl, or heteroaryl moiety; or is an epothilone, desoxyepothilone, or analogues thereof; or is
15 a polymer; carbohydrate; photoaffinity label; or radiolabel; wherein each occurrence of $R_{B'}$ is independently hydrogen; a protecting group; a linear or branched, substituted or unsubstituted, cyclic or acyclic, aliphatic, heteroaliphatic, aryl, heteroaryl, arylalkyl, arylalkenyl, arylalkynyl, heteroarylalkyl, heteroarylalkenyl, or heteroarylalkynyl
20 moiety;

reacting a compound of the formula:



with a carbene or carbenoid reagent.

25 56. The method of claim 55, wherein the carbene is CH₂N₂.