

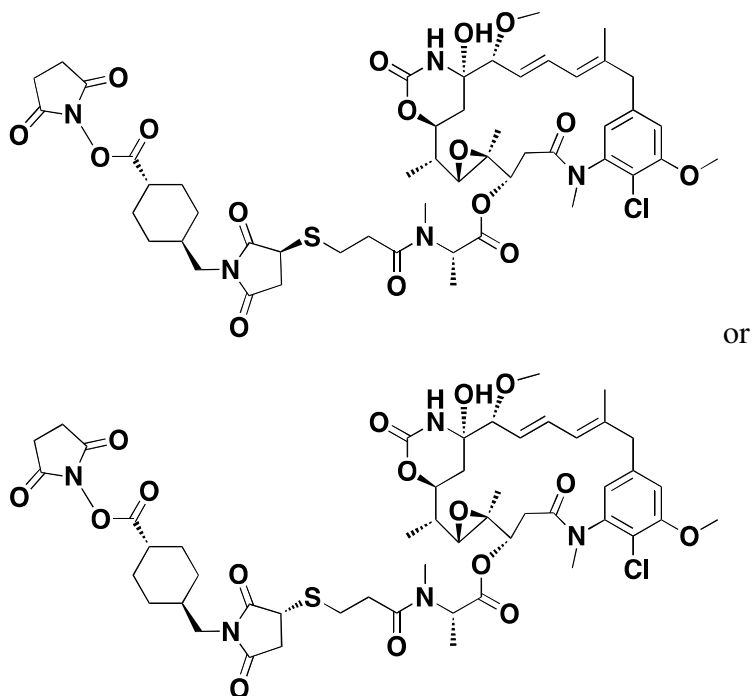
of the starting material having formula III and the compound of formula IV is from about 1:1 to about 1:3.

[0037] In certain embodiments, the disclosure provides a method for preparing compositions comprising a plurality of drug molecules of formula I, further comprising combining the compound of formula I with an antibody or antigen-binding fragment thereof to make an antibody-drug conjugate.

[0038] In certain embodiments, the disclosure provides a method for preparing compositions comprising a plurality of drug molecules of formula I, wherein the compound of formula I is attached to the antibody or antigen-binding fragment via an S, O, or NR₃.

[0039] In an embodiment, the disclosure provides a method for preparing a composition comprising a plurality of drug molecules of formula I, wherein the drug molecules of formula I are present in the composition in a diastereomeric excess of about 60% 70%, 80%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more.

[0040] In an embodiment, the disclosure provides a method for preparing compositions comprising a plurality of drug molecules of formula I represented by the following structure:



in a diastereoselective excess of greater than 50%. Although certain exemplary methods for preparing the compositions of the present disclosure are set forth in the working examples herein, other methods are contemplated within the scope of the present disclosure such as,

such as tamoxifen, prednisone, dacarbazine, mechlorethamine, cisplatin, methotrexate, 5-fluorouracil, and ara-C.

[0049] The present disclosure also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of an active agent and a pharmaceutically acceptable carrier. The term “pharmaceutically acceptable” means approved by a regulatory agency of the United States Federal or State government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term “carrier” refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in “Remington's Pharmaceutical Sciences” by E. W. Martin.

[0050] In an embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lidocaine to ease pain at the site of the injection. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0051] The active agents of the disclosure can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed

also be useful in the treatment of a medical disorder selected from autoimmune diseases and other immunological diseases and dysfunctions, inflammatory diseases, infectious diseases, neurodegenerative diseases, bone disorders, and cardiovascular diseases. Further, any disorder that can benefit from the targeted delivery of a toxic substance to particular cells, cell types, tissues and/or organs is within the scope of the present disclosure.

[0058] Finally, embodiments herein may include compositions comprising mixtures of compounds as represented by formula (I) and formula (II).

[0059] The references to certain embodiments made in the following description are considered illustrative only of the principles of the disclosure. Further, since numerous modifications and changes will readily be apparent to those skilled in the art, it is not intended to limit the disclosure to the exact construction and process shown as described herein. Accordingly, all suitable modifications and equivalents may be resorted to as falling within the scope of the disclosure and as defined by the claims that follow.

[0060] The words “comprise”, “comprising”, “include” and “including” when used in this specification and in the following claims are intended to specify the presence of the stated features, integers, components, or steps, but they do not preclude the presence or addition of one or more additional features, integers, components, or steps thereof.

[0061] General terms used in any of the embodiments herein can be defined as follows; however, the meaning stated should not be interpreted as limiting the scope of the term per se.

[0062] The term “conjugate” as used herein refers to compound having a Ligand, linker and Biologically Active Molecule. Illustrative examples include compounds of formula (II).

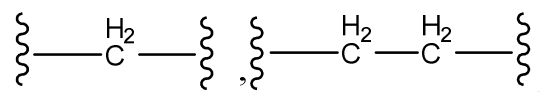
[0063] The term “spacer” as used herein refers to chemical building blocks of the linker used to spatially separate the Ligand from the Biologically Active Molecule and to allow for catabolism of the linker inside of cells.

[0064] The term “macrolide” as used herein refers to any Biologically Active Molecule having a macrolide ring.

[0065] The symbol  denotes the points of attachment.

[0066] The term "alkyl" as used herein refers to a hydrocarbon radical having a straight or branched chain or combinations thereof. Alkyl radicals can be a univalent, a bivalent or a cyclic radical. Examples of univalent alkyl radicals are methyl, ethyl, 1-propyl,

2-propyl, n-butyl, isobutyl, sec-butyl, t-butyl, pentyl, neopentyl, hexyl, isohexyl, and the like. As a way of illustration examples of bivalent alkyl radicals include



[0067] Examples of cyclic alkyl radicals include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and the like. Typical alkyl radicals have from one to ten carbon atoms, one to nine carbon atoms, one to eight carbon atoms, one to seven carbon atoms, one to six carbon atoms, one to five carbon atoms, one to four carbon atoms, one to three carbon atoms, one to two carbon atoms or one carbon atom. Typical cycloalkyl are 3 to 7 member monocyclic ring radicals.

[0068] The phrase “pharmaceutically acceptable salt” as used herein refers to both organic and inorganic salts of the conjugate compounds described herein, *e.g.*, compounds of formula (I), and (II). The salts are pharmaceutically acceptable and include: sulfate, citrate, nitrate, phosphate, ascorbate, bromide, gluconate, benzoate, oxalate, pantothenate, and the like. Note that pharmaceutically acceptable salts herein may include more than one charged atom in its structure as well as one or more counter ion. Preparation of conjugate compounds herein as pharmaceutically acceptable salts is well known to one of skill in the art.

[0069] The term "ligand" as used herein means any molecule or compound that specifically or selectively interacts with and/or binds to a second molecule or compound. In certain embodiments, a ligand is an antibody or antigen-binding fragment thereof. Other examples of ligands suitable for use in the context of the present disclosure include, *e.g.*, aptamers, peptides that specifically interact with a particular antigen (*e.g.*, peptibodies), receptor molecules, and antigen-binding scaffolds (*e.g.*, DARPin, HEAT repeat proteins, ARM repeat proteins, tetratricopeptide repeat proteins, and other scaffolds based on naturally occurring repeat proteins, etc., [*see, e.g.*, Boersma and Pluckthun, 2011, *Curr. Opin. Biotechnol.* 22:849-857, and references cited therein]).

[0070] Antibodies exist as intact immunoglobulins, or as a number of well-characterized fragments produced by digestion with various peptidases. For example, pepsin digests an antibody below the disulfide linkages in the hinge region to produce F(ab)₂, a dimer of Fab which itself is a light chain joined to V_H-C_H by a disulfide bond. The F(ab)₂ may be reduced under mild conditions to break the disulfide linkage in the hinge region, thereby converting the F(ab)₂ dimer into an Fab' monomer. The Fab' monomer is

essentially Fab with part of the hinge region. While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that such fragments may be synthesized de novo either chemically or by using recombinant DNA methodology. Thus, the term antibody, as used herein, also includes antibody fragments either produced by the modification of whole antibodies, or those synthesized de novo using recombinant DNA methodologies (*e.g.*, single chain Fv (scFv) single variable domains (Dabs)) or those identified using display libraries such as phage, *E. coli* or yeast display libraries (see, for example, McCafferty et al. (1990) Nature 348:552-554).

[0071] Methods for preparing antibodies are known to the art. See, for example, Kohler & Milstein (1975) Nature 256:495-497; Harlow & Lane (1988) *Antibodies: a Laboratory Manual*, Cold Spring Harbor Lab., Cold Spring Harbor, N.Y.). Antibodies that are isolated from organisms other than humans, such as mice, rats, rabbits, cows, can be made more human-like through chimerization or humanization.

[0072] The term “human antibody” as used herein is intended to include antibodies having variable and constant regions derived from human immunoglobulin sequences. The human mAbs of the disclosure may include amino acid residues not encoded by human immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs and in particular CDR3. However, the term “human antibody”, as used herein, is not intended to include mAbs in which CDR sequences derived from the germline of another mammalian species have been grafted onto human FR sequences.

