

INTERMEDIATES IN THE SYNTHESIS OF ERIBULIN AND RELATED METHODS OF SYNTHESIS

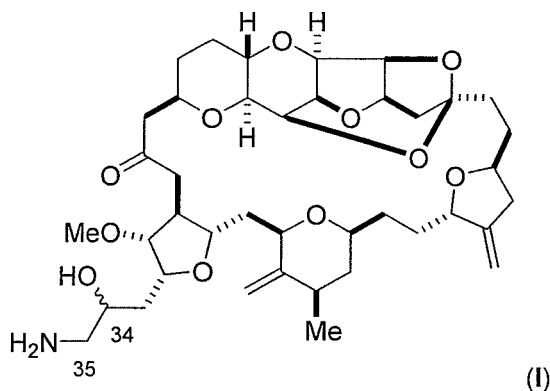
Background of the invention

Halichondrin B is a structurally complex, macrocyclic compound that was originally isolated from the marine sponge *Halichondria okadae*, and subsequently was found in *Axinella* sp., *Phakellia carteri*, and *Lissodendoryx* sp. Eribulin is a synthetic analog of halichondrin B. The mesylate salt of eribulin (eribulin mesylate, which is marketed under the trade name HALAVEN®) is approved for the treatment of patients with breast cancer who have previously received at least two chemotherapeutic regimens for the treatment of metastatic disease that included an anthracycline and a taxane in either the adjuvant or metastatic setting.

Summary of the invention

The invention provides eribulin and diastereomers thereof containing carbon-11, as well as methods of synthesizing eribulin by a two-step process including a nitro aldol reaction and a reduction.

In one example, the disclosure provides a compound according to formula (I),



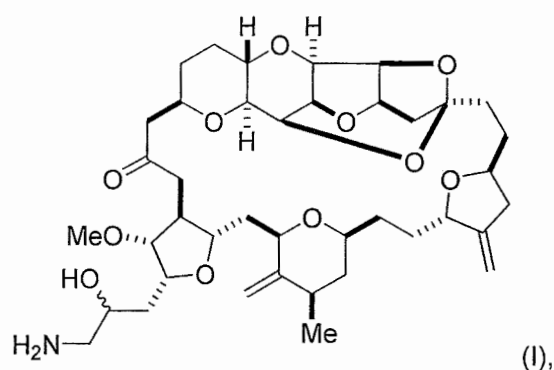
or a pharmaceutically acceptable salt thereof, wherein the compound contains carbon-11.

In some examples, the stereochemical configuration at position 34 of the compound is (*S*). In some examples, the stereochemical configuration at position 34 of the compound is (*R*). In some examples, the carbon-11 is located at position 35 of the compound of formula (I).

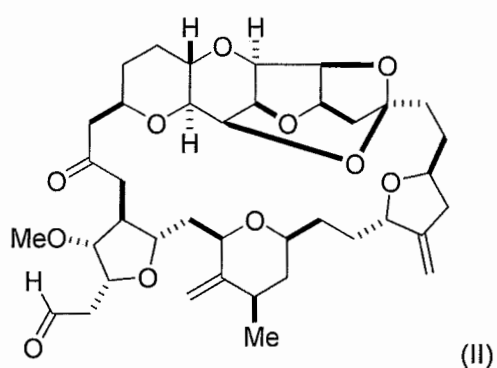
In some examples, the compound is the mesylate salt of formula (I).

The compound of formula (I) may also be in the form of an isotopically enriched composition, i.e., in carbon 11. The term "isotopically enriched," as used herein, refers to a composition including an isotope, e.g., ¹¹C, at a position in the compound in an abundance greater than other isotopes, e.g., ¹²C, at that same position. Typically and depending on the isotope, compositions enriched in a particular isotope may have an isotopic enrichment factor of at least 5, at least 10, at least 50, at least 500, at least 2000, at least 3000, at least 6000, or at least 6600, e.g., relative to ¹²C.

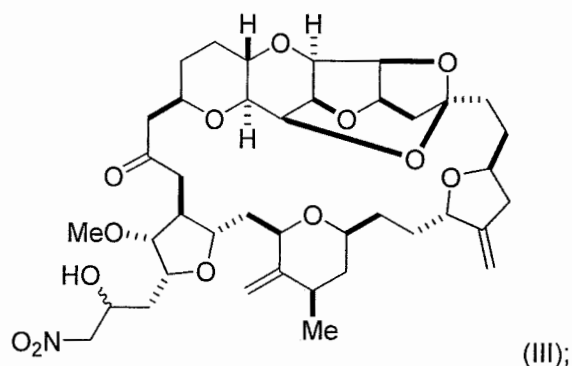
In one aspect, the present invention provides a method of synthesizing a compound according to formula (I):



or a pharmaceutically acceptable salt thereof, the method comprising reacting an aldehyde
5 according to formula (II):

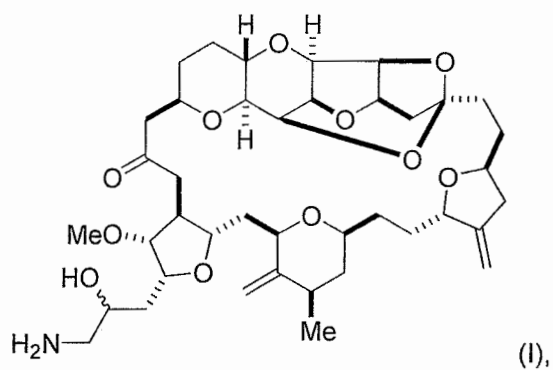


with nitromethane under Henry reaction conditions to form a compound according to formula (III):

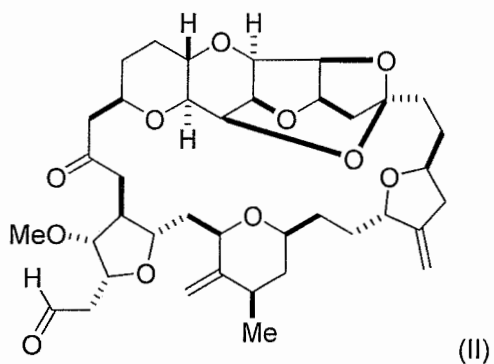


and reducing the compound according to formula (III) with a reducing agent to form the
10 compound according to formula (I).

