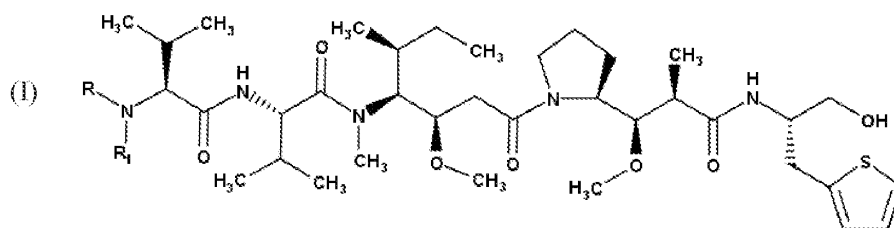


What Is Claimed Is:

1. A compound of formula (I):

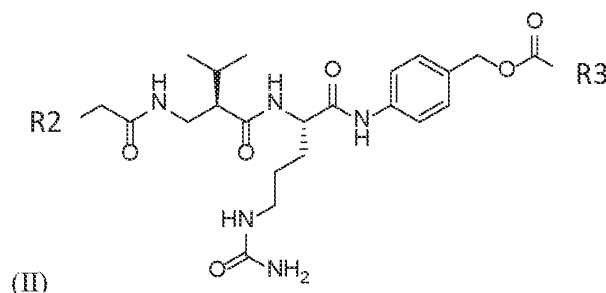


wherein R1 is a hydrogen or a C₁₋₆ alkyl group;

wherein R is selected from the group consisting of: a hydrogen, a C₁₋₆ alkyl, a linker, or a group X1-Y1-* wherein * is the point of attachment to the nitrogen; and

wherein Y1 is an oxycarbonyl group and X1 is a C₁₋₆ alkyl group, a 9-fluorenylmethyl group, a benzyl group, or a tert-butyl group.

2. The compound of claim 1, wherein R1 is a methyl group and R is a hydrogen.
3. The compound of claim 1, wherein X1-Y1 is a 9-fluorenylmethoxycarbonyl (Fmoc) group.
4. A compound of formula (II):



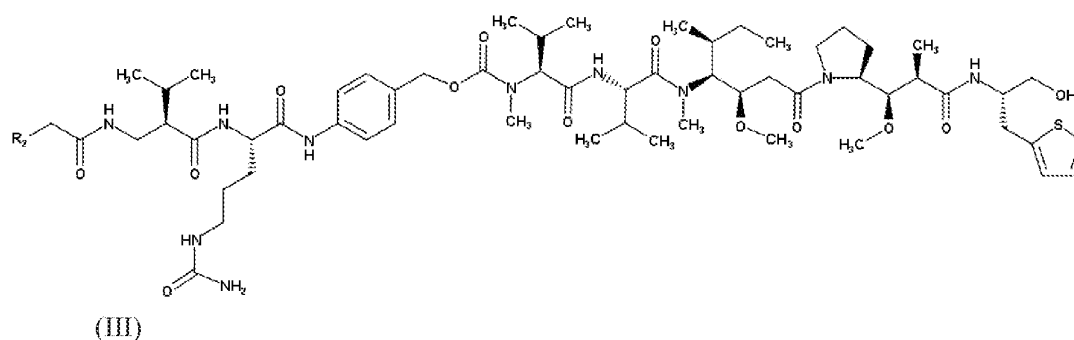
wherein R3 is an agent attached to formula (II) where the point of attachment is a nitrogen, sulfur, oxygen, or carbon atom; and

wherein R2 is a moiety attached to formula (II) wherein the point of attachment is selected from the group consisting of: a chlorine group, an iodine group, a bromine group, and a thiol group.

5. The compound of claim 4, wherein R₂ is a target-binding moiety, wherein the point of attachment at R₂ is a thiol group.

6. The compound of claim 5, wherein the thiol group is a side chain thiol group of a cysteine residue.

7. A compound of formula (III):



wherein R₂ is a moiety attached to formula (III) wherein the point of attachment is selected from the group consisting of: a chlorine group, an iodine group, a bromine group, and a thiol group.

8. The compound of claim 7, wherein R₂ is a target-binding moiety, wherein the point of attachment at R₂ is a thiol group.

