

**PHARMACEUTICAL COMPOSITIONS COMPRISING MACROLIDE
DIASTEREOMERS, METHODS OF THEIR SYNTHESIS AND
THERAPEUTIC USES**

[0001] Proliferative diseases are characterized by uncontrolled growth and spread of abnormal cells. If the spread of these cells is not controlled, it can result in death. Proliferative diseases, for example cancer, can be treated by surgery, radiation, chemotherapy, hormone-based therapy and/or immunotherapy. A number of these treatments, particularly the chemotherapy, are based on the use of anti-proliferative drugs which limit the spread of the abnormal cells. Anti-proliferative drugs, however, typically are indiscriminate in their ability to kill cells, affecting both normal and abnormal cells based simply on whether a cell is replicating. Regardless, most anti-proliferative drugs require a relatively high concentration at the locale of the abnormal cell proliferation to be effective. It is this combination of providing sufficient anti-proliferative drug at a site of abnormal cell growth without also causing significant death to normal cells systemically or in the vicinity of these cells that this disclosure addresses.

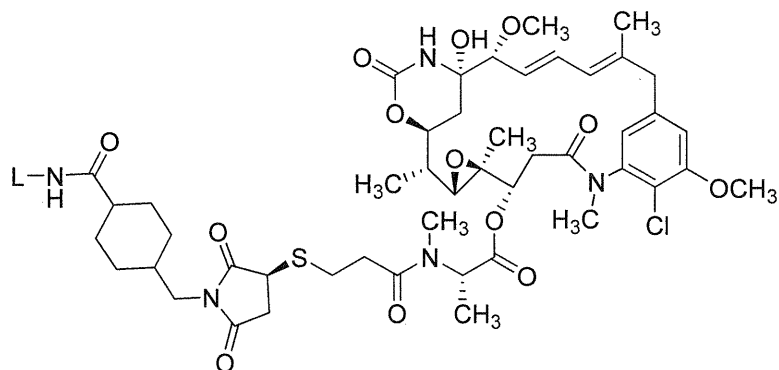
[0002] Various approaches to targeted drug delivery have been tried, including the use of conjugates of tumor targeted probes (such as antibodies or growth factors) with toxins such as pseudomonas or diphtheria toxins. Conjugates for use in the treatment of cancer thereby target the anti-proliferative drug to a population of abnormal cells. Recently, conjugates that include the toxin maytansine have been employed for the treatment of cancer. Maytansine has shown great effectiveness as an anti-proliferative agent but the compound's toxicity has proven problematic toward normal cells. A need exists for developing maytansine based conjugates that have sufficient activity for use as cancer therapeutics. The more active or effective the maytansine based conjugate is at inhibiting or killing a population of abnormal cells the lower the concentration of the conjugate is required, the benefit being a reduced overall risk of damaging normal cells.

[0002a] Summary: The present invention refers to subject matter as defined in the attached set of claims.

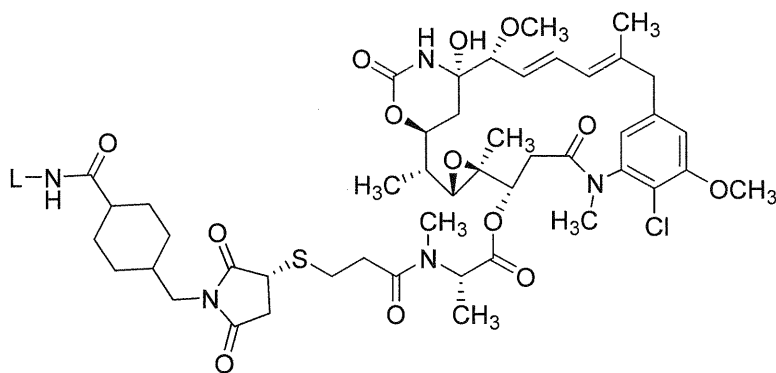
[0003] Many anti-proliferative compounds exhibit asymmetric structures, such as the Maytansinoid family of macrolides, and may therefore exist in the form of a racemic mixture, in the form of separate enantiomers with configuration "R" and "S", or (+) and (-), per stereogenic center, and various diastereomers. The present disclosure shows that targeted delivery of a single maytansinoid diastereomer exhibits improved inhibitory cell proliferation profile as compared to delivery of its respective mixture of diastereomers. Therefore,

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;

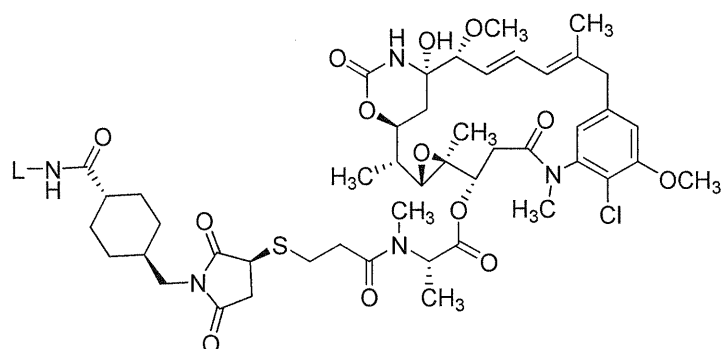
and wherein the compound of Formula IIa or IIb is present in a diastereomeric excess of greater than 50%.