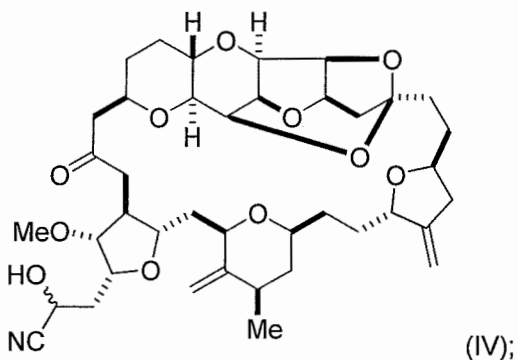
In another aspect, the present invention provides a method of synthesizing a compound according to formula (I):



or a pharmaceutically acceptable salt thereof, the method comprising reacting an aldehyde according to formula (II):

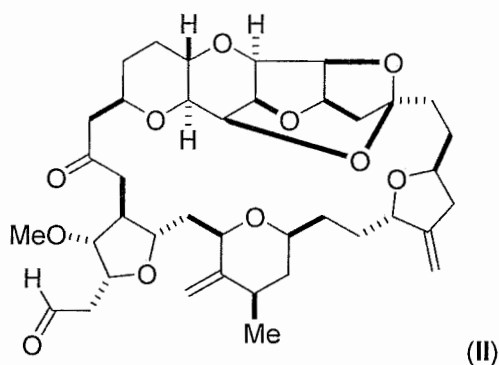


5 with a cyanide salt under conditions to form a cyanohydrin according to formula (IV):

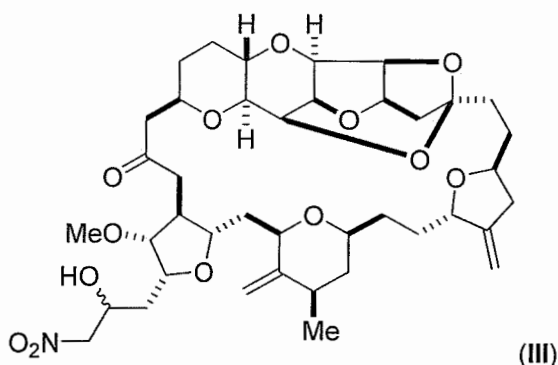


and reducing the cyanohydrin according to formula (IV) with a reducing agent to form the compound according to formula (I)

10 In an additional example, the disclosure relates to a method of synthesizing a compound according to formula (I), by reacting an aldehyde according to formula (II):



with nitromethane, e.g., containing carbon-11, under Henry reaction conditions to form a compound according to formula (III):



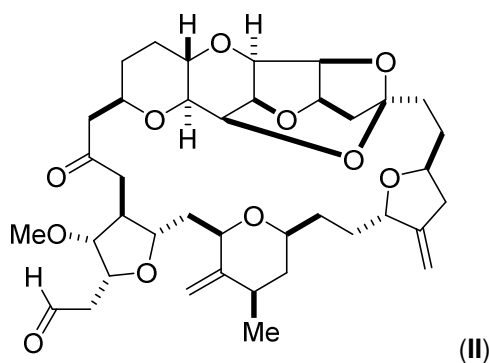
5 and reducing the compound according to formula (III) with a reducing agent to form the compound according to formula (I).

In some examples, the method includes dissolving the aldehyde in an alcohol, e.g., methanol, to form a solution. In some examples, the solution has a concentration of from 0.01 M to 0.5 M, e.g., about 0.1 M. In some examples, the method includes adding from 1 to 10,000 molar equivalents of the
10 nitromethane to the aldehyde, e.g., about 1000 molar equivalents of the nitromethane to the aldehyde.

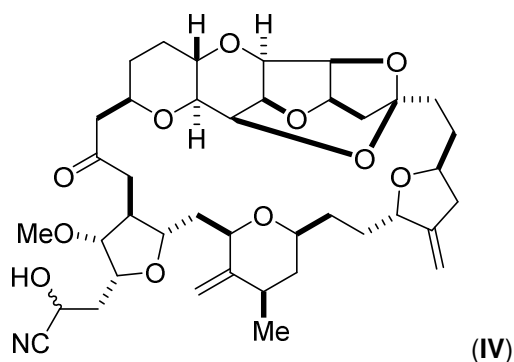
In some examples, the method includes adding a base, e.g., sodium hydroxide, to a mixture containing the aldehyde and the nitromethane.

In some examples, the compound according to formula (III) is reduced by a lanthanide salt, such as samarium (II) iodide. In some examples, the method includes dissolving the compound according to
15 formula (III) in an alcohol, e.g., methanol, to form a solution. In some examples, the solution has a concentration of compound according to formula (III) from 0.01 mM to 1 mM, e.g., about 0.7 mM. In some examples, the reducing agent is present in a solution at a concentration of from 0.01 M to 1 M, e.g., about 0.1 M. In some examples, the method includes mixing from 2 molar equivalents to 1000 molar equivalents of the reducing agent with the compound according to formula (III).

20 In another example, the disclosure provides a method of synthesizing a compound according to formula (I), by reacting an aldehyde according to formula (II):

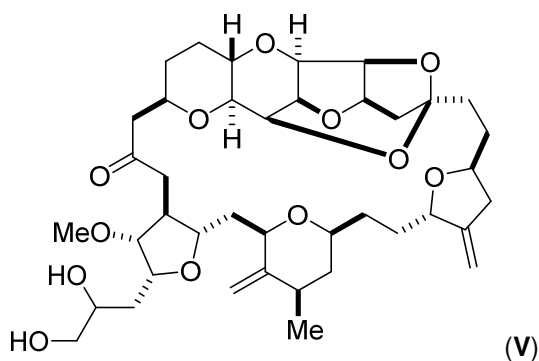


with a cyanide salt, e.g., potassium cyanide, under conditions to form a cyanohydrin according to formula (IV):



- 5 and reducing the cyanohydrin according to formula (IV) with a reducing agent to form the compound according to formula (I). In some examples, the reducing agent is a silane, such as triethylsilane, used, for example, in combination with a Lewis acid, such as tris(perfluorophenyl)borane. In some examples, the cyanide salt contains carbon-11 (e.g., $K^{11}CN$).