9. The compound of claim 8, wherein the thiol group is a cysteine side chain thiol group.

10. The compound of any one of claims 4 to 9, wherein the target-binding moiety is an isolated antibody or an antigen binding fragment thereof (AB) that specifically binds to the target.

11. The compound of any one of claims 4 to 9, wherein the target-binding moiety is an activatable antibody that, in an activated state, specifically binds to the target, the activatable antibody comprising:

an antibody or an antigen binding fragment thereof (AB) that specifically binds to the target;

a masking moiety (MM) coupled to the AB, wherein the MM inhibits the binding of the AB to the target when the activatable antibody is in an uncleaved state; and

a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease.

12. The compound of claim 11, wherein the MM has a dissociation constant for binding to the AB that is greater than the dissociation constant of the AB to its target.

13. The compound of claim 11 or claim 12, wherein the MM does not interfere or compete with the AB for binding to its target when the activatable antibody is in a cleaved state.

14. The compound of any one of claims 11 to 13, wherein the MM is a polypeptide of no more than 40 amino acids in length.

15. The compound of any one of claims 11 to 14, wherein the MM polypeptide sequence is different from that of the target sequence.

16. The compound of any one of claims 11 to 15, wherein the MM polypeptide sequence is no more than 50% identical to any natural binding partner of the AB.

17. The compound of any one of claims 10 to 16, wherein the target is selected from the group consisting of CD44, CD147, CD166, ITGa3, ITGb1, PSMA, and SLC34A2.

18. The compound of any one of claims 4 to 6, wherein the agent is selected from the group consisting of auristatin E, monomethyl auristatin F (MMAF), monomethyl auristatin E (MMAE), monomethyl auristatin D (MMAD), maytansinoid DM4, maytansinoid DM1, a calicheamicin, a duocarmycin, a pyrrolobenzodiazepine, and a pyrrolobenzodiazepine dimer.

19. The compound of any one of claims 1 to 3, wherein R is a linker.

20. The compound of claim 19, wherein the linker is a cleavable linker.

21. The compound of claim 19 or claim 20, wherein the linker is linked to a target-binding moiety.

22. The compound of claim 21, wherein the target-binding moiety is an isolated antibody or an antigen binding fragment thereof (AB) that specifically binds to the target.

23. The compound of claim 21, wherein the target-binding moiety is an activatable antibody that, in an activated state, specifically binds to the target, the activatable antibody comprising:

an antibody or an antigen binding fragment thereof (AB) that specifically binds to the target;

a masking moiety (MM) coupled to the AB, wherein the MM inhibits the binding of the AB to the target when the activatable antibody is in an uncleaved state; and

a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease.

24. The compound of any one of claims 21 to 23, wherein the target is selected from the group consisting of CD44, CD147, CD166, ITGa3, ITGb1, PSMA, and SLC34A2.

25. The compound of any one of claims 10 to 23, wherein the antibody or activatable antibody comprises a cysteine residue at Kabat position 328.

26. An IgG1 antibody, wherein position Kabat position 328 is a cysteine.

27. An activatable antibody comprising:

an antibody or an antigen binding fragment thereof (AB) that specifically binds to the target;

a masking moiety (MM) coupled to the AB, wherein the MM inhibits the binding of the AB to the target when the activatable antibody is in an uncleaved state; and

a cleavable moiety (CM) coupled to the AB, wherein the CM is a polypeptide that functions as a substrate for a protease,

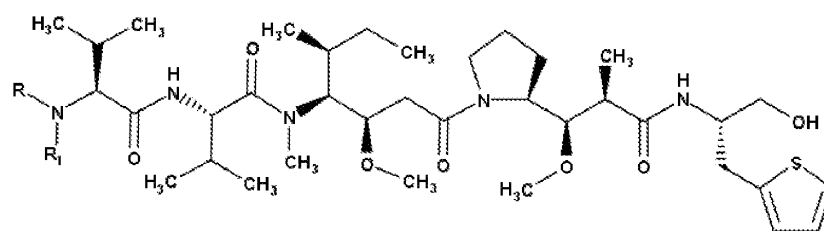
wherein position Kabat position 328 of the AB is a cysteine.