[0073] The term “therapeutically effective amount” as used herein refers to an amount that produces the desired effect for which it is administered. The exact amount will depend on the purpose of the treatment, and will be ascertainable by one skilled in the art using known techniques (see, for example, Lloyd (1999) *The Art, Science and Technology of Pharmaceutical Compounding*).

[0074] The term “racemate” as used herein means an equimolar mixture of a pair of enantiomers. The term racemate also refers to a racemic mixture.

[0075] The term “enantiomer” as used herein refers to compounds which non-superimposable with the mirror images of each other. Enantiomers may exist in either the (R) or (S) configuration.

[0076] The term “stereoselective synthesis” refers to a chemical reaction that leads to formation of a single stereoisomer or an enantiomer-enriched mixture of isomers from among two or more possible stereoisomers.

[0077] The term “diastereomeric excess” refers to the difference between the mole fraction of the desired single diastereomer as compared to the remaining diastereomers in a composition. Diastereomeric excess is calculated as follows:

$$(\text{amount of single diastereomer}) - (\text{amount of other diastereomers}) / 1$$

For example, a composition that contains 90% of 1 and 10% of 2, 3, 4, or a mixture thereof has a diastereomeric excess of 80% $[(90-10)/1]$. A composition that contains 95% of 1 and 5% of 2, 3, 4, or a mixture thereof has a diastereomeric excess of 90% $[(95-5)/1]$. A composition that contains 99% of 1 and 1% of 2, 3, 4, or a mixture thereof has a diastereomeric excess of 98% $[(99-1)/1]$. The diastereomeric excess can similarly be calculated for any one of 1, 2, 3, or 4.

[0078] The term “stereomerically pure” as used herein refers to a compound wherein the indicated stereoisomer is present to a greater extent than other stereoisomers of that compound, *e.g.*, the compound is present in diastereomeric excess. In some embodiments, the stereomerically pure compounds described herein comprise 80% or greater, 85% or greater, 90% or greater, 95% or greater, or 97% or greater by weight of one stereoisomer of the compound.

[0079] Conjugates in accordance with various embodiments described herein can be prepared by any known method in the art. An illustrative protocol for producing conjugates is provided in the Examples below.

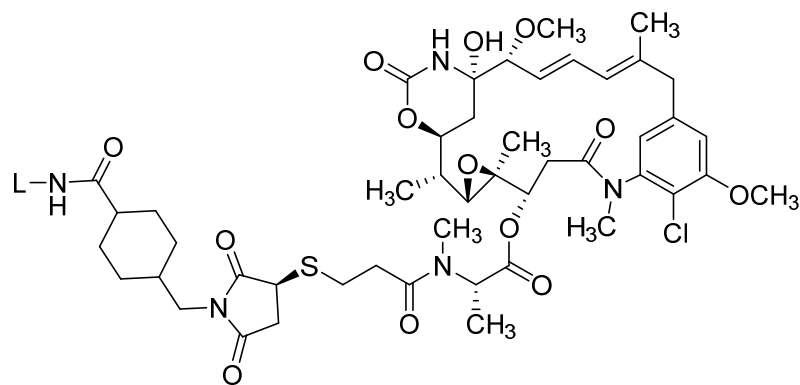
[0080] In one embodiment, the conjugates can be prepared by i) reacting a Ligand with drug molecules of formula (I) to form a conjugate of formula (II), and ii) purifying the conjugate.

[0081] In an alternative embodiment, the conjugates are prepared by reacting a Ligand, linker and biologically active macrolide in a single reaction. Once the conjugates in accordance with the disclosure are prepared they can be purified.

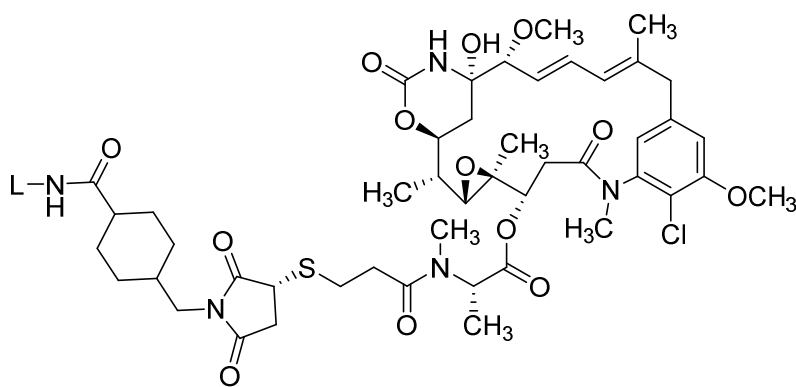
[0082] In one embodiment, the compositions comprising drug molecules of formula (I) and/or compositions comprising conjugate compounds of formula (II) described herein can be evaluated for their ability to suppress proliferation of various cancer cell lines *in vitro*. For example, compositions comprising drug molecules of formula (I) and/or compositions comprising conjugate compounds of formula (II) can be applied to *in vitro* plated cancer cells

for a predetermined number of days and surviving cells measured in assays by known methods.

[0082a] According to an aspect there is provided a composition comprising a compound of Formula IIa or IIb:



IIa



IIb

wherein:

L is an antibody or an antigen-binding fragment thereof;

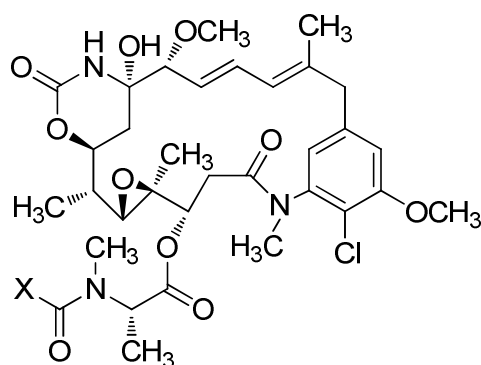
and wherein the compound of Formula IIa or IIb is present in a diastereomeric excess of greater than 50% and wherein the diastereomers have different stereochemical configurations at the chiral carbon that is bound to the sulfur atom of Formula IIa or IIb, wherein the diastereomeric excess is based on the amounts of at least two diastereomers having different stereochemical configurations at the chiral carbon that is bound to the sulfur atom of Formula IIa or IIb.

[0082b] According to another aspect there is provided use of the composition described herein for the manufacture of a medicament for treating cancer in a patient in need thereof.

[0082c] According to another aspect there is provided use of the composition described herein in the manufacture of a medicament for treating a disease or disorder associated with a tumor-associated antigen, wherein the tumor-associated antigen is selected from the group consisting of AFP, ALK, BAGE proteins, β -catenin, bcr-abl, BRCA1, BORIS, CA9, carbonic anhydrase IX, caspase-8, CD20, CD40, CDK4, CEA, CLEC12A, cMET, CTLA4, cyclin-B1, CYP1B1, EGFR, EGFRvIII, ErbB2/Her2, ErbB3, ErbB4, ETV6-AML, EphA2, Fra-1, FOLR1, GAGE proteins, GD2, GD3, GloboH, glypican-3, GM3, gp100, Her2, HLA/B-raf, HLA/k-ras, HLA/MAGE-A3, hTERT, IGF1R, LGR5, LMP2, MAGE proteins, MART-1, mesothelin, ML-IAP, Muc1, Muc16 or CA-125, MUM1, NA17, NY-BR1, NY-BR62, NY-BR85, NY-ESO1, OX40, p15, p53, PAP, PAX3, PAX5, PCTA-1, PDGFR- α , PDGFR- β , PDGF-A, PDGF-B, PDGF-C, PDGF-D, PLAC1, PRLR, PRAME, PSMA, FOLH1, RAGE proteins, Ras, RGS5, Rho, SART-1, SART-3, Steap-1, Steap-2, survivin, TAG-72, TGF- β , TMPRSS2, Tn, TNFRSF17, TRP-1, TRP-2, tyrosinase, and uroplakin-3, said composition comprising a therapeutically effective amount of the compound present in a diastereomeric excess.