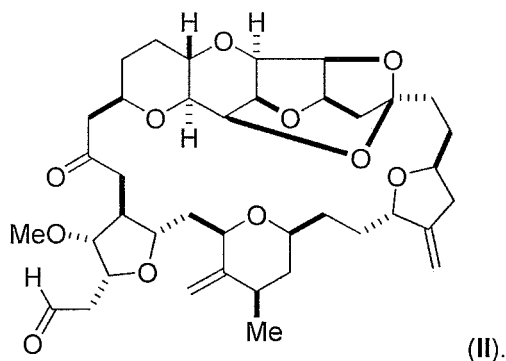
In some examples, the aldehyde is synthesized by reacting a diol according to formula (V):



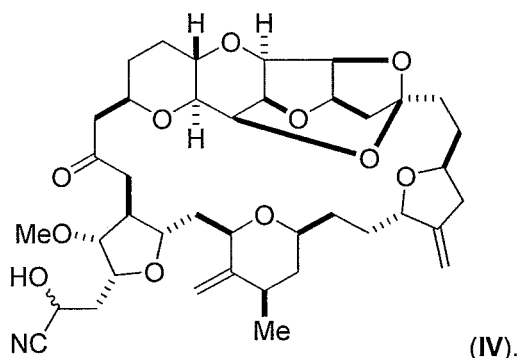
10 with an oxidizing agent, e.g., sodium periodate.

In some examples, any of the methods may further include salifying the compound of formula (I) to produce a pharmaceutically acceptable salt thereof, e.g., the mesylate salt. The methods may also include separating the diastereomers of the compound of formula (I) to isolate eribulin, or a pharmaceutically acceptable salt thereof, e.g., the mesylate salt.

15 In another example, the disclosure features a compound according to formula (II):



In another aspect, the invention features a compound according to formula (IV):



The disclosure further provides a pharmaceutical composition comprising an effective amount of a carbon-11 containing compound of formula (I) or an isotopically enriched composition of a compound of formula (I) and a pharmaceutically acceptable carrier.

In an additional example, the disclosure provides a method of using a carbon-11 containing compound of formula (I) to image a subject, e.g., a human subject. In some examples, the method includes administering the compound to the subject and detecting the presence of the compound. In some examples, the detecting includes analyzing the subject by positron emission tomography.

Brief Description of the Figures

Figure 1 shows a series of chromatograms tracing the conversion of compound (II) to compound (III) as described in Example 1. From top to bottom, the chromatograms correspond to the reaction mixture sampled at about 5 minutes, about 30 minutes, about 1 hour and 50 minutes, and about 3 hours from the beginning of the addition of nitromethane and sodium hydroxide to compound (II) as outlined in Example 1.

Figure 2 shows a series of chromatograms tracing the conversion of compound (III) to compound (I) as described in Example 2. From bottom to top, the chromatograms correspond to the reaction mixture sampled at the start of the reaction, about 17 minutes following the addition of SmI_2 to the nitro adduct (III), and following the addition of Rochelle salt solution to the reaction mixture.

Figure 3A is a chromatogram obtained from analysis of the reaction mixture following the inverse addition of nitro adduct to reducing agent as described in Example 3.

Figure 3B is a chromatogram obtained from analysis of the reaction mixture following the addition of reducing agent to nitro adduct as described in Example 2.

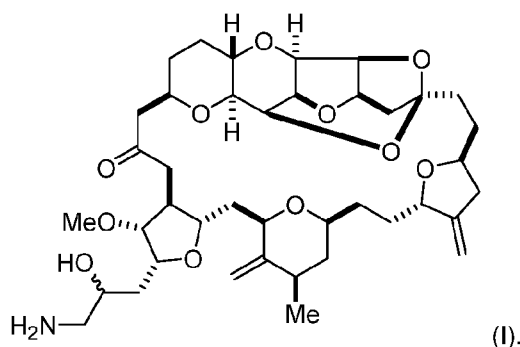
Figure 4 shows a series of chromatograms tracing the conversion of compound (II) to compound (I) as described in Example 4. From bottom to top, the chromatograms correspond to starting material, (i) reaction mixture following the addition of nitromethane and sodium hydroxide, (ii) reaction mixture following quenching in acetic acid, (iii) and (iv) reaction mixture following addition of Sml_2 , and (v) reaction mixture after work up.

Figure 5A is a chromatogram obtained from HPLC analysis of product obtained by the reaction of equimolar quantities of compound (II) and nitromethane using a tandem nitro aldol/reduction process as described in Example 4. Eribulin was separated from its C34 diastereomer by this process.

Figure 5B is a chromatogram obtained from HPLC analysis of product obtained by the reaction of compound (II) with 0.2 molar equivalents of nitromethane using a tandem nitro aldol/reduction process as described in Example 4. Eribulin was separated from its C34 diastereomer by this process.

Detailed Description

The invention provides eribulin and diastereomers thereof, represented by formula (I), below, that contain carbon-11, as well as related methods of synthesis.

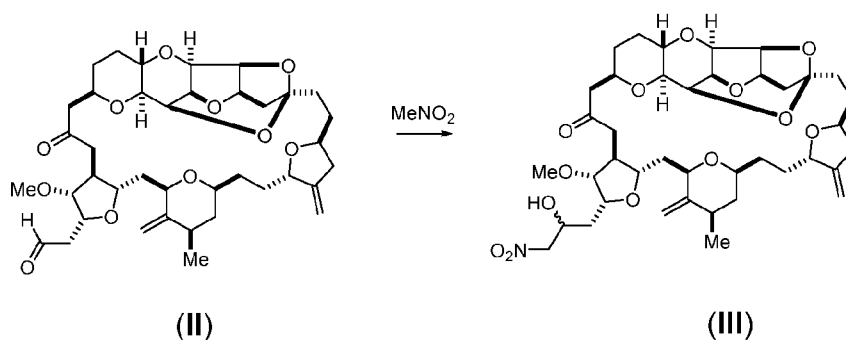


The invention further features a pharmaceutically acceptable salt of the compound of formula (I), e.g., the mesylate salt.

The compounds produced by the methods of the invention, such as eribulin or diastereomer thereof containing carbon-11 at position 35 of the molecule, can be used for a variety of purposes. Eribulin is a known chemotherapeutic agent, and eribulin synthesized by the methods of the invention can be administered to a human patient suffering from cancer, optionally in combination with additional chemotherapeutic agents, in order to treat the cancer. Additionally, as carbon-11 is a well-established radiolabel for positron emission tomography, eribulin containing carbon-11 can be administered to a patient in order to visualize a sample within a subject, such as a particular organ or tissue within the subject. For instance, eribulin containing carbon-11 may be administered to a patient in order to image one or more solid tumors within a subject, e.g., that is undergoing chemotherapeutic treatment.