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,

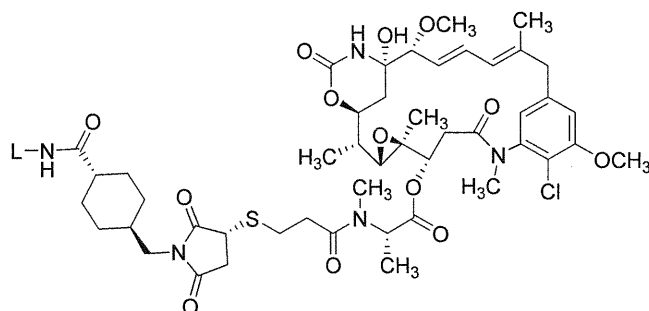
CD40, CDK4, CEA, CLEC12A, cMET, CTLA4, cyclin-B1, CYP1B1, EGFR, EGFRvIII, ErbB2/Her2, ErbB3, ErbB4, ETV6-AML, EphA2, Fra-1, FOLR1, GAGE proteins, GD2, GD3, GloboH, glypican-3, GM3, gp100, Her2, HLA/B-raf, HLA/k-ras, HLA/MAGE-A3, hTERT, IGF1R, LGR5, LMP2, MAGE proteins, MART-1, mesothelin, ML-IAP, Muc1, 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%.
7. Use of the composition of claim 1 for the manufacture of a medicament for treating cancer in a patient in need thereof.

8. The composition of any of claims 1-6, wherein the compound present in a diastereomeric excess is a compound of Formula IIa having the following structure:



9. The composition of any of claims 1-6, wherein the compound present in a diastereomeric excess is a compound of Formula IIb having the following structure:

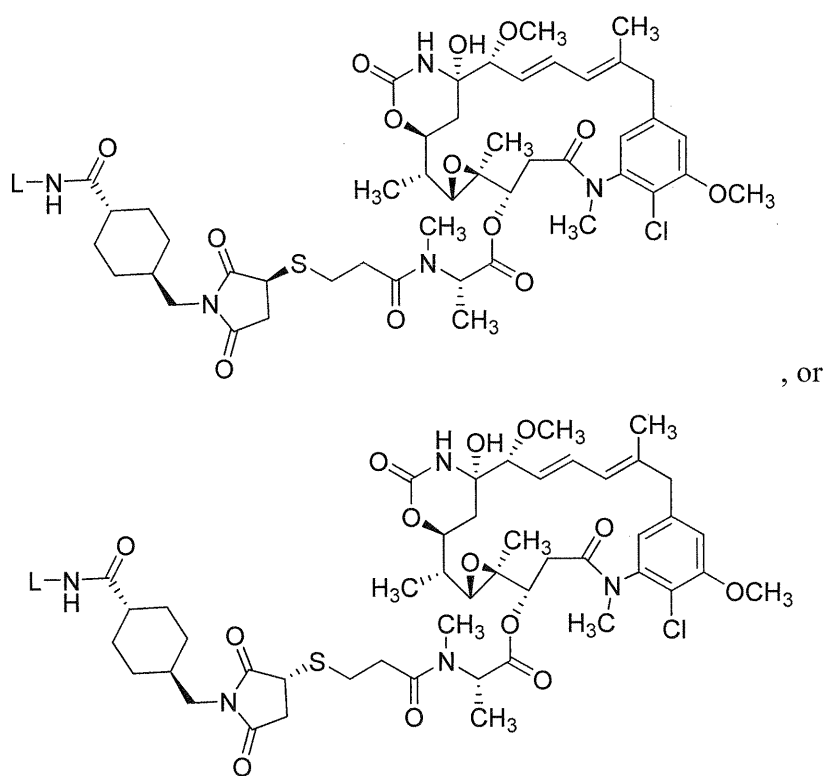


10. Use of the composition of claim 1 in the manufacture of a medicament for treating a disease or disorder associated with a tumor-associated antigen, wherein the tumor-associated antigen is selected from the group consisting of AFP, ALK, BAGE proteins, β -catenin, bcr-abl, BRCA1, BORIS, CA9, carbonic 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.

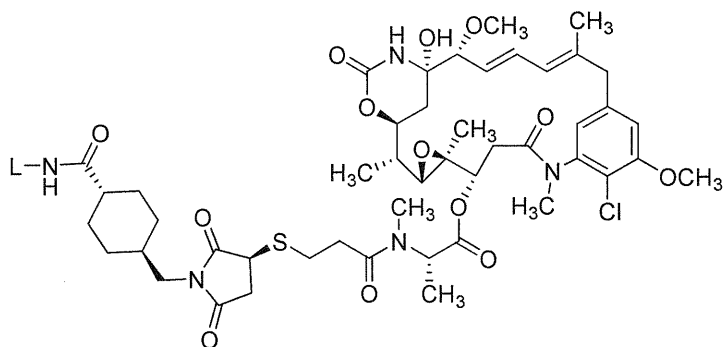
11. A pharmaceutical composition comprising the composition of claim 1, further comprising a pharmaceutically acceptable excipient.

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

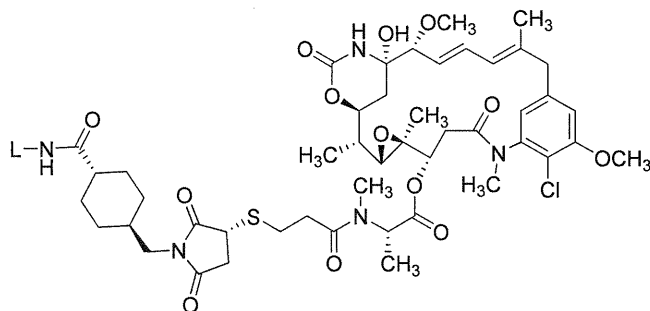


13. The pharmaceutical composition of claim 12, wherein the compound of Formula IIa or IIb is present in a diastereomeric excess of greater than 95%.