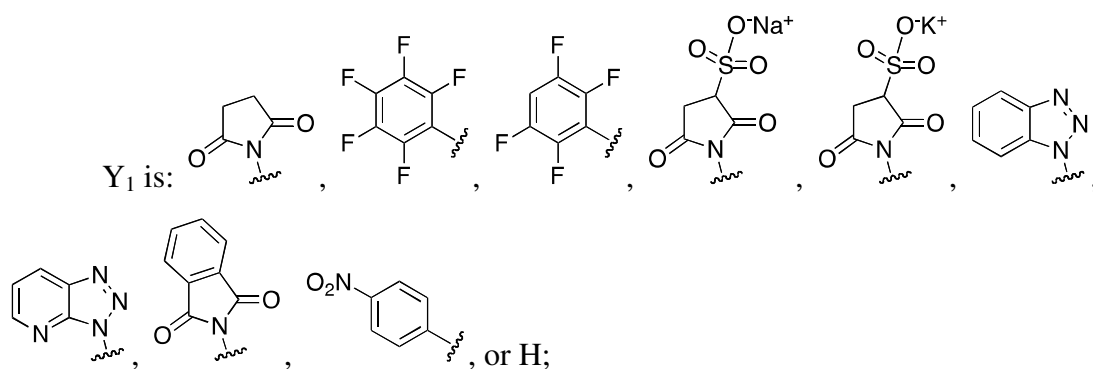
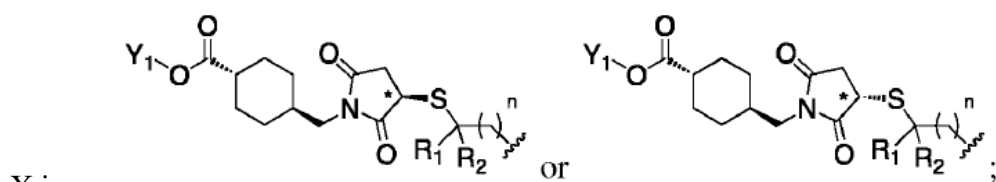
[0082d] According to another aspect there is provided a pharmaceutical composition comprising the composition described herein, further comprising a pharmaceutically acceptable excipient.

[0082e] According to another aspect there is provided a composition comprising a compound of Formula I:

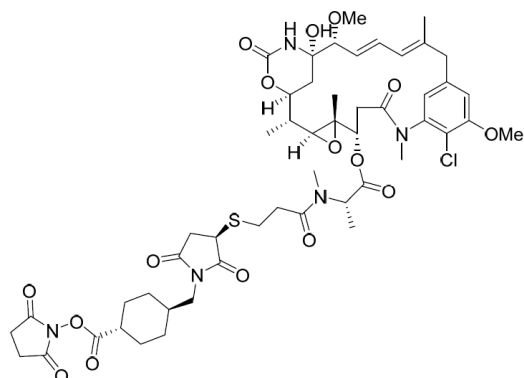


I

wherein:

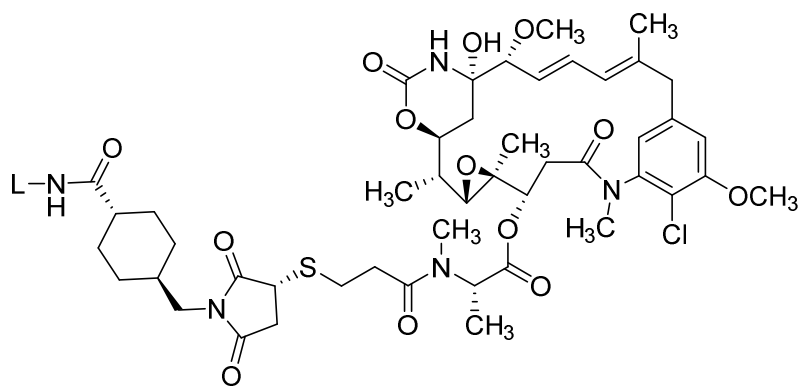


wherein the antibody-drug conjugate is made by combining a compound of Formula (I) having the following structure:



with an antibody or antigen-binding fragment thereof.

[0082g] According to another aspect there is provided an antibody-drug conjugate of



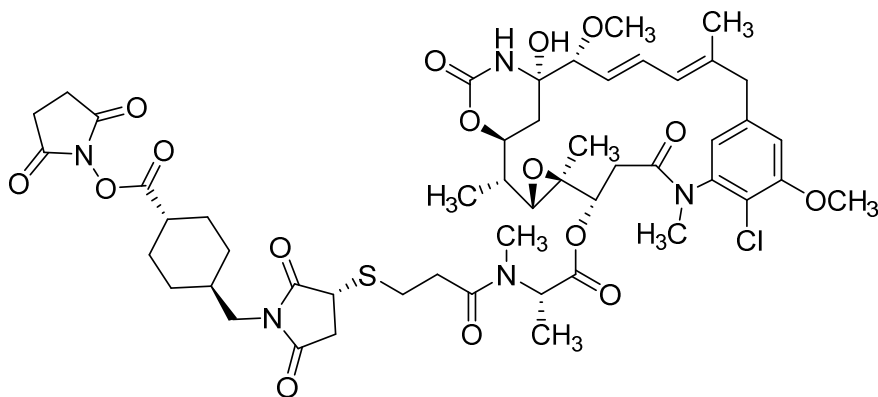
structure

IIb

wherein:

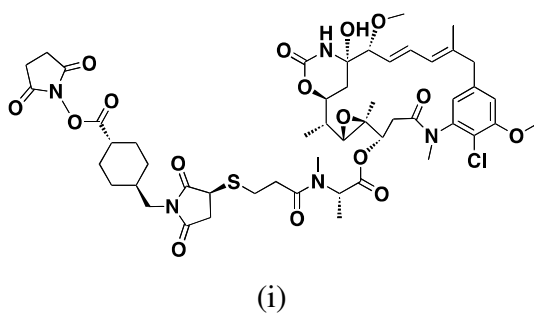
L is an antibody or an antigen-binding fragment thereof;

and wherein the antibody-drug conjugate is made by combining a compound of Formula (I) having the following structure:



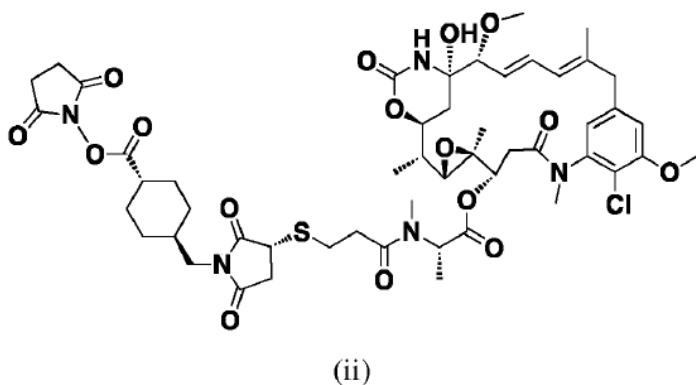
with an antibody or antigen-binding fragment thereof.

[0082h] According to another aspect there is provided a compound of Formula (i):



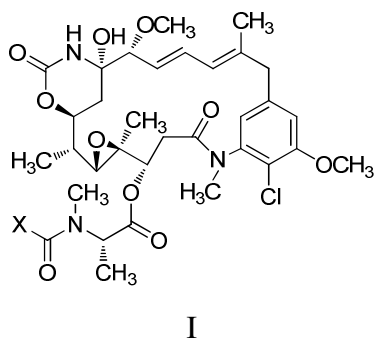
wherein the compound is stereomerically pure.

[0082i] According to another aspect there is provided a compound of formula (ii)

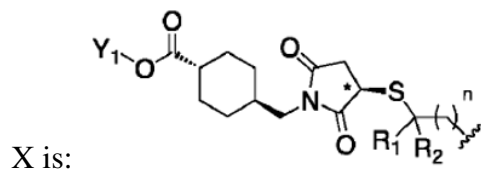


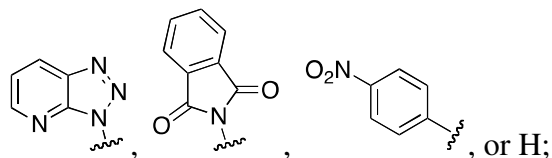
wherein the compound is stereoisomerically pure.

[0082j] According to another aspect there is provided a process for preparing a compound of Formula I:



wherein:

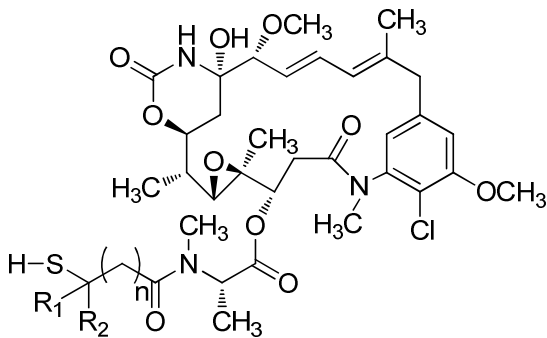




n is independently 0 or an integer from 1 to 50;

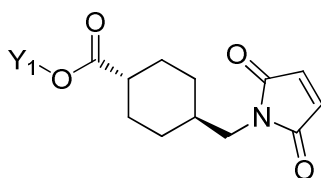
(a) contacting:

(i) a compound of Formula III:



III;

(ii) a compound of formula IV:



IV

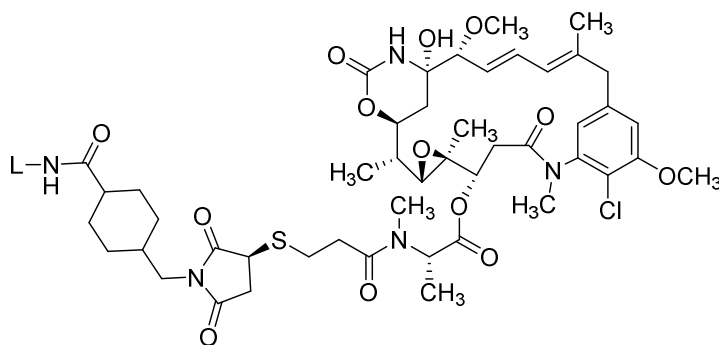
(iii) silica gel; and

(iv) a diluent comprising an organic solvent and water; and

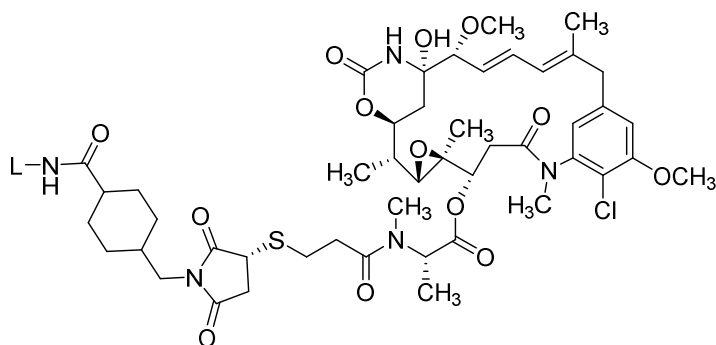
(b) isolating the compound of Formula I, wherein the compound of Formula I is present in a diastereomeric excess of greater than 50% and wherein the diastereomers have different stereochemical configurations at the chiral carbon represented by (*) in formula X of Formula I, wherein said chiral carbon atom is a carbon atom that is bound to a sulfur atom, wherein the diastereomeric excess is based on the amounts of at least two diastereomers

having different stereochemical configurations at the chiral carbon that is bound to the sulfur atom of Formula I.

[0082k] According to another aspect there is provided a process for preparing a compound of Formula IIa or IIb:



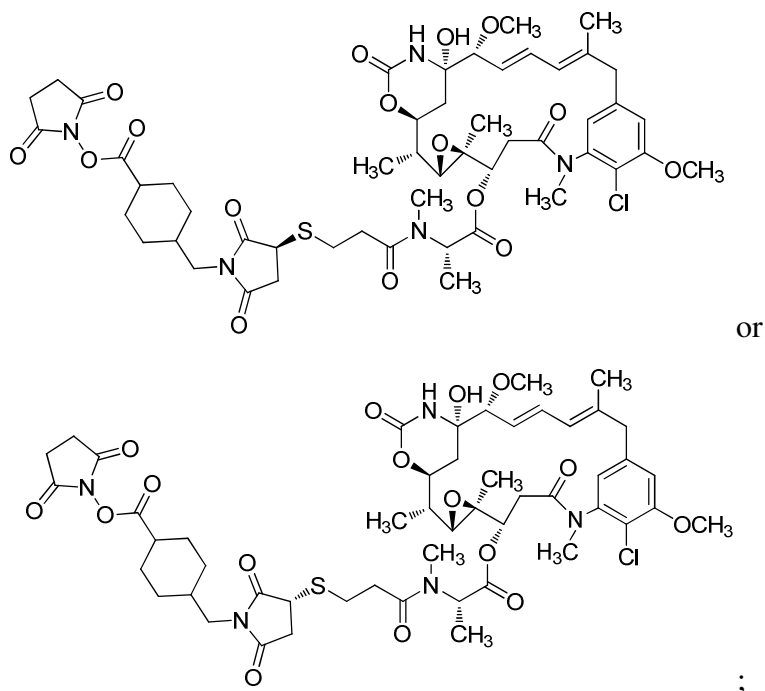
IIa



IIb

wherein L is an antibody or an antigen-binding fragment thereof; and
 wherein the compound of Formula IIa or IIb is present in a diastereomeric excess of greater than 50%, wherein the diastereomers have different stereochemical configurations at the chiral carbon that is bound to the sulfur atom of Formula IIa or IIb, and wherein the diastereomeric excess is based on the amounts of at least two diastereomers having different stereochemical configurations at the chiral carbon that is bound to the sulfur atom of Formula IIa or IIb, comprising:

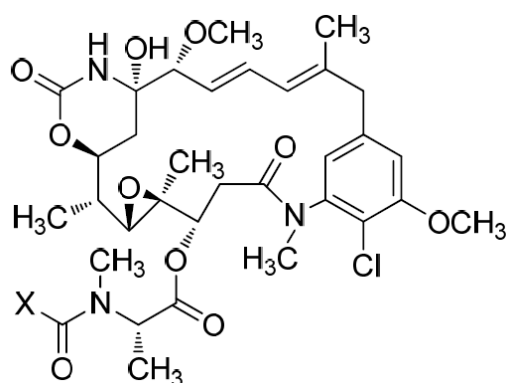
(a) reacting an antibody or antigen-binding fragment thereof with a compound of Formula I, wherein the compound is:



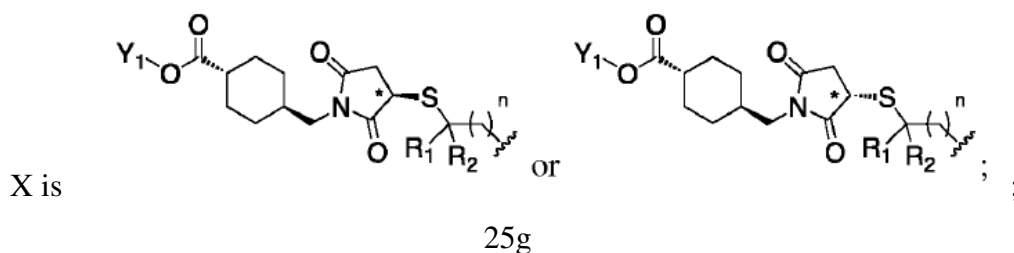
wherein the compound of Formula I is present in a diastereomeric excess of greater than 50% and wherein the diastereomers have different stereochemical configurations at the chiral carbon that is bound to the sulfur atom of Formula I; and

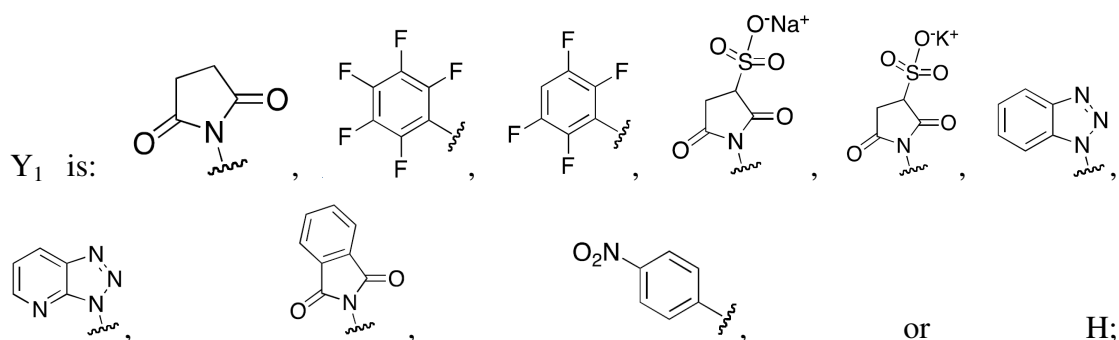
(b) purifying the compound of Formula IIa or IIb.

[00821] According to another aspect there is provided a compound of Formula I:



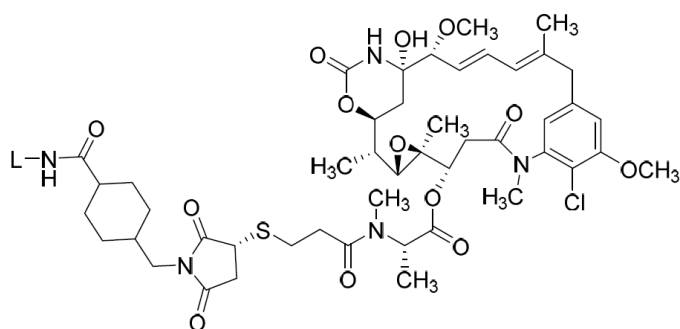
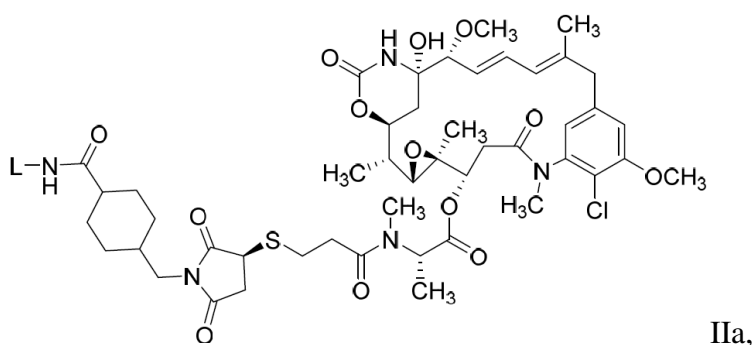
wherein:





R₁ and R₂ are independently selected from H or alkyl; and n is independently 0 or an integer from 1 to 50; prepared by the process described herein wherein the compound of Formula I is present in a diastereomeric excess of greater than 50% and wherein the diastereomers have different stereochemical configurations at the chiral carbon represented by (*) in formula X of Formula I, wherein said chiral carbon atom is a carbon atom that is bound to a sulfur atom, and wherein the diastereomeric excess is based on the amounts of at least two diastereomers having different stereochemical configurations at the chiral carbon that is bound to the sulfur atom of Formula I.