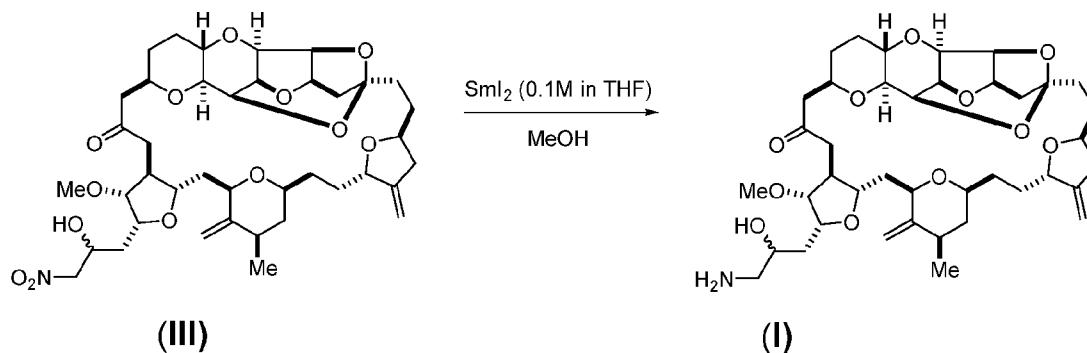
Using the methods of the invention, eribulin can be synthesized under Henry reaction conditions, e.g., as follows:

Scheme 1.



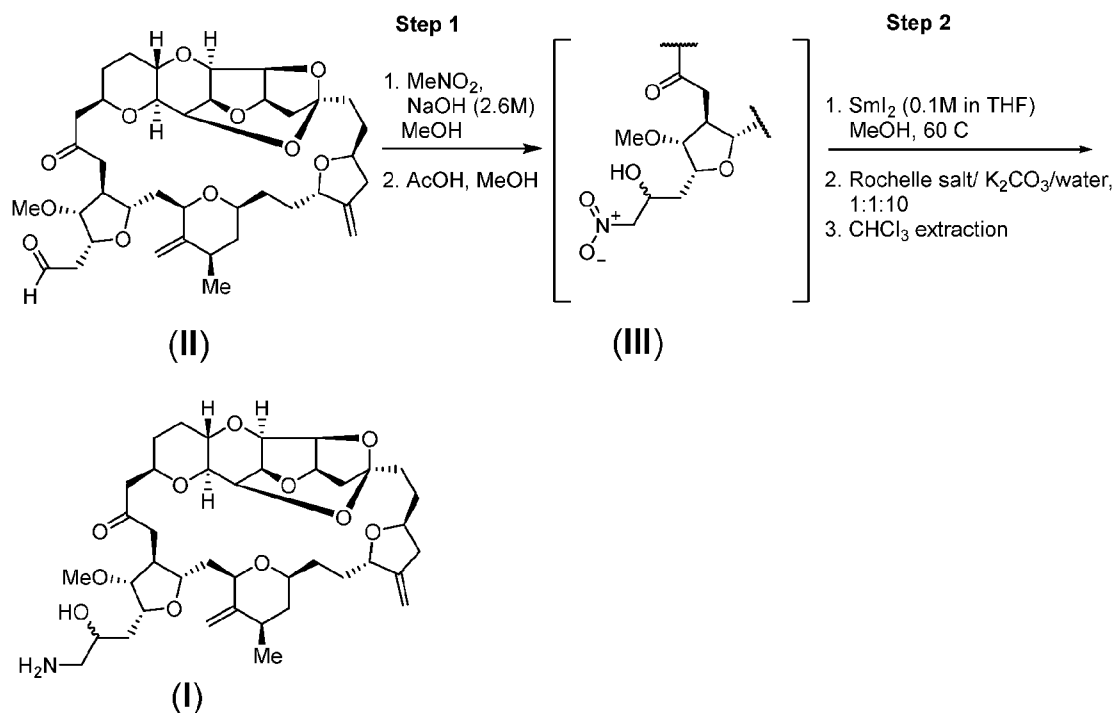
According to the methods of the invention, compound (III) can be reduced, e.g., using lanthanide salt, such as samarium (II) iodide. Other suitable reducing agents are known in the art. For instance, compound (III) can be reduced according to the following reaction scheme:

Scheme 2.



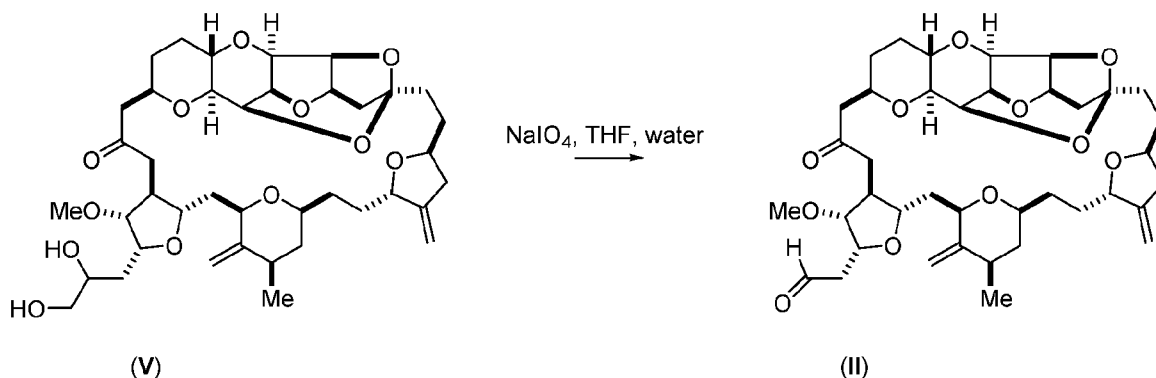
Optionally, eribulin can be synthesized using a one- or two-pot procedure, e.g., as described in Scheme 3, below. In this process, compound (II) is transformed to compound (III) by a nitro aldol process using nitromethane. A reducing agent, such as SmI₂, can then be mixed directly with the reaction mixture containing compound (III). As shown in Scheme 3, below, and as described in further detail in the Examples, this process can be performed either by adding the reducing agent directly to the reaction mixture following the Henry reaction, or by transferring the reaction mixture containing compound (III) to a vessel containing the reducing agent. Acidic workup may also be performed with pivalic acid instead of acetic acid.

Scheme 3.