28. The antibody or claim 26 or the activatable antibody of claim 27, wherein the antibody or the AB specifically binds to a target selected from the group consisting of CD44, CD147, CD166, ITGa3, ITGb1, PSMA, and SLC34A2.

29. A pharmaceutical composition comprising:

the compound, antibody, or activatable antibody of any one of claims 1 to 28; and a suitable carrier.

30. A method of conjugating a compound to a polypeptide, the method comprising: conjugating a compound of formula (I) to a polypeptide:



(I)

wherein R1 is a hydrogen or a C₁₋₆ alkyl group;

wherein R is selected from the group consisting of: a hydrogen, a C₁₋₆ alkyl, a linker, or a group X1-Y1-* wherein * is the point of attachment to the nitrogen; and

wherein Y1 is an oxycarbonyl group and X1 is a C₁₋₆ alkyl group, a 9-fluorenylmethyl group, a benzyl group, or a tert-butyl group;

wherein at least one equivalent of the compound of formula (I) or a derivative thereof is conjugated to the polypeptide.

31. The method of claim 30, wherein R1 is a methyl group and R is a hydrogen.

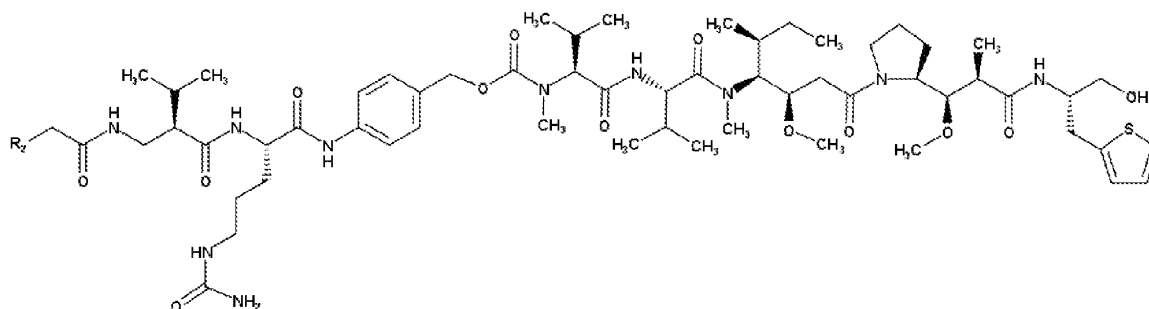
32. The method of claim 30, wherein X1-Y1 is a 9-fluorenylmethoxycarbonyl (Fmoc) group.

33. The method of claim 30, wherein R is a linker.

34. The method of claim 33, wherein the linker is a cleavable linker.

35. A method of conjugating a compound to a polypeptide, the method comprising:
conjugating a compound formula (III) to a polypeptide:

(III),



wherein R₂ is a moiety attached to formula (III) wherein the point of attachment is selected from the group consisting of: a chlorine group, an iodine group, a bromine group, and a thiol group.

36. The method of claim 35, wherein the R₂ is a halogen group.

37. The method of claim 36, wherein the R₂ is an iodine group,

38. The method of claim 36, wherein the R₂ is a bromine group.

39. The method of claim 36, wherein the R₂ is a chlorine group.

40. The method of any one of claims 30 to 39, wherein at least one compound of formula (I) or (III) is conjugated to the polypeptide via a thiol group on the polypeptide.

41. The method of claim 40, wherein the thiol group is a side chain thiol group of a cysteine residue of the polypeptide.

42. The method of any one of claims 30 to 41, wherein the polypeptide comprises a target-binding moiety.

43. The method of any one of claims 30 to 42, wherein the polypeptide comprises an antibody or an antigen binding fragment thereof (AB) that specifically binds to a target.

44. The method of claim 43, wherein the cysteine residue is at Kabat position 328 of the AB.

45. The method of any one of claims 30 to 44, wherein the method comprises the steps of:

(i) reducing the polypeptide with a reducing agent, wherein at least one disulfide group is reduced to a free thiol group;

(ii) re-oxidizing the polypeptide with an oxidizing agent without oxidizing the free thiol group; and

(iii) conjugating the compound of formula (I) or (III) to the free thiol group.

