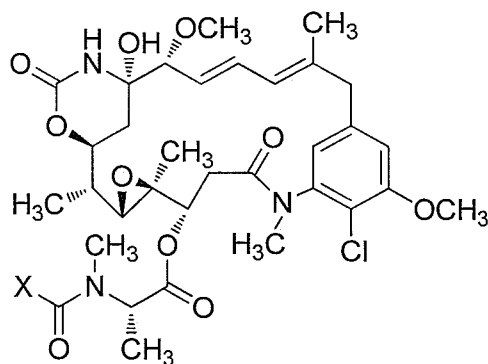


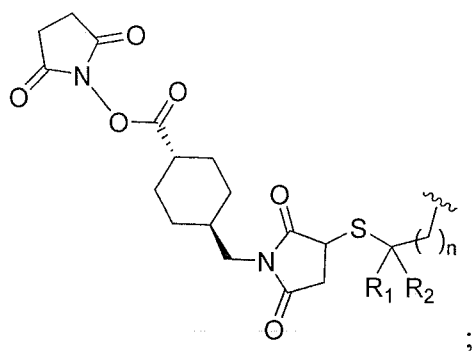
the diastereomers have different stereochemical configurations at the chiral carbon that is bound to the sulfur atom of Formula IIa or IIb, and wherein the diastereomeric excess is based on the amounts of at least two diastereomers having different stereochemical configurations at the chiral carbon that 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 diastereomer of a compound of Formula I characterized by a ^1H NMR substantially as shown in Figure 1:



I

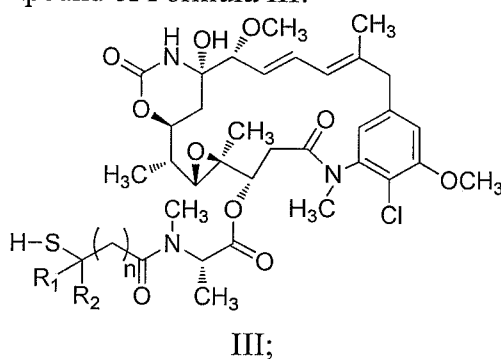
wherein:



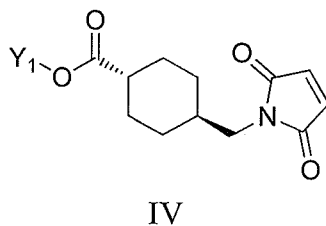
R_1 and R_2 are H; and
 n is 1.

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

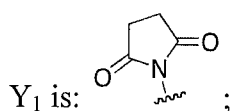
- (a) contacting:
 (i) a compound of Formula III:



- (ii) a compound of formula IV:



- (iii) silica gel; and
 (iv) a diluent comprising an organic solvent and water;



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.

[0082p] 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.

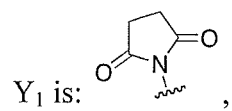
BRIEF DESCRIPTION OF THE FIGURES

[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.

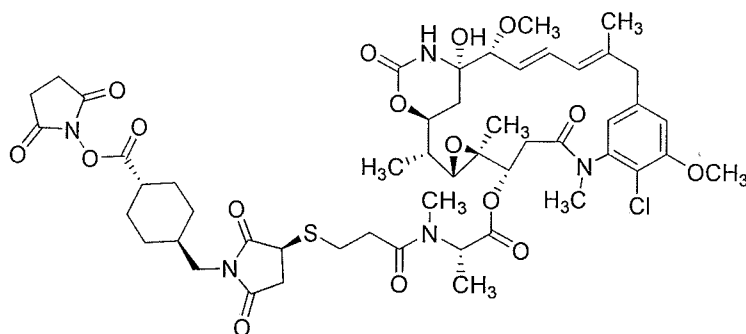
50. The process of any one of claims 29-49, wherein