[0082m] According to another aspect there is provided a compound of Formula IIa or IIb:

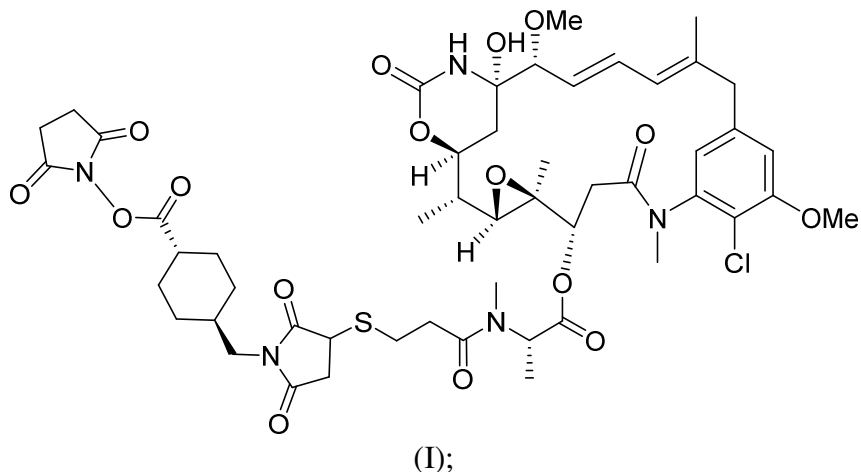


IIb

wherein: L is an antibody or an antigen-binding fragment thereof; and wherein the compound of Formula IIa or IIb is present in a diastereomeric excess of greater than 50% and wherein

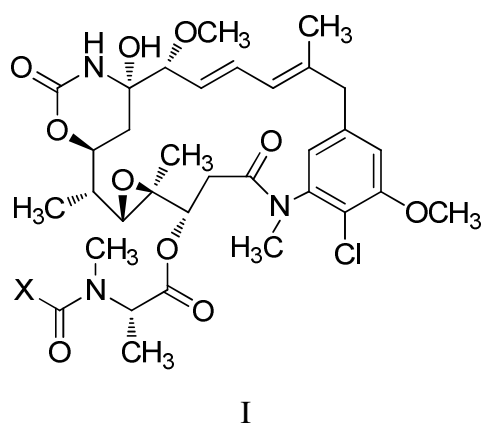
the diastereomers have different stereochemical configurations at the chiral carbon that is bound to the sulfur atom of Formula IIa or IIb, and wherein the diastereomeric excess is based on the amounts of at least two diastereomers having different stereochemical configurations at the chiral carbon that is bound to the sulfur atom of Formula IIa or IIb; prepared by the process described herein.

[0082n] According to another aspect there is provided a composition comprising a compound of Formula I:

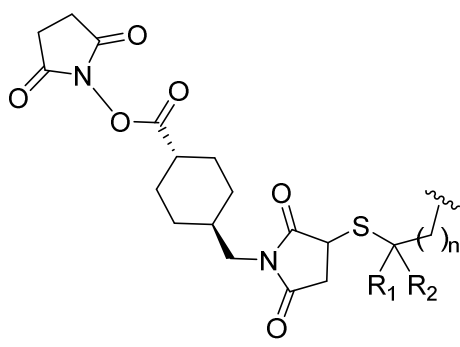


wherein the compound of Formula I is characterized by a ^1H NMR substantially as shown in Figure 1.

[0082o] According to another aspect there is provided a diastereomer of a compound of Formula I characterized by a ^1H NMR substantially as shown in Figure 1:



wherein:



X is:

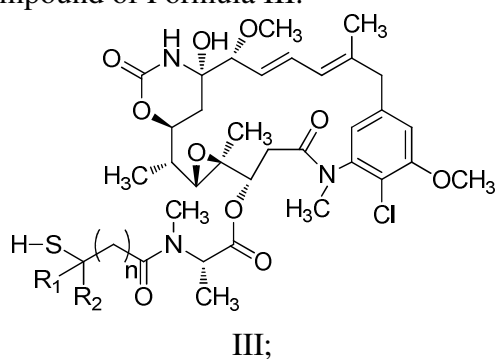
R_1 and R_2 are H; and

n is 1.

[0082p] According to another aspect there is provided a compound prepared by a process comprising the steps of:

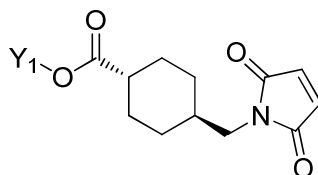
(a) contacting:

(i) a compound of Formula III:



III;

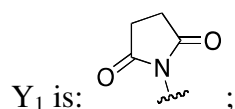
(ii) a compound of formula IV:



IV

(iii) silica gel; and

(iv) a diluent comprising an organic solvent and water;



Y_1 is:

R_1 and R_2 are independently selected from H or alkyl; and

n is independently 0 or an integer from 1 to 50

and

(b) isolating the compound.

[0082q] According to another aspect there is provided an antibody drug conjugate prepared by contacting the compound of step (b) in of the process described herein with an antibody or antigen-binding fragment thereof.

[0083] The above specification, examples and data provide a complete description of the manufacture and use of the composition of the invention. Since many embodiments of the invention can be made without departing from the spirit and scope of the invention, the invention resides in the claims hereinafter appended.

[0084] All references cited herein and in the Examples that follow, including patents, published patent applications, and literature references, are expressly incorporated by reference in their entireties.

[0085] The description and Examples presented infra are provided to illustrate the subject invention. One of skill in the art will recognize these Examples are provided by way of illustration only and are not included for the purpose of limiting the invention.

[0086] Embodiments disclosed herein are illustrated in greater detail by means of the non-limiting examples described below.

BRIEF DESCRIPTION OF THE FIGURES

[0087] Figure 1 illustrates an ¹H-NMR spectrum of Maytansin-3-N-methyl-L-alanine-propanamidyl-3-thio-3-succinimidyl-N-methylcyclohexyl-4-trans-carboxysuccinate (**5**). The ¹H-NMR spectrum is consistent with a single diastereomer present in at least 95% diastereomeric excess since the spectrum is not complicated by resonances attributable to the other diastereomer. (For comparative purposes Example 2 sets forth the ¹H-NMR spectrum of the mixture of diastereomers).

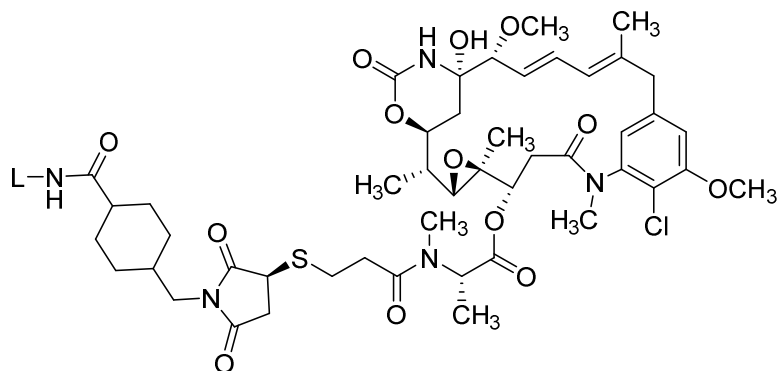
[0088] Figure 2 illustrates an ¹H-NMR spectrum of mixture of diastereomers of Maytansin-3-N-methyl-L-alanine-propanamidyl-3-thio-3-succinimidyl-N-methylcyclohexyl-4-trans-carboxysuccinate (**6**).

[0089] Figure 3 illustrates that in SKBR3 cells the single diastereomer compound conjugate HER2-5 (*in vitro* and *in vivo* lots) possessed an IC₅₀ value of 0.3 nM versus 0.9 nM for the mixture of diastereomer conjugate HER2-6.

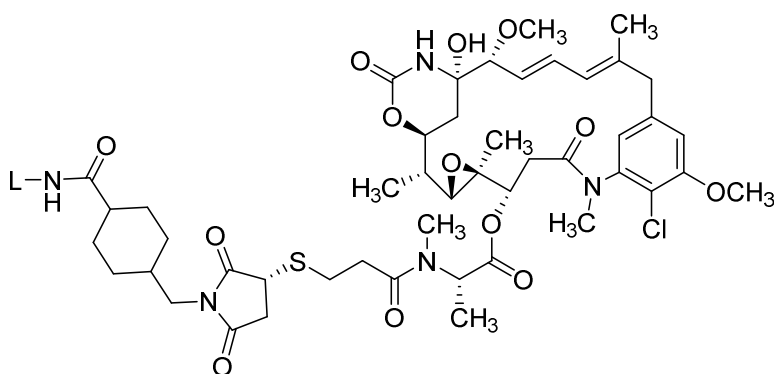
[0090] Figure 4 illustrates that in BT474 cells the single diastereomer compound conjugate HER2-**5** (*in vitro*) in possessed an IC₅₀ value of 4.6 nM while the HER2-**5** (*in vivo*) lot had an IC₅₀ value of 4.0 nM versus 11.6 nM for the mixture of diastereomer conjugate HER2-**6**.