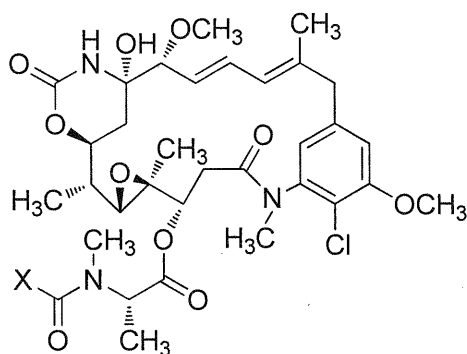
14. The pharmaceutical composition of claim 11, wherein the compound present in a diastereomeric excess is a compound of Formula IIa having the following structure:



15. The pharmaceutical composition of claim 11, wherein the compound present in a diastereomeric excess is a compound of Formula IIb having the following structure:



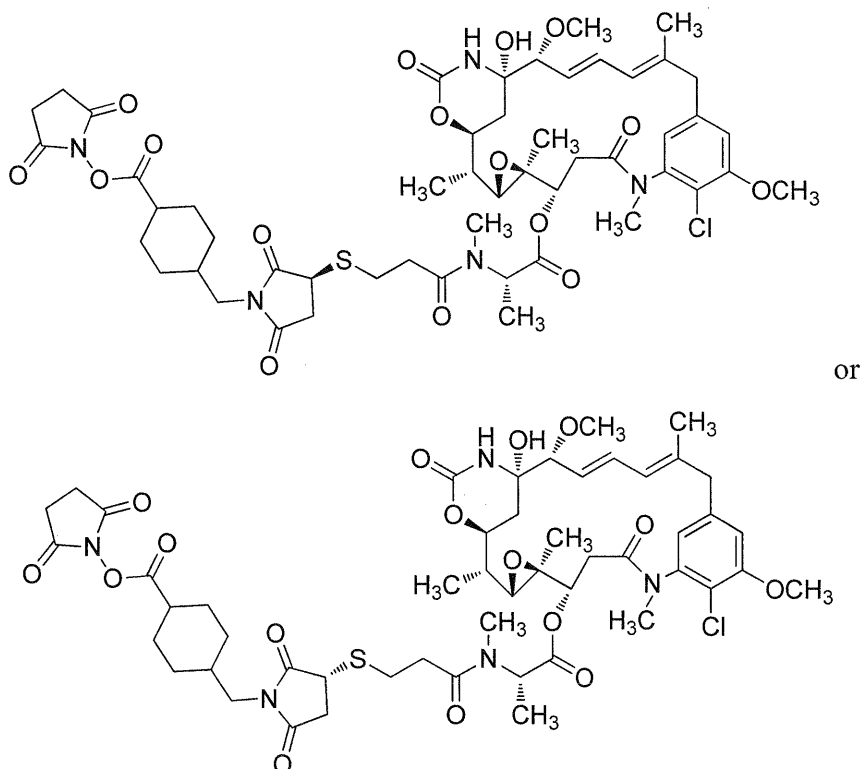
16. A composition comprising a compound of Formula I:



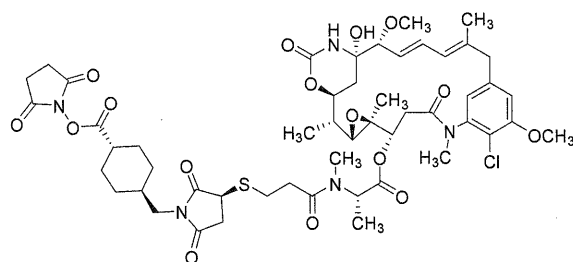
I

wherein:

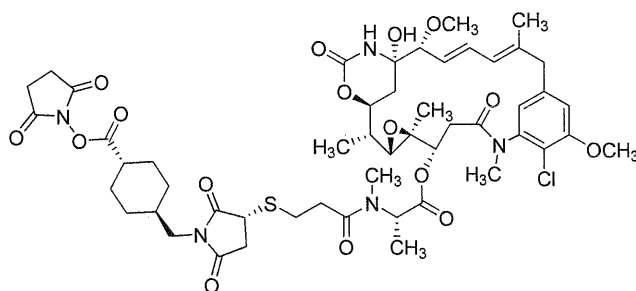
20. The composition of any of claim 16-19, wherein the compound of Formula I is



21. The composition of claim 20, wherein the compound of Formula I is:

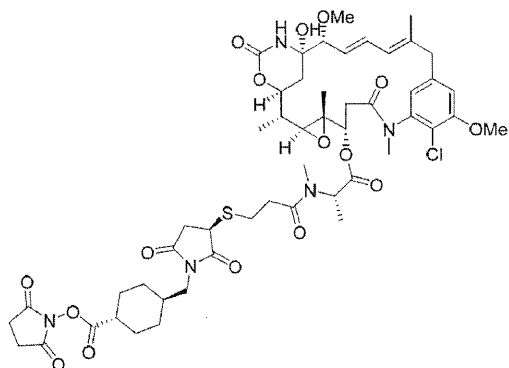


22. The composition of claim 20, wherein the compound of Formula I is:



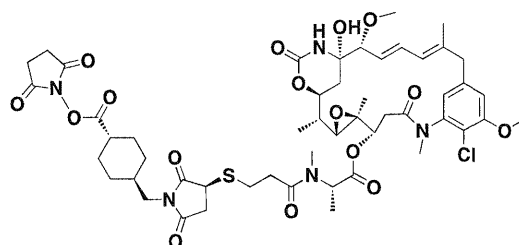
23. The composition of any of claims 16-22, wherein the diastereomeric excess is at least 95%.