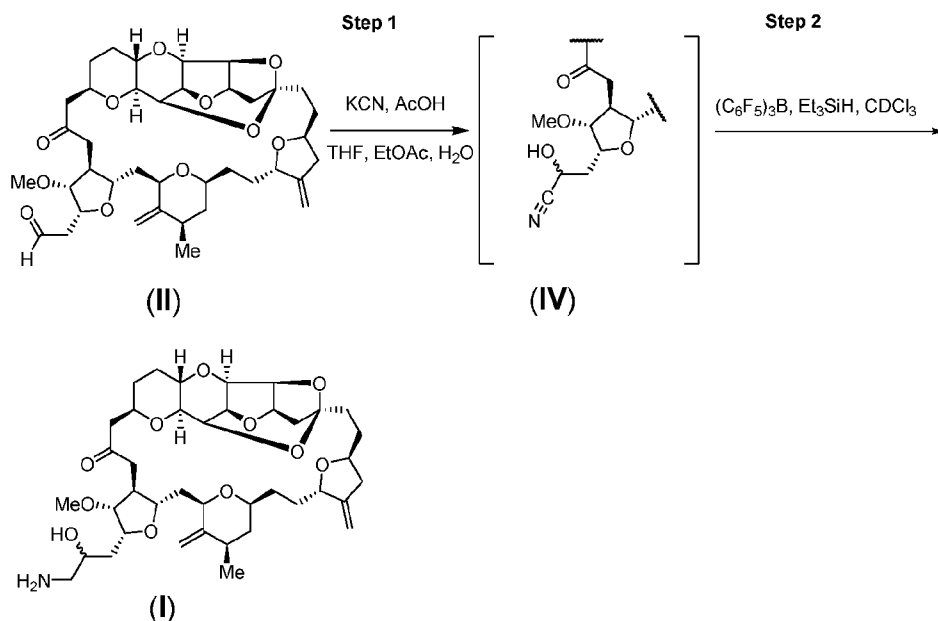
Using the methods of the invention, compound (II) can be synthesized by the oxidation of a diol according to formula (V). For instance, compound (II) can be prepared by sodium periodate-mediated oxidation of diol (V), as illustrated in reaction scheme 4, below.

Scheme 4.



Using the methods of the invention, eribulin can alternatively be synthesized by reacting aldehyde (II) with a cyanide salt, such as potassium cyanide, in order to form a cyanohydrin represented by formula (IV), below. The cyanohydrin thus obtained can subsequently be reduced to generate eribulin. This two-step process is depicted in Scheme 5, below. The nitrile substituent of cyanohydrin (IV) can be reduced to an amine, e.g., by reaction of the cyanohydrin with a reducing agent, such as a silane, e.g., diethylsilane or triethylsilane, in the presence of a Lewis acid, such as tris(perfluorophenyl)borane (Chang et al. J. Org. Chem. 2015, 80, 7281–7287).

Scheme 5.



Eribulin can be separated from its C-34 diastereomer using standard techniques, such as HPLC.

Salification reaction conditions are known in the art. Salification of eribulin can afford a pharmaceutically acceptable salt of eribulin (e.g., eribulin mesylate). In particular, the salification reaction can involve contacting eribulin with a Brønsted acid (e.g., a pharmaceutically acceptable Brønsted acid (e.g., methanesulfonic acid)) to afford a pharmaceutically acceptable salt of eribulin (e.g., *Handbook of Pharmaceutical Salts: Properties, Selection and Use*, ed.: Stahl and Wermuth, Wiley-VCH/VHCA, Weinheim/Zurich, 2002). Pharmaceutically acceptable salts of eribulin, e.g., eribulin mesylate, can be formed by methods known in the art, e.g., in situ during the final isolation and purification of the compound or separately by reacting the free base group with a suitable organic acid. In one example, eribulin is treated with a solution of MsOH and NH₄OH in water and acetonitrile. The mixture is concentrated. The residue is dissolved in DCM-pentane, and the solution is added to anhydrous pentane. The resulting precipitate is filtered and dried under high vacuum to provide eribulin mesylate.

Formulations

Compounds or isotopically enriched compositions of the invention can be formulated as pharmaceutically acceptable salts, which are a salt within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and animals without undue toxicity, irritation, allergic response and the like and commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, pharmaceutically acceptable salts are described in: Berge et al., *J. Pharmaceutical Sciences* 66:1-19, 1977 and in *Pharmaceutical Salts: Properties, Selection, and Use*, (Eds. P.H. Stahl and C.G. Wermuth), Wiley-VCH, 2008. Representative acid addition salts include acetate, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate,

borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptonate, glycerophosphate, hemisulfate, heptonate, hexanoate, hydrobromide, hydrochloride, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, toluenesulfonate, undecanoate, valerate salts and the like. A preferred salt is the mesylate salt.

Compounds or isotopically enriched compositions of the invention can also be formulated as pharmaceutical compositions, e.g., by combining an effective amount of the compound or isotopically enriched composition with a pharmaceutically acceptable carrier. An effective amount is typically the amount needed to image a subject by positron emission topography.

Pharmaceutical compositions can be prepared using standard methods known in the art, or can be obtained from commercial sources. A compound of formula (I), e.g., eribulin, is typically provided in liquid form, for intravenous administration.

Pharmaceutical compositions used in the invention can be prepared by, for example, mixing or dissolving the active ingredient(s), having the desired degree of purity, in a physiologically acceptable carrier (see, e.g., Remington's Pharmaceutical Sciences (20th edition), ed. A. Gennaro, 2000, Lippincott, Williams & Wilkins, Philadelphia, PA). Acceptable carriers include water and saline, optionally including buffers such as phosphate, citrate, or other organic acids; antioxidants including butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone, amino acids such as glycine, glutamine, asparagines, arginine or lysine; monosaccharides, disaccharides, or other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN[™], PLURONICS[™], or PEG.

Optionally, the formulations of the invention contain a pharmaceutically acceptable preservative. In some embodiments the preservative concentration ranges from 0.1 to 2.0%, typically v/v. Suitable preservatives include those known in the pharmaceutical arts, such as benzyl alcohol, phenol, m-cresol, methylparaben, and propylparaben. Further, the formulations of a compound of formula (I), e.g., eribulin, can optionally include a pharmaceutically acceptable salt, such as sodium chloride at, for example, about physiological concentrations. Thus, in one example, the compound of formula (I), e.g., eribulin (e.g., eribulin mesylate), is formulated in 0.9% Sodium Chloride Injection (USP).

The formulations noted above (and others) can be used for parenteral administration of the drugs. Thus, the drugs can be administered by routes including intravenous, intra-tumoral, peri-tumoral, intra-arterial, intra-dermal, intra-vesical, ophthalmic, intramuscular, intradermal, intraperitoneal, pulmonary, subcutaneous, and transcutaneous routes. Other routes can also be used including, for example, transmucosal, transdermal, inhalation, intravaginal, rectal, and oral administration routes.