R₁ and R₂ are H,

n is 1; and

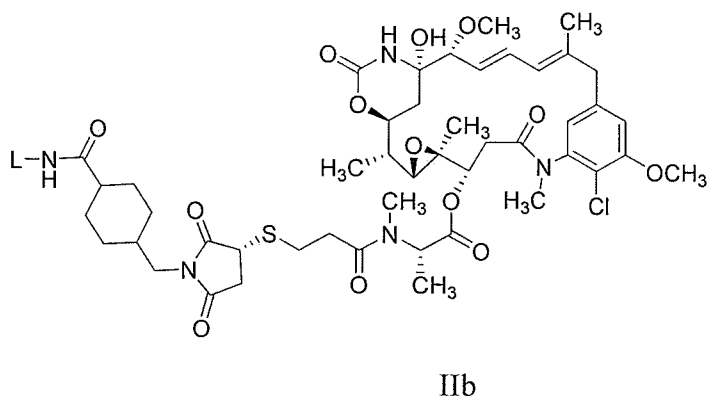
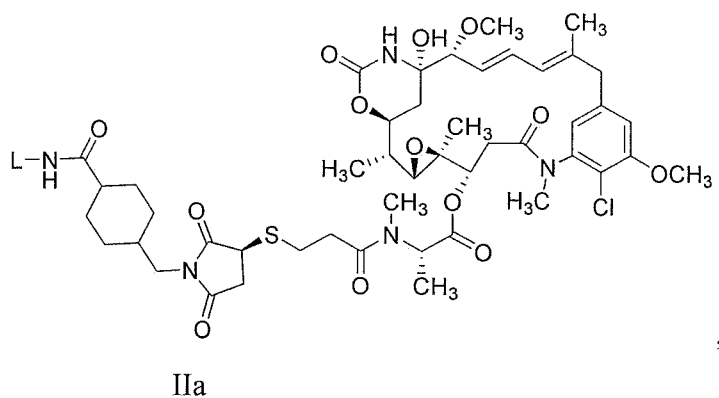
the compound of Formula I is:



51. The process of claim 48 or 49, wherein the 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.
52. The process of claim 51, wherein the GAGE proteins are selected from the group consisting of GAGE-1 and GAGE-2.
53. The process of claim 51, wherein the MAGE proteins are selected from the group consisting of MAGE-1, MAGE-2, MAGE-3, MAGE-4, MAGE-6, and MAGE-12.

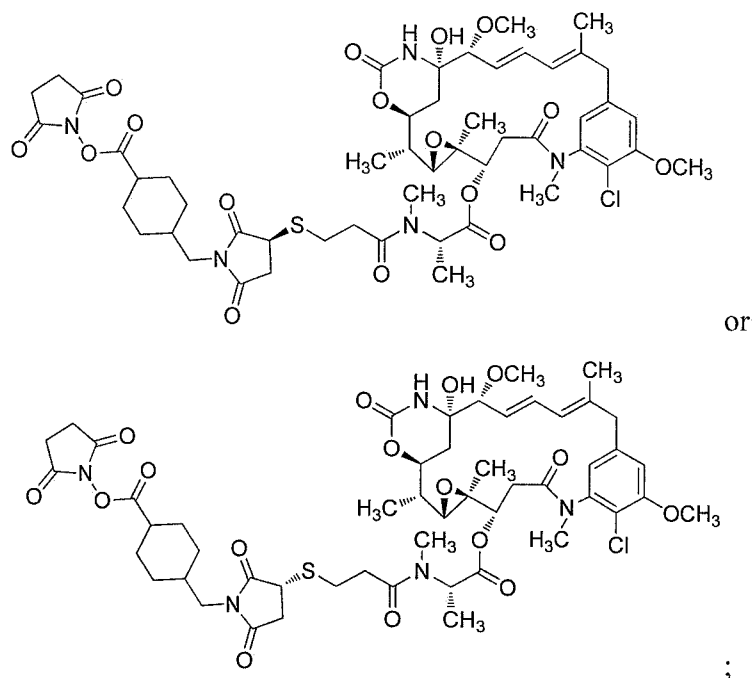
54. The process of claim 51, wherein the tumor-associated antigen is selected from the group consisting of EGFRvIII, Her2, and Muc16.