What is claimed is:

1. A composition comprising a compound of Formula IIa or IIb:



IIa



IIb

wherein:

L is an antibody or an antigen-binding fragment thereof;

and wherein the compound of Formula IIa or IIb is present in a diastereomeric excess of greater than 50% and wherein the diastereomers have different stereochemical configurations at the chiral carbon that is bound to the sulfur atom of Formula IIa or IIb, wherein the diastereomeric excess is based on the amounts of at least two diastereomers having different stereochemical configurations at the chiral carbon that is bound to the sulfur atom of Formula IIa or IIb.

2. The composition of claim 1, wherein L is an antibody, or antigen-binding fragment thereof that specifically binds a tumor-associated antigen and further wherein the tumor-associated antigen is selected from the group consisting of AFP, ALK, BAGE proteins,

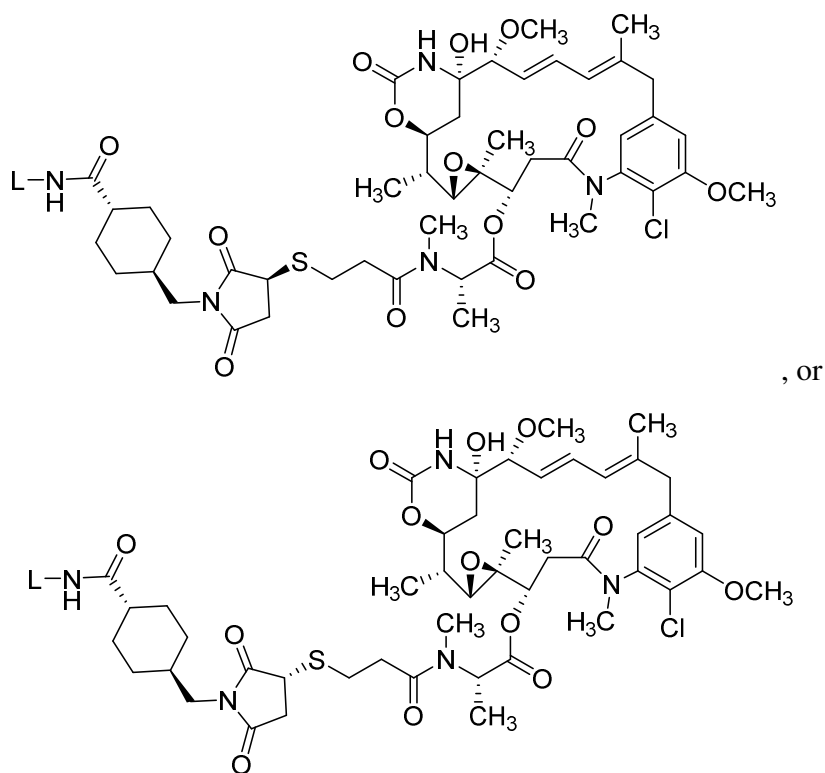
β -catenin, bcr-abl, BRCA1, BORIS, CA9, carbonic anhydrase IX, caspase-8, CD20, CD40, CDK4, CEA, CLEC12A, cMET, CTLA4, cyclin-B1, CYP1B1, EGFR, EGFRvIII, ErbB2/Her2, ErbB3, ErbB4, ETV6-AML, EphA2, Fra-1, FOLR1, GAGE proteins, GD2, GD3, GloboH, glypican-3, GM3, gp100, Her2, HLA/B-raf, HLA/k-ras, HLA/MAGE-A3, hTERT, IGF1R, LGR5, LMP2, MAGE proteins, MART-1, mesothelin, ML-IAP, Muc1, Muc16 or CA-125, MUM1, NA17, NY-BR1, NY-BR62, NY-BR85, NY-ESO1, OX40, p15, p53, PAP, PAX3, PAX5, PCTA-1, PDGFR- α , PDGFR- β , PDGF-A, PDGF-B, PDGF-C, PDGF-D, PLAC1, PRLR, PRAME, PSMA, FOLH1, RAGE proteins, Ras, RGS5, Rho, SART-1, SART-3, Steap-1, Steap-2, survivin, TAG-72, TGF- β , TMPRSS2, Tn, TNFRSF17, TRP-1, TRP-2, tyrosinase, and uroplakin-3.

3. The composition of claim 2, wherein the GAGE proteins are selected from the group consisting of GAGE-1 and GAGE-2.
4. The composition of claim 2 wherein the MAGE proteins are selected from the group consisting of MAGE-1, MAGE-2, MAGE-3, MAGE-4, MAGE-6, and MAGE-12.
5. The composition of claim 2, wherein the tumor-associated antigen is selected from the group consisting of EGFRvIII, Her2, and Muc16.
6. The composition of claim 5, wherein the compound of Formula IIa or IIb is present in a diastereomeric excess of more than 95% and wherein the diastereomers have different stereochemical configurations at the chiral carbon that is bound to the sulfur atom of Formula IIa or IIb, wherein the diastereomeric excess is based on the amounts of at least two diastereomers having different stereochemical configurations at the chiral carbon that is bound to the sulfur atom of Formula IIa or IIb.
7. Use of the composition of claim 1 for the manufacture of a medicament for treating cancer in a patient in need thereof.

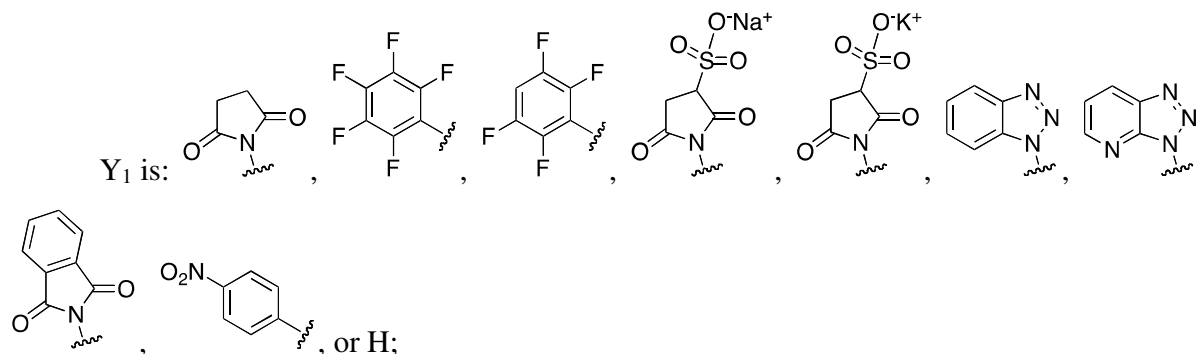
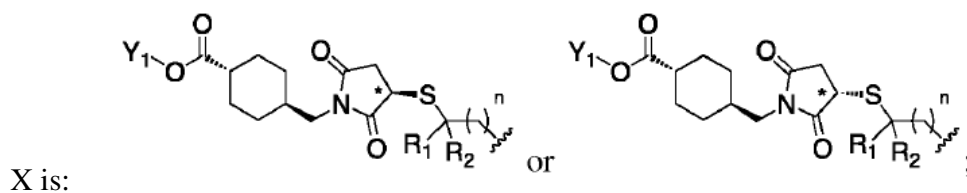
composition comprising a therapeutically effective amount of the compound present in a diastereomeric excess.

11. A pharmaceutical composition comprising the composition of claim 1, further comprising a pharmaceutically acceptable excipient.

12. The pharmaceutical composition of claim 11, wherein the compound of Formula IIa or IIb is:



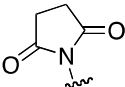
13. The pharmaceutical composition of claim 12, wherein the compound of Formula IIa or IIb is present in a diastereomeric excess of greater than 95% wherein the diastereomers have different stereochemical configurations at the chiral carbon that is bound to the sulfur atom of Formula IIa or IIb, wherein the diastereomeric excess is based on the amounts of at least two diastereomers having different stereochemical configurations at the chiral carbon that is bound to the sulfur atom of Formula IIa or IIb.



R₁ and R₂ are independently selected from H or alkyl; and

n is independently 0 or an integer from 1 to 50;

wherein the compound of Formula I is present in a diastereomeric excess of greater than 50%; and wherein the diastereomers have different stereochemical configurations at the chiral carbon represented by (*) in formula X of Formula I, wherein said chiral carbon atom is a carbon atom that is bound to a sulfur atom, wherein the diastereomeric excess is based on the amounts of at least two diastereomers having different stereochemical configurations at the chiral carbon that is bound to the sulfur atom of Formula I; wherein alkyl is selected from methyl, ethyl, 1-propyl, 2-propyl, n-butyl, isobutyl, sec-butyl, t-butyl, pentyl, neopentyl, hexyl, and isohexyl.