24. An antibody-drug conjugate 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.

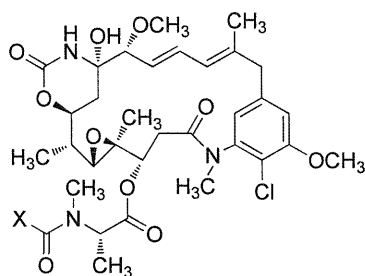
25. A compound of Formula (i):



(i)

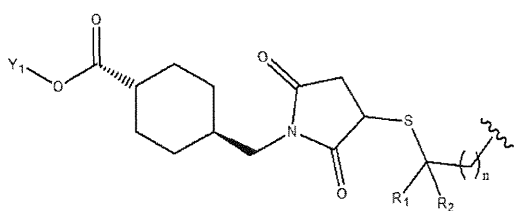
wherein the compound is stereomerically pure.

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



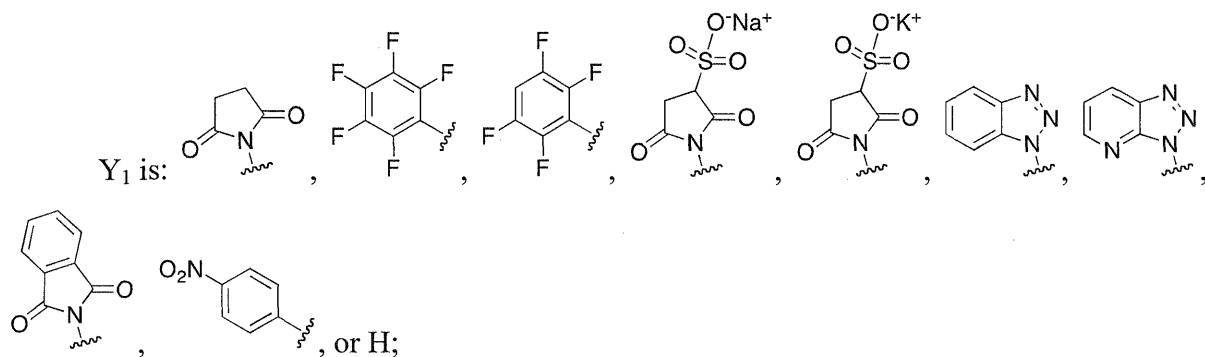
I

wherein:



X is:

or;



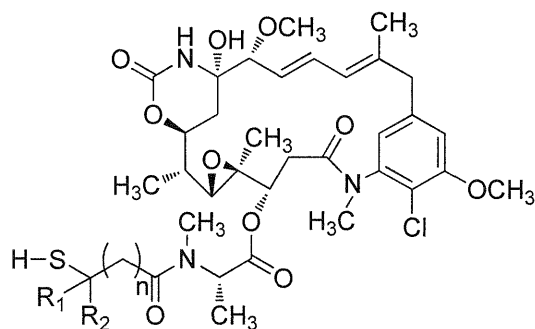
R₁ and R₂ are independently selected from H or alkyl; and

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

comprising the steps of

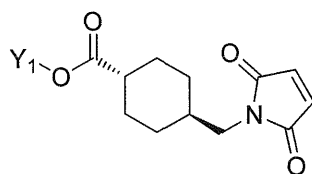
(a) contacting:

(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%.

27. The process of claim 26, wherein the organic solvent comprises a polar aprotic solvent.

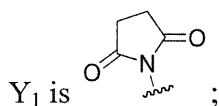
28. The process of claim 27, wherein the polar aprotic solvent comprises acetonitrile.

29. The process of claim 26, 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.

30. The process of claim 26, wherein the molar ratio of the compound of Formula III and the compound of formula IV is from about 1:1 to about 1:3.

31. The process of claim 26, wherein n is 1, and R₁ and R₂ are each independently hydrogen.

32. The process of claim 26, wherein:



R₁ and R₂ are H; and

n is 1.

33. The process of any of claims 26-32, wherein the contacting is performed under argon.

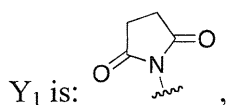
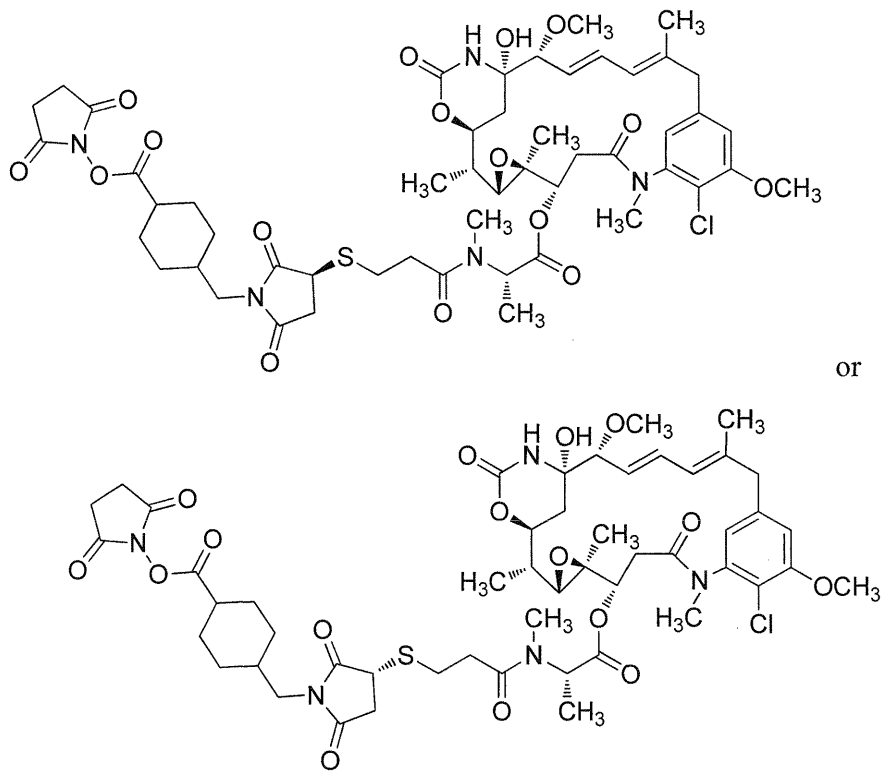
34. The process of any of claims 26-33, wherein components (i)-(v) are stirred.