55. A process for preparing 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%, 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

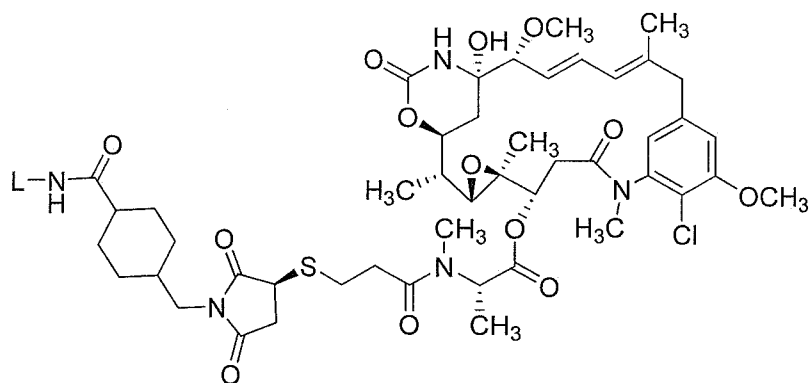
(b) purifying the compound of Formula IIa or IIb.

56. The process of claim 55, 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 (*e.g.*, MAGE-1, -2, -3, -4, -6, and -12), 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,

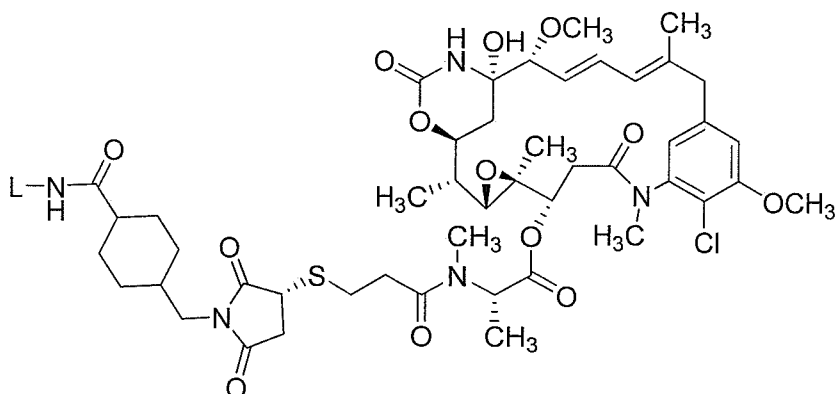
PRAME, PSMA, FOLH1, RAGE proteins, Ras, RGS5, Rho, SART-1, SART-3, Steap-1, Steap-2, survivin, TAG-72, TGF- β , TMPRSS2, Tn, TNFRSF17, TRP-1, TRP-2, tyrosinase, and uroplakin-3.

57. The process of claim 56, wherein the GAGE proteins are selected from the group consisting of GAGE-1 and GAGE-2.
58. The process of claim 56, wherein the MAGE proteins are selected from the group consisting of MAGE-1, MAGE-2, MAGE-3, MAGE-4, MAGE-6, and MAGE-12.
59. The process of claim 56, wherein the tumor-associated antigen is selected from the group consisting of EGFRvIII, Her2, and Muc16.
60. The process of claim 55, 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.
61. The process of claim 55, 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.