17. The composition of claim 16, wherein Y₁ is .

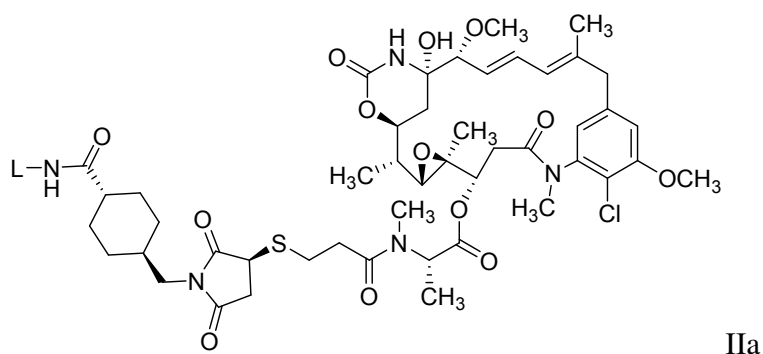
18. The composition of claim 16 or 17, wherein R₁ and R₂ are H.

19. The composition of any of claims 16-18, wherein n is an integer from 1 to 10.

23. The composition of any of claims 16-22, wherein the diastereomeric excess is at least 95% and wherein the diastereomers have different stereochemical configurations at the chiral carbon represented by (*) in formula X of Formula I, wherein said chiral carbon atom is a carbon atom that is bound to a sulfur atom, wherein the diastereomeric excess is based on the amounts of at least two diastereomers having different stereochemical configurations at the chiral carbon that is bound to the sulfur atom of Formula I.

24. The composition of claim 16, wherein the compound of Formula I is characterized by a ¹HNMR spectrum substantially as shown in Figure 1.

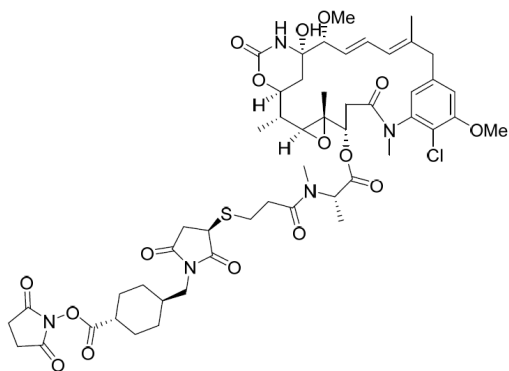
25. An antibody-drug conjugate



wherein:

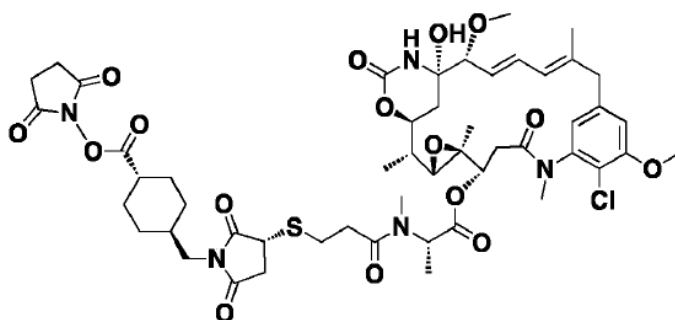
L is an antibody or an antigen-binding fragment thereof; and

wherein the antibody-drug conjugate is made by combining a compound of Formula (I) having the following structure:



with an antibody or antigen-binding fragment thereof.

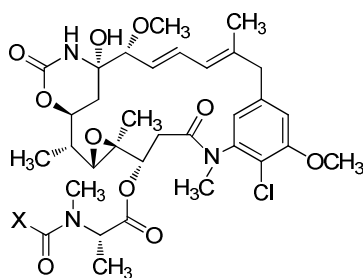
28. A compound of formula (ii)



(ii)

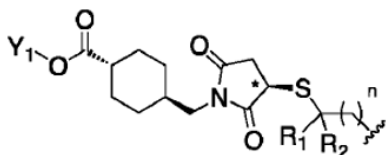
wherein the compound is stereoisomerically pure.

29. A process for preparing a compound of Formula I:

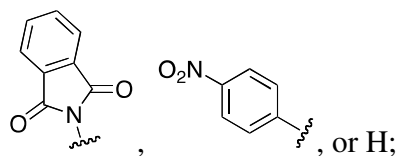
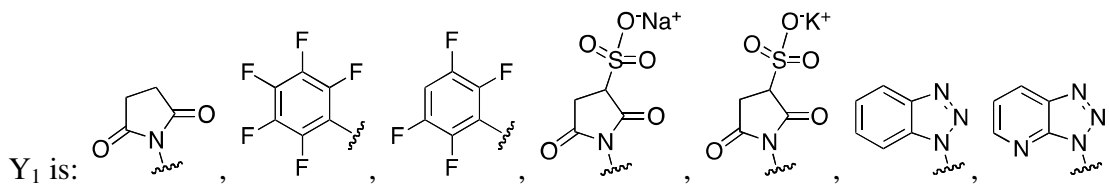


I

wherein:



X is:

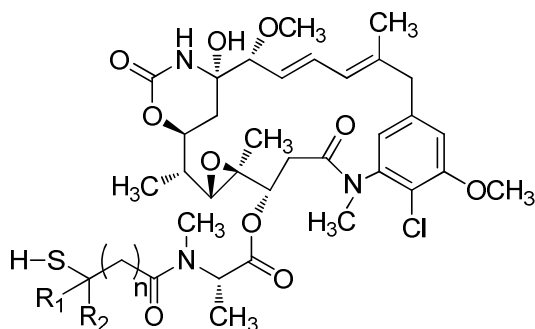


R₁ and R₂ are independently selected from H or alkyl; and

n is independently 0 or an integer from 1 to 50;
 comprising the steps of

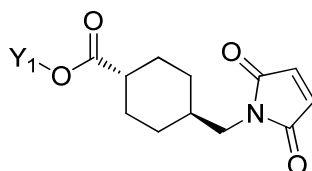
(a) contacting:

(i) a compound of Formula III:



III;

(ii) a compound of formula IV:



IV

(iii) silica gel; and

(iv) a diluent comprising an organic solvent and water; and

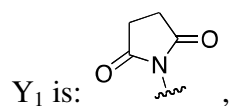
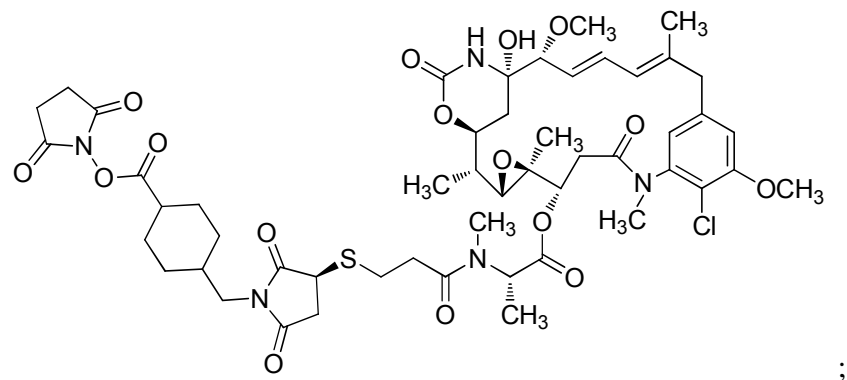
(b) isolating the compound of Formula I, wherein the compound of Formula I is present in a diastereomeric excess of greater than 50% and wherein the diastereomers have different stereochemical configurations at the chiral carbon represented by (*) in formula X of Formula I, wherein said chiral carbon atom is a carbon atom that is bound to a sulfur atom, wherein the diastereomeric excess is based on the amounts of at least two diastereomers having different stereochemical configurations at the chiral carbon that is bound to the sulfur atom of Formula I.

30. The process of claim 29, wherein the organic solvent comprises a polar aprotic solvent.

31. The process of claim 30, wherein the polar aprotic solvent comprises acetonitrile.

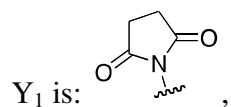
32. The process of claim 29, wherein the organic solvent and the water are present in volume ratio from about 1:1 to about 4:1 or from about 1:1 to about 10:1.

50. The process of any one of claims 29-49, wherein the compound of Formula I is:



R₁ and R₂ are H, and
n is 1.