The dosage of compound of formula (I), e.g., eribulin, administered can differ markedly depending on the type of target disease, the choice of delivery method, as well as the age, sex, and weight of the patient, the severity of the symptoms, along with other factors.

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Examples

The following methods illustrate the synthesis of compounds of formula (I) using reagents with the natural abundance of carbon isotopes. These processes may be modified by use of ^{11}C -labeled nitromethane or ^{11}C -labeled cyanide to produce ^{11}C labeled compounds of formula (I).

10 **Example 1. Synthesis of compound of formula (III) by nitro aldol reaction**

The aldehyde of formula (II) (278 mg, 0.398 mmol) was dissolved in methanol (2.780 mL), and nitromethane (21.45 μL , 1 eq., 0.398 mmol) and sodium hydroxide (2.6 M, 161 μL , 1.05 eq., 0.418 mmol) were subsequently added to the aldehyde solution. The ensuing reaction was allowed to proceed for about 3 hours, and the reaction mixture was periodically monitored by liquid chromatography/mass spectrometry (LC/MS). After 5 minutes, the ratio of product, compound of formula (III), to starting material was approximately 2:1. After about 30 minutes, only 5-10% of the aldehyde remained, as the reaction mixture contained predominantly the nitro-containing compound of formula (III). After about 1 hour and 50 minutes, no change in reaction mixture composition was observed relative to the reaction mixture analyzed after 30 minutes. Additionally, after about 3 hours, no change in reaction mixture composition was observed relative to the reaction mixture analyzed after 30 minutes (Figure 1).

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The reaction was subsequently quenched by adding acetic acid (23.91 μL , 0.418 mmol) followed by the drop-wise addition of water (7 mL) to obtain a white suspension. The suspension was filtered and dried under a stream of N_2 to yield 235 mg of compound of formula (III) as a white powder (0.31 mmol, 78%).

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Example 2. Reduction of compound of formula (III) by standard addition process

Compound of formula (III) (6.40 mg, 8.422 μmol) was dissolved in methanol (1.28 mL) and was degassed by N_2 sparge for 2 minutes. The solution was then gradually warmed to 55-60° C. A solution of SmI_2 (0.1 M in THF, 0.84 mL, 10 eq.) was then added to the solution of compound of formula (III) over the course of 2 minutes. The temperature of the reaction mixture at the beginning of the addition was 40° C and was steadily increased to 50° C during the addition. After 8 minutes from the beginning of the addition of the SmI_2 solution to compound of formula (III), the reaction was observed to be complete. No change in reaction composition was observed after 10 additional minutes. A solution containing Rochelle salt (potassium sodium tartrate), K_2CO_3 , and water (1:1:10 by mole, 1 mL) was subsequently added to the reaction mixture, and the resulting suspension was stirred for 2 minutes. Chloroform (1 mL) was then added, and the reaction was stirred for an additional 2 minutes (Figure 2). The bottom layer was removed, and the aqueous layer was extracted twice with chloroform. Thin layer chromatography (TLC) analysis of the extracts revealed that compound (I) was contained within the first two chloroform

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solutions. The extracts were combined and concentrated to yield 9 mg crude product as a pale yellow solid.

Example 3. Reduction of compound of formula (III) by inverse addition process

Compound of formula (III) (6.40 mg, 8.422 μ mol) was dissolved in methanol (1.28 mL) and was degassed by N₂ sparge for 2 minutes. In a separate flask, a solution of Sml₂ was prepared (0.1 M in THF, 0.84 mL, 10 eq.) and was gradually warmed to 55-60°C. The solution of compound of formula (III) was then added to the solution of Sml₂ over the course of 3 minutes. The temperature of the reaction mixture at the beginning of the addition was 64°C and was 60°C at the end of the addition. The reaction mixture after 12 minutes from the beginning of the addition of the Sml₂ solution to compound of formula (III) was monitored by LC/MS, and the results were compared to the LC/MS trace obtained following the standard addition process described in Example 2. This comparison is shown in Figures 3A and 3B.

After 12 minutes from the beginning of the addition of the Sml₂ solution to compound of formula (III), the mixture was cooled to room temperature. A solution containing Rochelle salt, K₂CO₃, and water (1:1:10 by mole, 1 mL) was subsequently added to the reaction mixture, and the resulting suspension was stirred for 2 minutes. Chloroform (1 mL) was then added, and the reaction was stirred for an additional 2 minutes. The bottom layer was removed, and the aqueous layer was extracted twice with chloroform. Thin layer chromatography (TLC) analysis of the extracts revealed that compound of formula (I) was contained within the first two chloroform solutions. The extracts were combined and concentrated to yield 16 mg crude product as a pale yellow solid.

Example 4. One-pot procedure for synthesis of compound of formula (I) by a tandem nitro aldol/reduction process

Step 1: A stock solution of carbon-11-labelled nitromethane (3.5 μ L) was added to methanol (1.5 mL) sparged with N₂. Compound of formula (II) (15.00 mg, 0.02 mmol) was dissolved in the nitromethane/methanol solution (0.15 mL solution, 0.262 mg nitromethane, 4.293 μ mol, 0.2 eq.) in a N₂ purged, 1.5-mL vial equipped with stir bar and silicon polytetrafluoroethylene (PTFE) septum. A 5-10 μ L aliquot of the resulting mixture was removed and diluted in 0.25 mL methanol for analysis. A sodium hydroxide solution (2.6 M, 1.65 μ L, 4.29 μ mol) was then added to the mixture, and the ensuing reaction was allowed to proceed for 16 minutes. Acetic acid (12.28 μ L, 0.215 mmol, 10 eq.) was then added to the reaction mixture, which was then diluted with N₂-sparged methanol (3 mL).

Step 2: A solution of Sml₂ (0.1 M in THF, 2.15 mL, 0.215 mmol) was added to a N₂-purged, 7-mL conical microwave vial equipped with silicon PTFE crimp cap and stir bar. The mixture was warmed to 60°C. The nitro adduct formed in step 1 was then added to the Sml₂ solution slowly over the course of 2 minutes. The temperature of the ensuing mixture at the beginning of the addition process was 38°C, and the temperature of the mixture at the end of the addition process was 45°C and rising. After 12 minutes from the start of the addition process, the reduction was observed to be complete. The reaction mixture

was then gradually cooled to room temperature, and the methanol was evaporated by N₂ stream. A solution containing Rochelle salt, K₂CO₃, and water (1:1:10 by mole, 3 mL) was then added to the reaction mixture. After 5 minutes, chloroform (2 mL) was added to the mixture, which was then vigorously stirred. The layers were allowed to partition, and the bottom layer (chloroform) was removed. The chloroform extraction was repeated a second time, and the extracts were analyzed by TLC. It was observed that the product (compound of formula (I)) was predominantly in the first extract, with trace quantities of product observed in the second extract. The aqueous layer was found to contain no product. LC/MS traces recorded during various stages of Step 1 and Step 2 are shown in Figure 4.