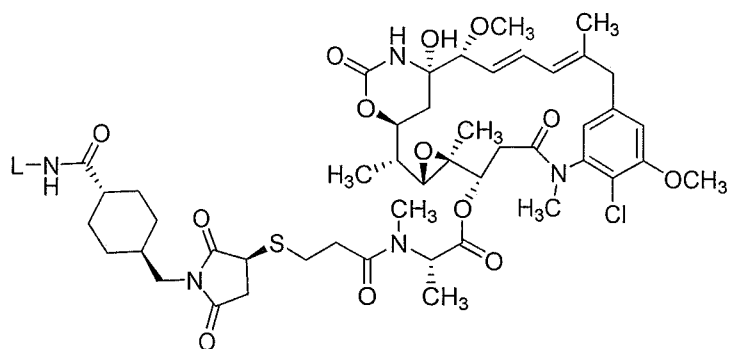
62. The process of claim 55, wherein the compound present in a diastereomeric excess is a compound of Formula IIa having the following structure:



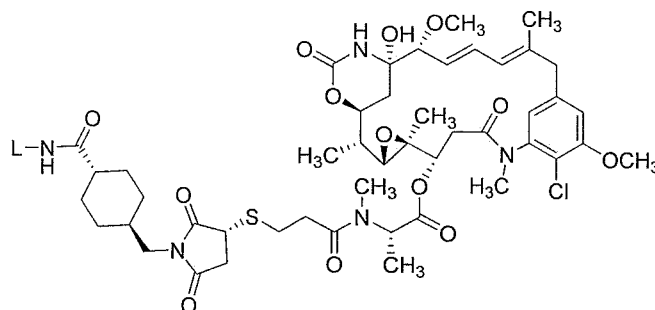
63. The process of claim 55, wherein the compound present in a diastereomeric excess is a compound of Formula IIb having the following structure:



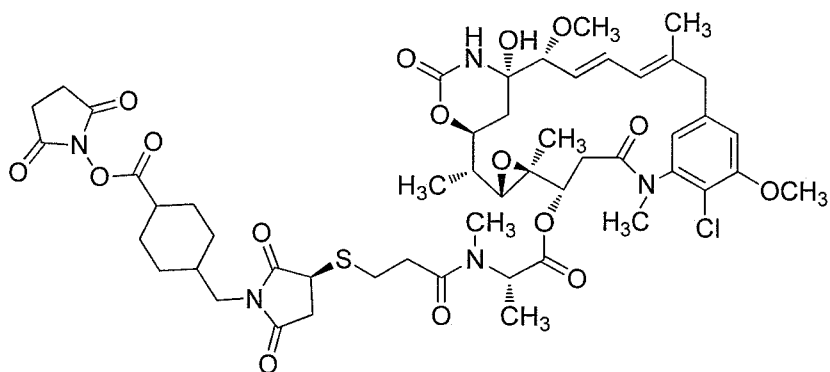
64. The process of claim 55, wherein the compound present in a diastereomeric excess is a compound of Formula IIa having the following structure:



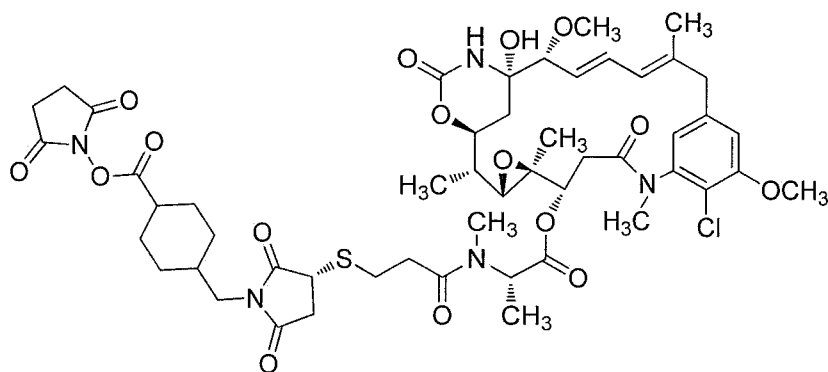
65. The process of claim 55, wherein the compound present in a diastereomeric excess is a compound of Formula IIb having the following structure:



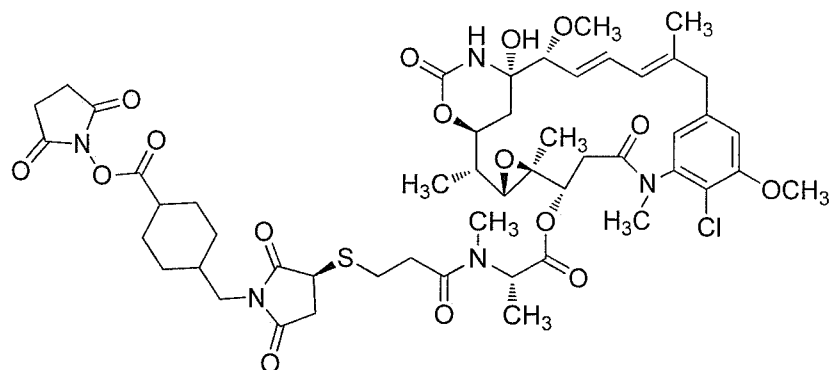
66. The process of claim 55, wherein the compound of Formula I is



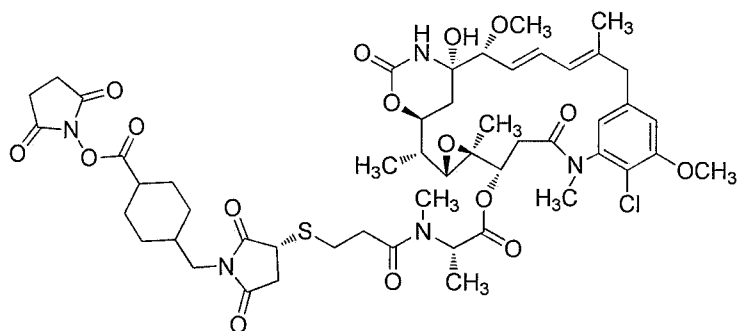
or



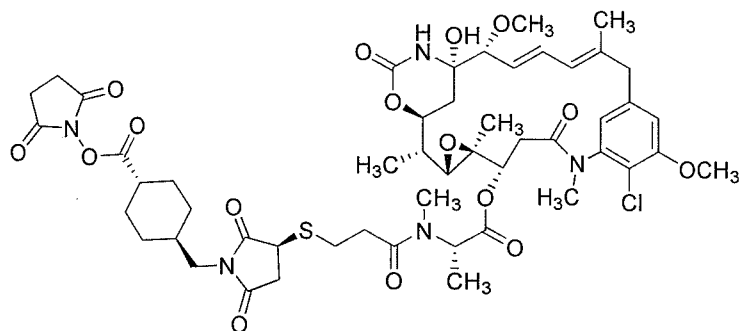
67. The process of claim 55, wherein the compound of Formula I is:



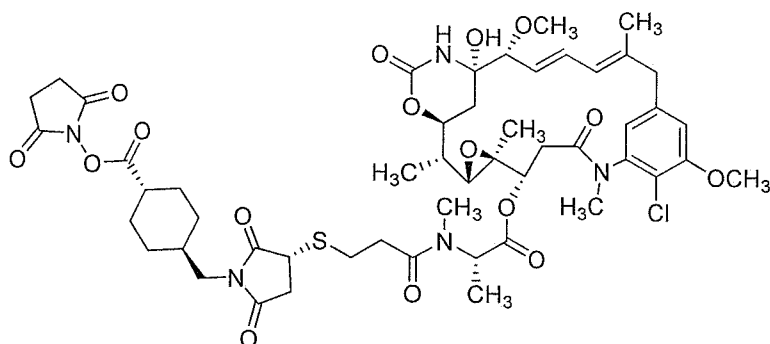
68. The process of claim 55, wherein the compound of Formula I is:



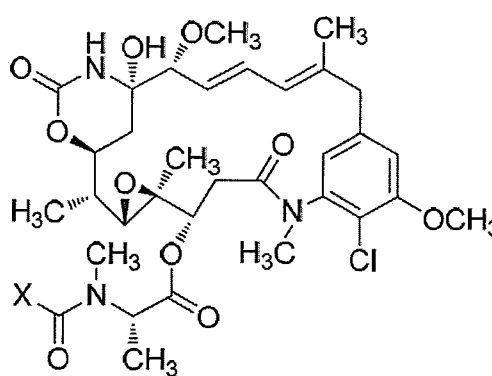
69. The process of claim 55, wherein the compound of Formula I is:



70. The process of claim 55, wherein the compound of Formula I is:

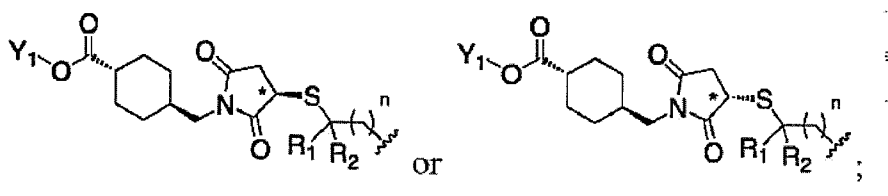


71. A compound of Formula I:

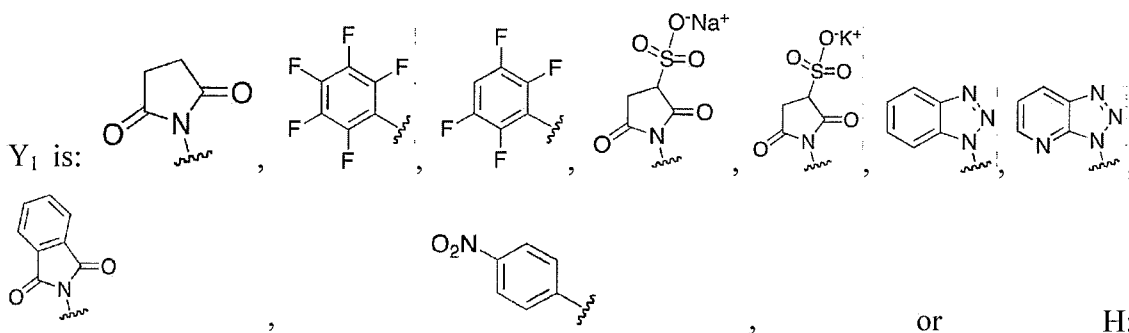


I;

wherein:



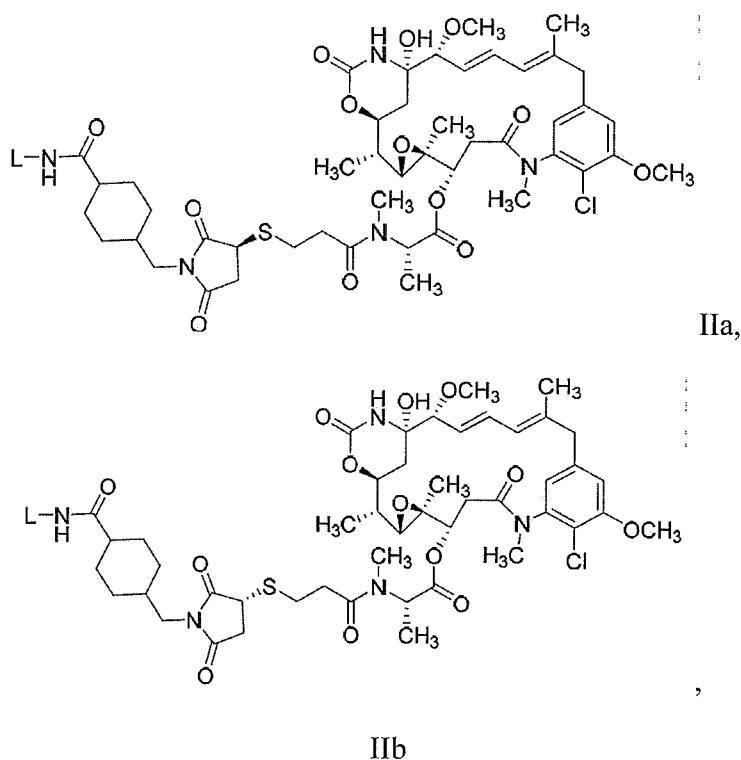
X is



R_1 and R_2 are independently selected from H or alkyl; and n is independently 0 or an integer from 1 to 50; prepared by the process of any one of claims of 29-47, or 50

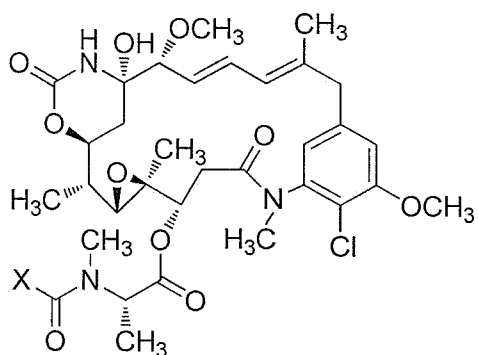
wherein the compound of Formula I is present in a diastereomeric excess of greater than 50% and wherein the diastereomers have different stereochemical configurations at the chiral carbon represented by (*) in formula X of Formula I, wherein said chiral carbon atom is a carbon atom that is bound to a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

72. A compound of Formula IIa or IIb:



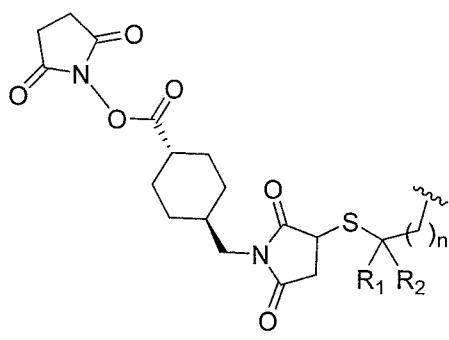
wherein: L is an antibody or an antigen-binding fragment thereof; and wherein the compound of Formula IIa or IIb is present in a diastereomeric excess of greater than 50% and wherein the diastereomers have different stereochemical configurations at the chiral carbon that is bound to the sulfur atom of 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 55-70.

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



I

wherein:



X is:

;

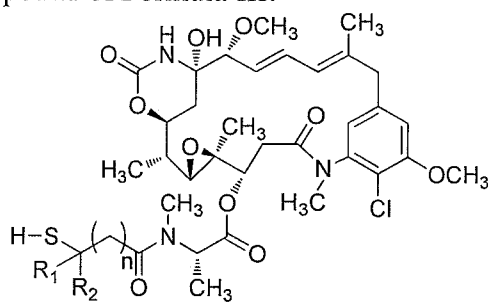
R₁ and R₂ are H; and

n is 1.

74. A compound prepared by a process comprising the steps of:

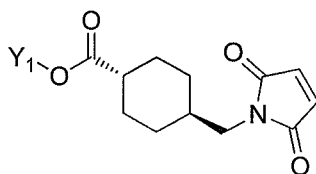
(a) contacting:

(i) a compound of Formula III:



III;

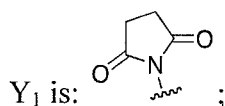
(ii) a compound of formula IV:



IV

(iii) silica gel; and

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



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

n is independently 0 or an integer from 1 to 50

and

(b) isolating the compound.

75. An antibody drug conjugate prepared by contacting the compound of step (b) in claim 74 with an antibody or antigen-binding fragment thereof.

76. The compound of claim 74, wherein the compound in step (b) is characterized by a ¹H NMR substantially as shown in Figure 1.