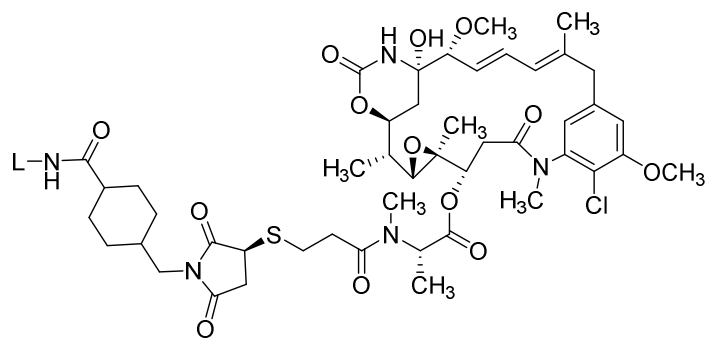
51. The process of any one of claims 29-50, wherein



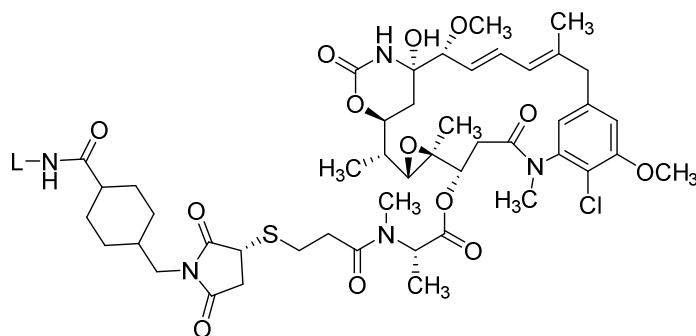
R₁ and R₂ are H,
n is 1; and

55. The process of claim 52, wherein the tumor-associated antigen is selected from the group consisting of EGFRvIII, Her2, and Muc16.

56. A process for preparing a compound of Formula IIa or IIb:



IIa



IIb

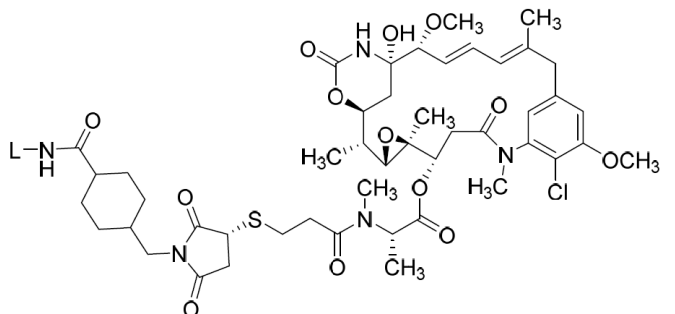
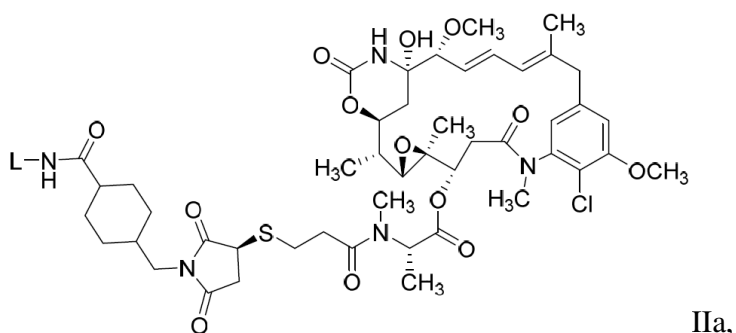
wherein L is an antibody or an antigen-binding fragment thereof; and
 wherein the compound of Formula IIa or IIb is present in a diastereomeric excess of greater than 50%, wherein the diastereomers have different stereochemical configurations at the chiral carbon that is bound to the sulfur atom of Formula IIa or IIb, and wherein the diastereomeric excess is based on the amounts of at least two diastereomers having different stereochemical configurations at the chiral carbon that is bound to the sulfur atom of Formula IIa or IIb, comprising:

PRAME, PSMA, FOLH1, RAGE proteins, Ras, RGS5, Rho, SART-1, SART-3, Steap-1, Steap-2, survivin, TAG-72, TGF- β , TMPRSS2, Tn, TNFRSF17, TRP-1, TRP-2, tyrosinase, and uroplakin-3.

58. The process of claim 57, wherein the GAGE proteins are selected from the group consisting of GAGE-1 and GAGE-2.
59. The process of claim 57, wherein the MAGE proteins are selected from the group consisting of MAGE-1, MAGE-2, MAGE-3, MAGE-4, MAGE-6, and MAGE-12.
60. The process of claim 57, wherein the tumor-associated antigen is selected from the group consisting of EGFRvIII, Her2, and Muc16.
61. The process of claim 56, wherein the compound of Formula IIa or IIb is present in a diastereomeric excess of more than 95% and wherein the diastereomers have different stereochemical configurations at the chiral carbon that is bound to the sulfur atom of Formula IIa or IIb, and wherein the diastereomeric excess is based on the amounts of at least two diastereomers having different stereochemical configurations at the chiral carbon that is bound to the sulfur atom of Formula IIa or IIb.
62. The process of claim 56, wherein the compound of Formula I is present in a diastereomeric excess of more than 95% and wherein the diastereomers have different stereochemical configurations at the chiral carbon represented by (*) in formula X of Formula I, wherein said chiral carbon atom is a carbon atom that is bound to a sulfur atom, and wherein the diastereomeric excess is based on the amounts of at least two diastereomers having different stereochemical configurations at the chiral carbon that is bound to the sulfur atom of Formula I.

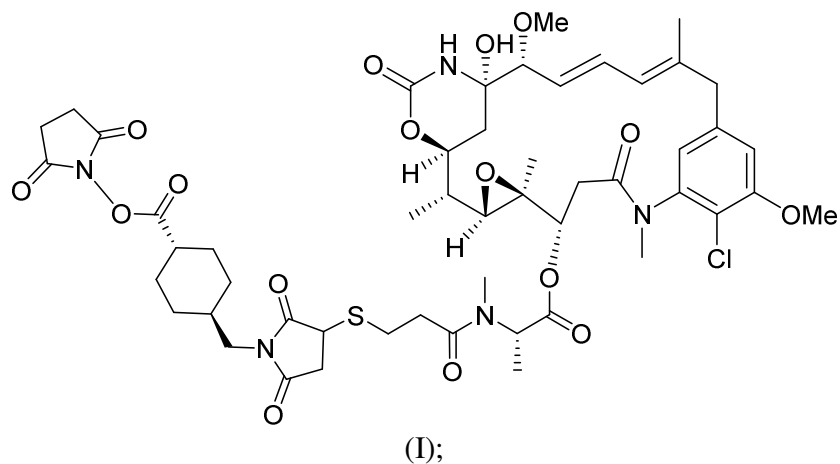
wherein the compound of Formula I is present in a diastereomeric excess of greater than 50% and wherein the diastereomers have different stereochemical configurations at the chiral carbon represented by (*) in formula X of Formula I, wherein said chiral carbon atom is a carbon atom that is bound to a sulfur atom, and wherein the diastereomeric excess is based on the amounts of at least two diastereomers having different stereochemical configurations at the chiral carbon that is bound to the sulfur atom of Formula I.

73. A compound of Formula IIa or IIb:



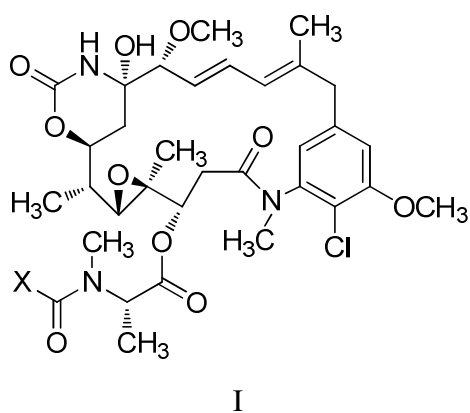
wherein: L is an antibody or an antigen-binding fragment thereof; and wherein the compound of Formula IIa or IIb is present in a diastereomeric excess of greater than 50% and wherein the diastereomers have different stereochemical configurations at the chiral carbon that is bound to the sulfur atom of Formula IIa or IIb, and wherein the diastereomeric excess is based on the amounts of at least two diastereomers having different stereochemical configurations at the chiral carbon that is bound to the sulfur atom of Formula IIa or IIb; prepared by the process of any one of claims of 56-71.

74. A composition comprising a compound of Formula I:

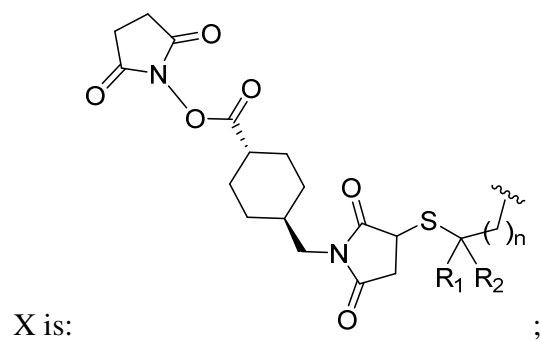


wherein the compound of Formula I is characterized by a ^1H NMR substantially as shown in Figure 1.

75. A diastereomer of a compound of Formula I characterized by a ^1H NMR substantially as shown in Figure 1:



wherein:



n is 1.

(a) contacting:

III;

O=C1C=CC(=O)N1C[C@H]2CCCC[C@@H]2C(=O)OY1

IV

(iv) a diluent comprising an organic solvent and water;

Y₁ is:  ;

n is independently 0 or an integer from 1 to 50

and

77. An antibody drug conjugate prepared by contacting the compound of step (b) in claim 76 with an antibody or antigen-binding fragment thereof.

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