35. The process of any of claims 26-34, wherein the contacting is performed at ambient temperature.

36. The process of any of claims 26-35, wherein components (i)-(v) are contacted for about 18 hours.
37. The process of any of claims 26-36, wherein the compound of Formula IV is in stoichiometric excess with respect to the compound of Formula III.
38. The process of any of claims 26-37, further comprising contacting an additional amount of a compound of Formula IV after step (a).
39. The process of any of claims 26-38, wherein the molar ratio of a compound of Formula IV to the compound of Formula III is about 2.3:1 in step (a).
40. The process of claim 26, wherein the molar ratio of the compound of Formula IV to the compound of Formula III is about 1.5:1 in step (a).
41. The process of any of claims 26-36, wherein the isolating of the compound of Formula I in step (b) is accomplished by filtration.
42. The process of claim 41, wherein the filtration is filtration through celite.
43. The process of any of claims 26-42, further comprising purifying the compound of Formula I by column chromatography on silica gel.
44. The process of claim 43 further comprising drying the compound of Formula I.
45. The process of any one of claims 26-44, further comprising combining the compound of Formula I with an antibody or antigen-binding fragment thereof to make an antibody-drug conjugate.

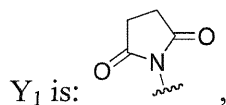
46. The process of any one of claims 45, wherein the compound of Formula I is attached to the antibody or antigen-binding fragment via an S, O, or NR₃; wherein R₃ is H or alkyl.

47. The process of any one of claims 26-46, wherein the compound of Formula I is:



R_1 and R_2 are H, and
n is 1.

48. The process of any one of claims 26-47, wherein



R_1 and R_2 are H,
n is 1; and

COC1=CC=C(C=C1C(=O)N(C)C)CC(C)=C/C=C/[C@H](O)N(C(=O)O[C@H]2C[C@@H](C)[C@H](OC(=O)N3C(=O)CCC3=O)[C@H](OC(=O)N4C(=O)CCC4=O)C2)C

Y₁ is:


O=C1CC(=O)N1

n is 1; and

COC1=CC=C(C=C1C(=O)N(C)C(C)C(=O)NCCSC2CC(=O)N(C2)CC3CCCCC3C(=O)ON4C(=O)CCC4=O)C/C=C/C(=C/C(=O)N[C@@H]5C[C@H](OC)[C@H](C)[C@H]5O)C

50

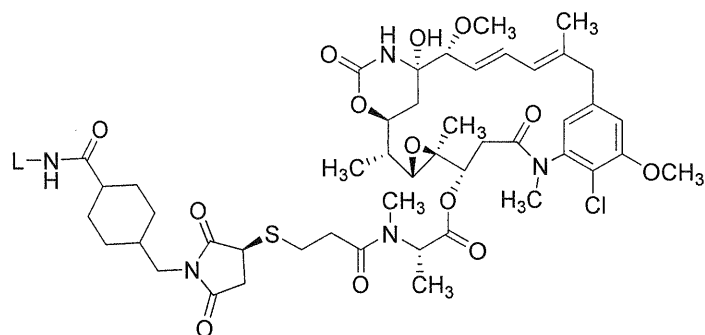
A, PDGF-B, PDGF-C, PDGF-D, PLAC1, PRLR, PRAME, PSMA, FOLH1, RAGE proteins, Ras, RGS5, Rho, SART-1, SART-3, Steap-1, Steap-2, survivin, TAG-72, TGF- β , TMPRSS2, Tn, TNFRSF17, TRP-1, TRP-2, tyrosinase, and uroplakin-3.

51. The process of claim 50, wherein the GAGE proteins are selected from the group consisting of GAGE-1 and GAGE-2.

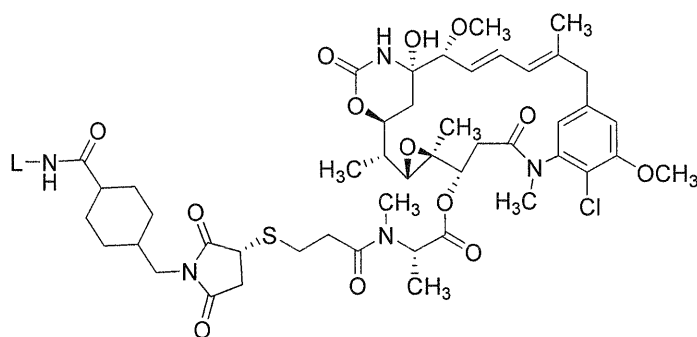
52. The process of claim 50, wherein the MAGE proteins are selected from the group consisting of MAGE-1, MAGE-2, MAGE-3, MAGE-4, MAGE-6, and MAGE-12.

53. The process of claim 50, wherein the tumor-associated antigen is selected from the group consisting of EGFRvIII, Her2, and Muc16.