Example 5. Synthesis of compound of formula (II) by oxidation of a diol precursor

The diol of formula (V) (126 mg, 0.172 mmol) was dissolved in THF (1.6 mL) and water (0.88 mL). Sodium periodate (55.3 mg, 0.259 mmol) was added to form a mixture, which was subsequently stirred at room temperature. The progress of the ensuing oxidation reaction was monitored by LCMS analysis. A white precipitate formed within 10 minutes of mixing. After 2 hours, water (15 mL) was added, and the mixture was extracted with ethyl acetate (3 x 15 mL). Ethanol 1 (mL) was added to the mixture causing layers to partition. The combined extracts were subsequently washed with water and then concentrated to provide the aldehyde of formula (II) as white solid (115 mg, 0.9 wt, 95% yield).

Example 6. Purification of eribulin by reverse-phase high pressure liquid chromatography (RP-HPLC)

Following the one-pot procedure described in Example 4, compound of formula (I) was purified by RP-HPLC using a two-component mobile phase and gradient elution profile as described in Tables 1 and 2, below. C18 columns were obtained from ACE® (Aberdeen, Scotland). Solvent A: 760 mL water and 240 mL acetonitrile mixed together, 7.0 ± 0.2 g ammonium trifluoromethanesulfonate, 3.0 mL of 1.0 M tetrabutylammonium dihydrogenphosphate aqueous solution added, and pH adjusted to between 6.9 and 7.1 with either 5.6% ammonium hydroxide solution or 1 M HCl. Solvent B: 300 mL of water, 7000 mL of acetonitrile, and 20 mL of 2-propanol mixed together, 7.0 ± 0.2 g ammonium trifluoromethanesulfonate, 3.0 mL of 1.0 M tetrabutylammonium dihydrogenphosphate aqueous solution added, and pH adjusted to between 6.9 and 7.1 with either 5.6% ammonium hydroxide solution or 1 M HCl.

Table 1. Summary of parameters used for HPLC analysis

HPLC column	3.0 mm x 15 cm column containing 3 µm packing (L1/USP) (e.g., ACE® C18, 3 µm particle size)
Column temperature	Constant temperature of approximately 40° C
Gradient profile	Linear gradient (see below)

Injection	5 μ L
Detection (UV)	UV absorbance at 200 nm
Run time	90 minutes

Table 2. HPLC gradient profile

Time (min)	% Solvent A	% Solvent B	Flow rate (mL/min)
0	100	0	0.50
55	100	0	0.50
75	0	100	0.63
85	0	100	0.63
86	100	0	0.63
105	Stop	Stop	Stop

Sample chromatograms obtained from RP-HPLC purification are shown in Figure 5A (one-pot process, 1:1 aldehyde:nitromethane by mole) and Figure 5B (one-pot process, 5:1 aldehyde:nitromethane by mole).

Example 7. Purification of compound of formula (I) by silica gel chromatography

Crude compound of formula (I) (mixture of diastereomers, prepared using the one-pot procedure as described in Example 4) was purified by silica gel column chromatography (230-400 SiO₂, 0.5 cm d x 5.5 cm h). Compound of formula (I) was eluted with 25 mL each of 3:2 heptane:ethyl acetate and ethyl acetate, 40 mL of 4:1 acetonitrile:water containing 0.2% NH₄OH. The silica column was conditioned with 3:2 heptane:ethyl acetate and crude product mixture was loaded with dichloromethane/heptane. The heptane:ethyl acetate and ethyl acetate eluants were collected in one fraction each (fractions 1 and 2). The acetonitrile:water eluant was collected in 4 x 10 mL fractions (fractions 3-6). Each fraction was subsequently analyzed by TLC and LC/MS. Fraction 4 was concentrated to provide a 1:1 mixture of eribulin and its C-34 diastereomer (2.5 mg, 3.56 μ mol, 83% based on nitromethane, contained minor amide impurity).

Example 8. Synthesis of compound of formula (I) via cyanohydrin

Compound of formula (II) (78 mg, 0.112 mmol) was dissolved in (EtOAc:THF:AcOH:water; 2:1:1.6:0.4 (v/v), 25 V). Potassium cyanide (25 mg, 0.384 mmol) in water (0.17mL) was added, and the solution stirred at room temperature and monitored by TLC (4:1 toluene/acetonitrile) and LCMS. When the reaction was deemed complete, water (20 mL) was added, and the reaction was stirred. The organic layer was removed, and the aqueous layer was extracted with EtOAc (3 x 15 mL). The combined

extracts were washed with 10% aqueous sodium bicarbonate (20 mL). The aqueous layer was back-extracted with DCM (10 mL). The combined organic layers were concentrated and azeotroped with toluene to provide crude product diastereomers of formula (IV). The residue was purified by flash chromatography (6:1 to 4:1 toluene/acetonitrile) to provide the β -isomer (4 mg) and mixture of α - (epi-34) and β -isomers (19 mg).

A stock solution of tris(perfluorophenyl)borane and triethylsilane was prepared by dissolving tris(perfluorophenyl)borane (10.6 mg) and triethylsilane (0.275 mL) in D-chloroform (0.75 mL). 70 μ L reagent solution (10 eq. silane, 0.03 eq. tris(perfluorophenyl)borane) were added to the α -isomer (5.00 mg, 6.888 μ mol) at room temperature, and the reaction was monitored by LCMS. After 10 minutes, HCl (0.01 N, 0.2 mL) was added, and the reaction was stirred for 5 minutes. The reaction mixture was extracted with DCM (0.2 mL) to remove lipophilic impurities. The aqueous layer was treated with sodium bicarbonate (0.5 mL) and then extracted with DCM (2 x 0.4 mL). The combined extracts were concentrated to provide the compound of formula (I) (epi-34 eribulin).