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



IIa

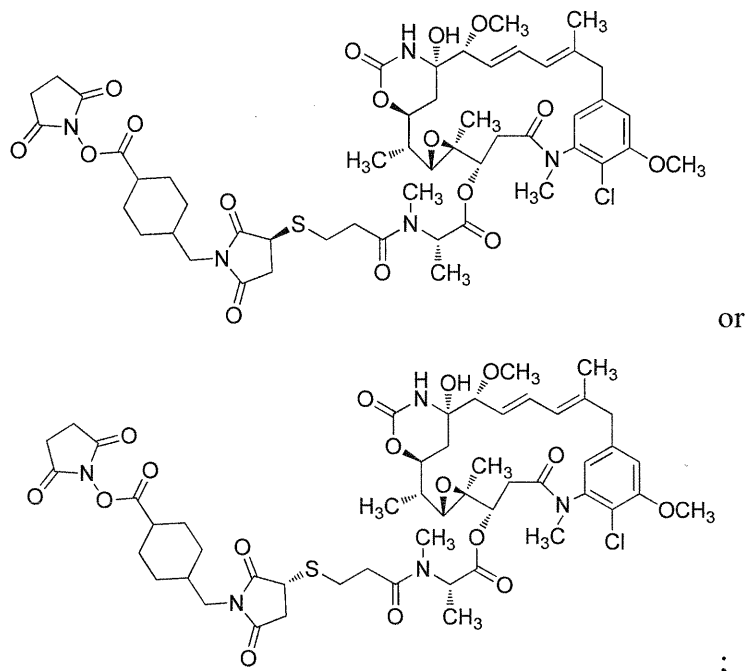


IIb

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%, comprising:

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



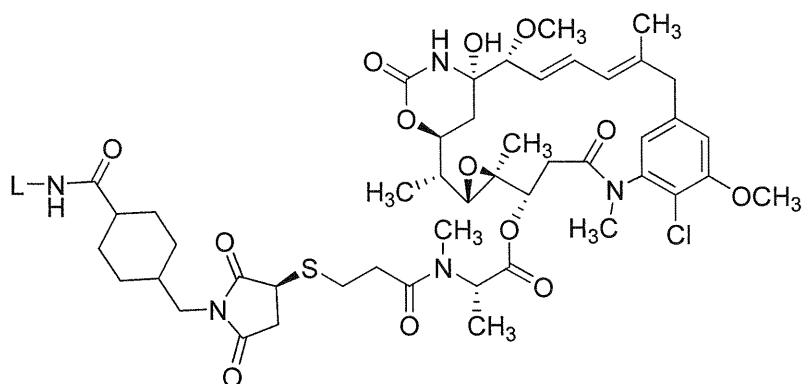
wherein the compound of Formula I is present in a diastereomeric excess of greater than 50%; and

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

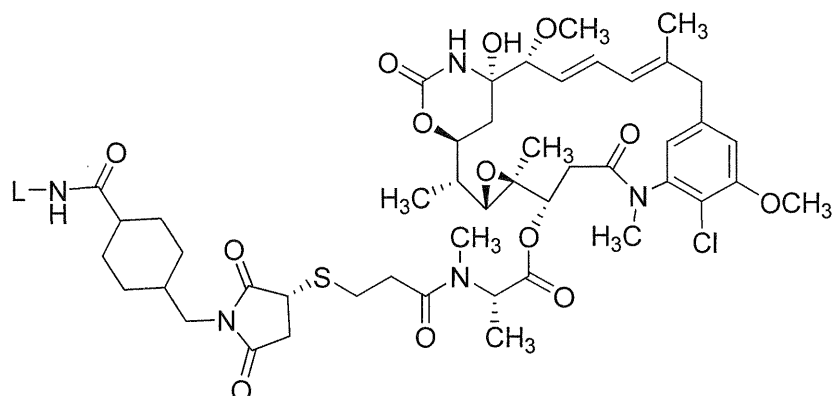
55. The process of claim 54, wherein L is an antibody, or antigen-binding fragment thereof that specifically binds a tumor-associated antigen and further wherein the tumor-associated antigen is selected from the group consisting of AFP, ALK, BAGE proteins, β -catenin, bcr-abl, BRCA1, BORIS, CA9, carbonic 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.

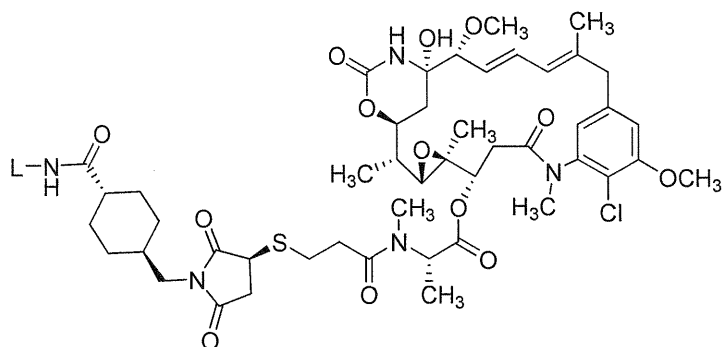
56. The process of claim 55, wherein the GAGE proteins are selected from the group consisting of GAGE-1 and GAGE-2.
57. The process of claim 55, wherein the MAGE proteins are selected from the group consisting of MAGE-1, MAGE-2, MAGE-3, MAGE-4, MAGE-6, and MAGE-12.
58. The process of claim 55, wherein the tumor-associated antigen is selected from the group consisting of EGFRvIII, Her2, and Muc16.
59. The process of claim 54, wherein the compound of Formula IIa or IIb is present in a diastereomeric excess of more than 95%.
60. The process of claim 54, wherein the compound of Formula I is present in a diastereomeric excess of more than 95%.
61. The process of claim 54, wherein the compound present in a diastereomeric excess is a compound of Formula IIa having the following structure:



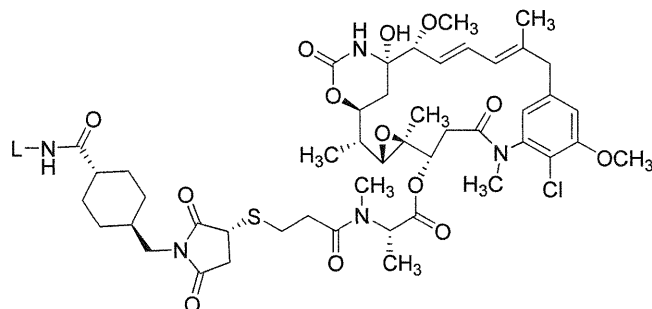
62. The process of claim 54, wherein the compound present in a diastereomeric excess is a compound of Formula IIb having the following structure:



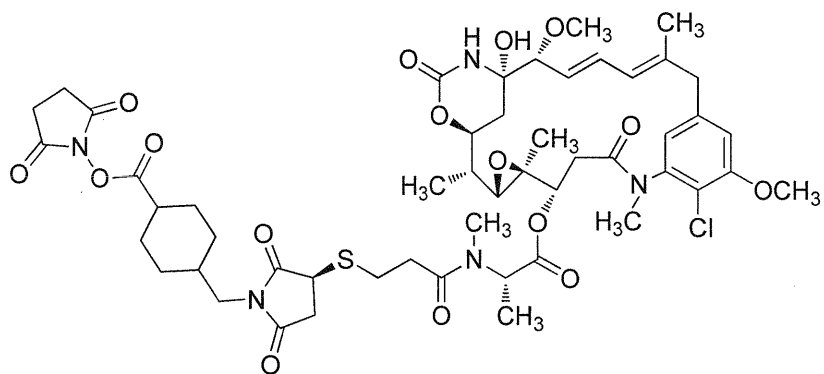
63. The process of claim 54, wherein the compound present in a diastereomeric excess is a compound of Formula IIa having the following structure:



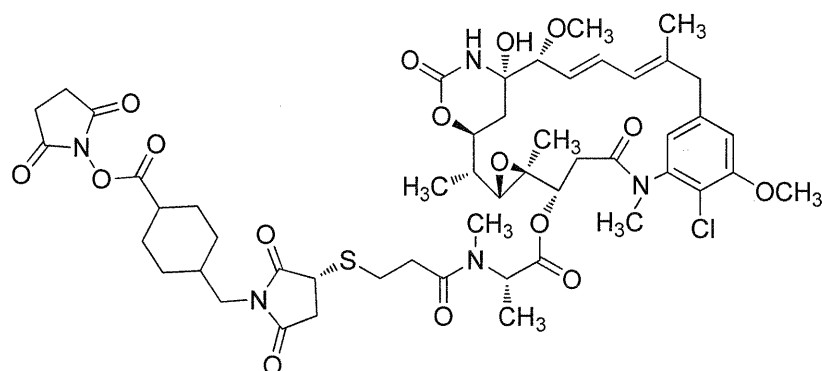
64. The process of claim 54, wherein the compound present in a diastereomeric excess is a compound of Formula IIb having the following structure:



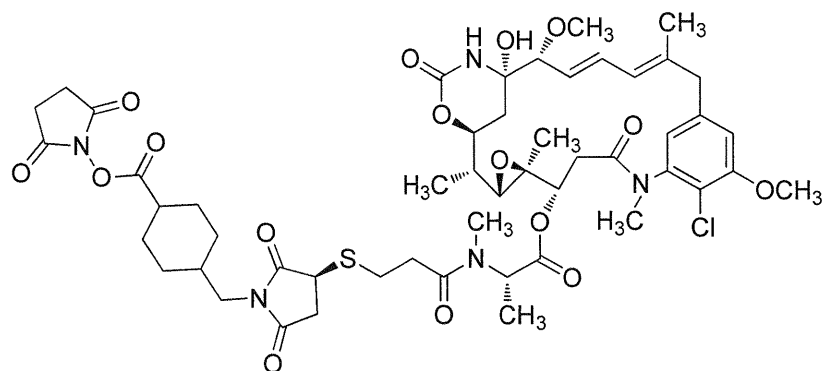
65. The process of claim 54, wherein the compound of Formula I is



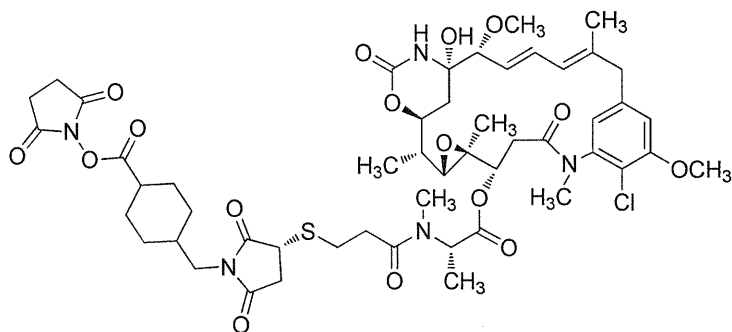
or



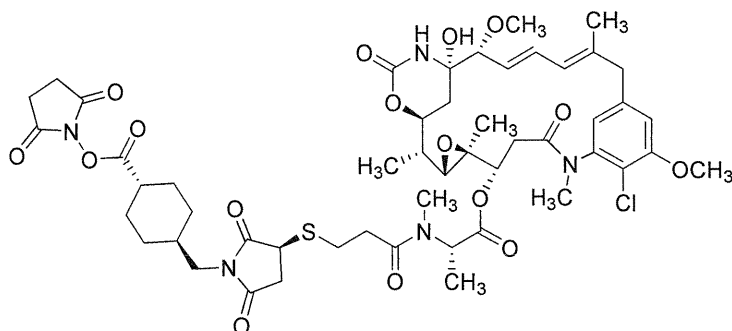
66. The process of claim 54, wherein the compound of Formula I is:



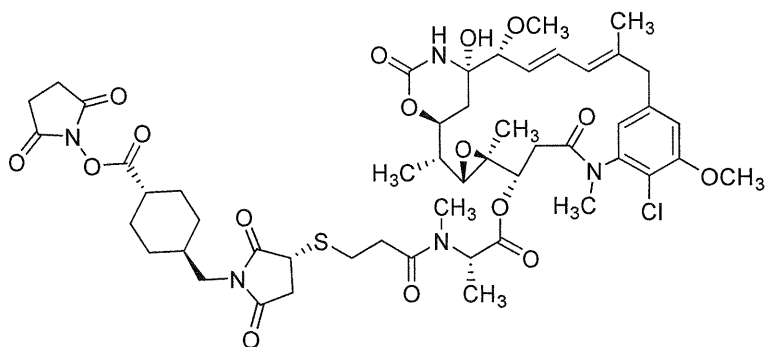
67. The process of claim 54, wherein the compound of Formula I is:



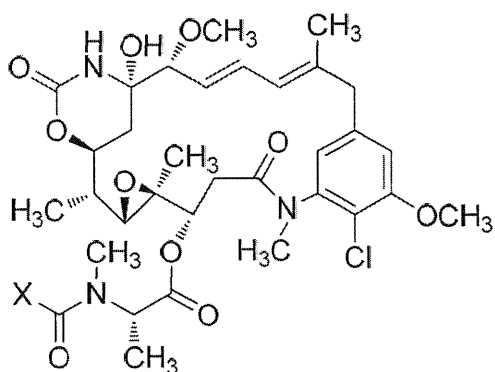
68. The process of claim 54, wherein the compound of Formula I is:



69. The process of claim 54, wherein the compound of Formula I is:

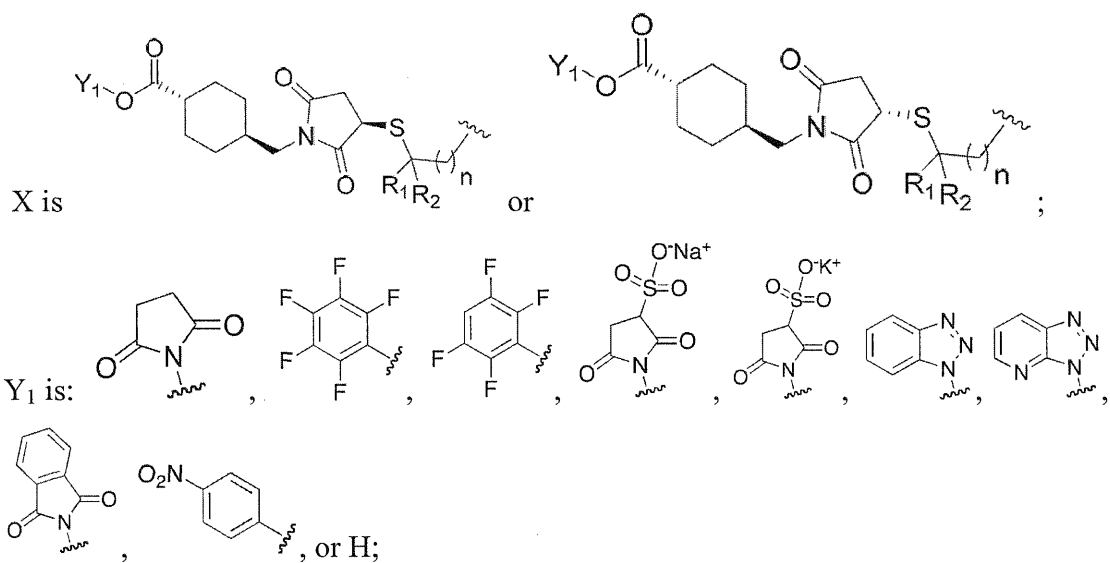


70. A compound of Formula I:



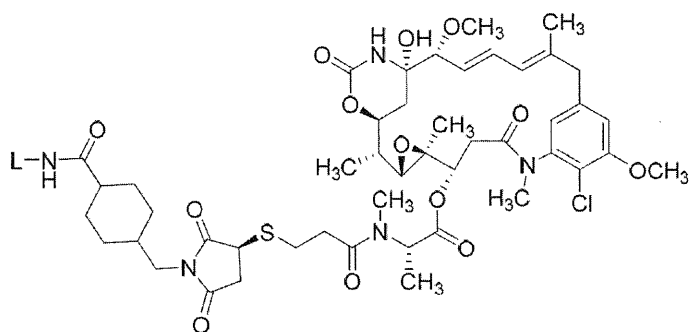
I;

wherein:

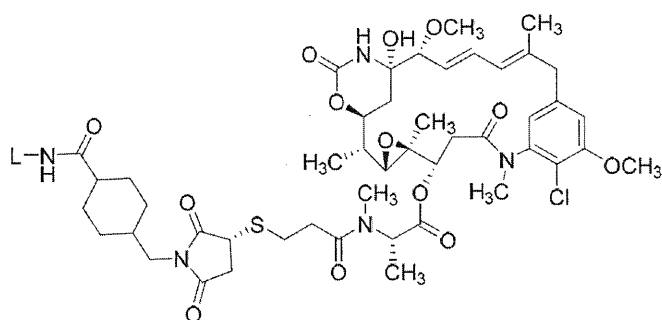


R₁ and R₂ are independently selected from H or alkyl; and n is independently 0 or an integer from 1 to 50; prepared by the process of any one of claims of 34-48, or 51-53.

71. 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%; prepared by the process of any one of claims of 54-69.