Other Embodiments

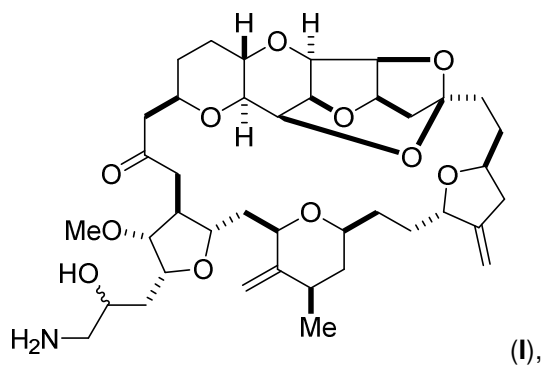
All publications, patents, and patent applications mentioned in this specification are incorporated herein by reference to the same extent as if each independent publication or patent application was specifically and individually indicated to be incorporated by reference.

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the invention that come within known or customary practice within the art to which the invention pertains and may be applied to the essential features hereinbefore set forth, and follows in the scope of the claims.

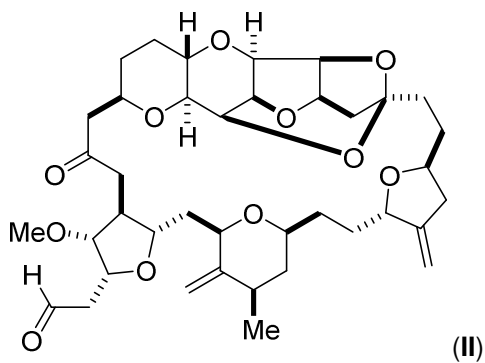
Other embodiments are within the claims.

CLAIMS

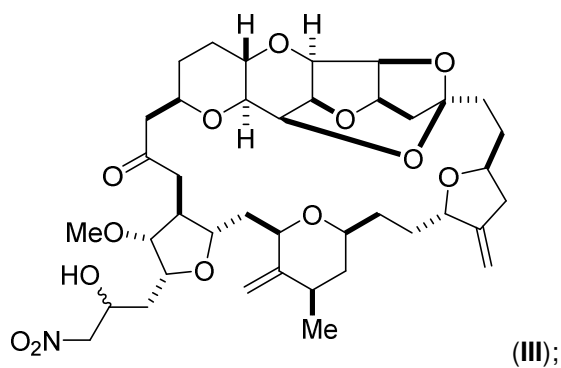
1. A method of synthesizing a compound according to formula (I):



or a pharmaceutically acceptable salt thereof, the method comprising reacting an aldehyde according to formula (II):



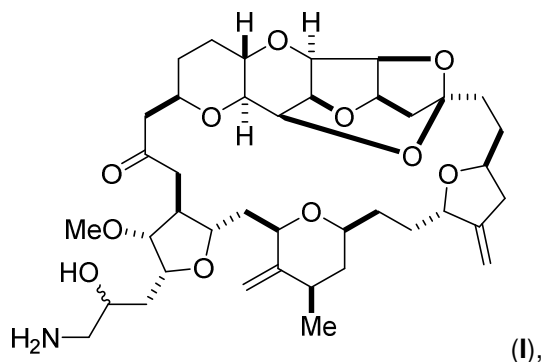
with nitromethane under Henry reaction conditions to form a compound according to formula (III):



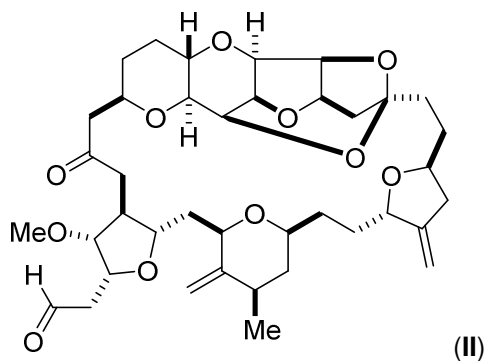
and reducing the compound according to formula (III) with a reducing agent to form the compound according to formula (I).

2. The method of claim 1, further comprising adding a base to a mixture comprising the aldehyde and the nitromethane.
3. The method of claim 2, wherein the base is sodium hydroxide.

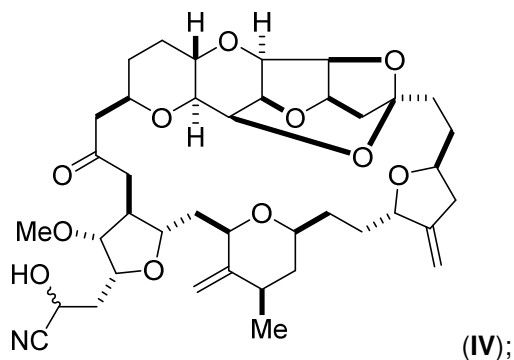
4. The method of any one of claims 1-3, wherein the reducing agent is a lanthanide salt.
5. The method of claim 4, wherein the lanthanide salt is samarium (II) iodide.
6. A method of synthesizing a compound according to formula (I):



or a pharmaceutically acceptable salt thereof, the method comprising reacting an aldehyde according to formula (II):



with a cyanide salt under conditions to form a cyanohydrin according to formula (IV):



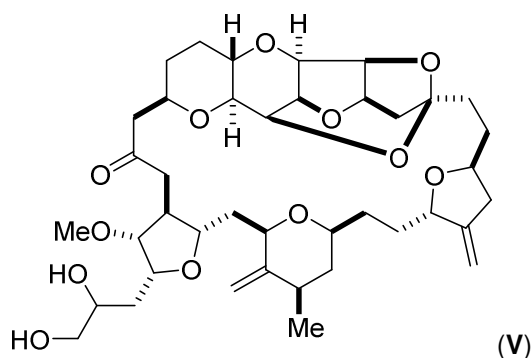
and reducing the cyanohydrin according to formula (IV) with a reducing agent to form the compound according to formula (I).

7. The method of claim 6, wherein the cyanide salt is potassium cyanide.
8. The method of claim 6 or 7, wherein the reducing agent is diethylsilane or triethylsilane.

9. The method of any one of claims 6-8, further comprising adding a Lewis acid to a mixture of the cyanohydrin and the reducing agent.

10. The method of claim 9, wherein the Lewis acid is tris(perfluorophenyl)borane.

11. The method of any one of claims 1-10, further comprising synthesizing the aldehyde according to formula (II) by reacting a diol according to formula (V):



with an oxidizing agent to form the aldehyde.

12. The method of claim 11, wherein the oxidizing agent is sodium periodate.

13. The method of any one of claims 1-12, further comprising salifying the compound of formula (I) to produce a pharmaceutically acceptable salt thereof.

14. The method of claim 13, wherein the pharmaceutically acceptable salt is a mesylate salt.

15. A compound according to formula (IV):