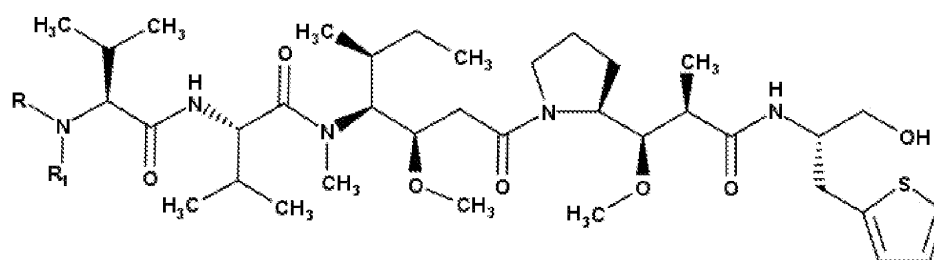
46. The method of claim 45, wherein the reducing agent is TCEP.

47. A conjugated polypeptide having the formula:



wherein [T] is a target-binding moiety and [L] is a linker moiety; and

wherein [C] is a compound comprising a compound of formula (I):



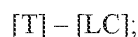
(I)

wherein R1 is a hydrogen or a C₁₋₆ alkyl group; and

wherein R is the point of attachment to [L].

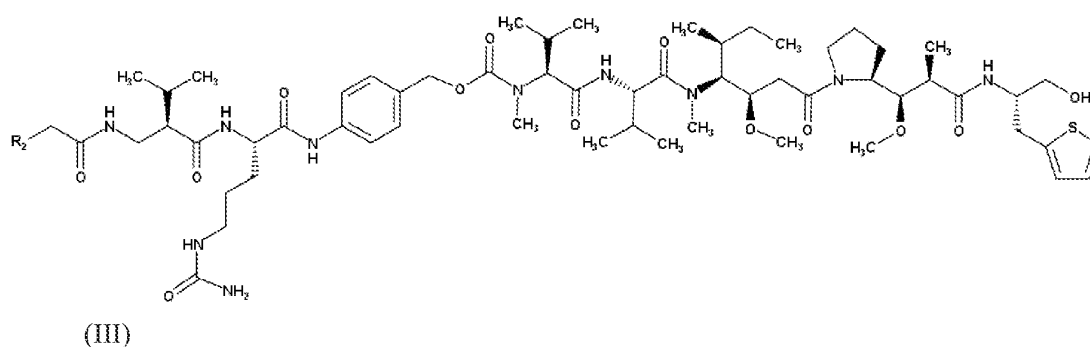
48. The conjugated polypeptide of claim 47, wherein R1 is a methyl group.

49. A conjugated polypeptide having the formula:



wherein [T] is a target-binding moiety and [LC] is a linker-toxin; and

wherein [LC] is a compound comprising a compound of formula (III):



wherein R2 is the point of attachment to [T].

50. The conjugated polypeptide of claim 47 or claim 48, wherein the linker [L] is a cleavable linker.

51. The conjugated polypeptide of any one of claims 47 to 50, wherein the linker [L] or the linker-toxin [LC] is coupled to the target-binding moiety [T] via a thiol group on the target-binding moiety.

52. The conjugated polypeptide of claim 51, wherein the thiol group is a side chain thiol group of a cysteine residue on the target-binding moiety.

53. The conjugated polypeptide of any one of claims 47 to 52, wherein the target-binding moiety [T] comprises an antibody or an antigen binding fragment thereof (AB) that specifically binds to a target.

54. The conjugated polypeptide of claim 53, wherein the cysteine residue is a cysteine residue at Kabat position 328 of the AB.

55. A method of treating a subject with a disease or disorder comprising:

administering to a subject in need thereof an effective amount of a composition comprising the compound of any one of claims 1 to 25, the pharmaceutical composition of claim 29, or the conjugated polypeptide of any one of claims 47 to 54.

56. Use of a compound of any one of claims 1 to 25, a pharmaceutical composition of claim 29, or a conjugated polypeptide of any one of claims 47 to 54 for treating a disease or disorder.

57. A compound of any one of claims 1 to 25, a pharmaceutical composition of claim 29, or a conjugated polypeptide of any one of claims 47 to 54 for use in the preparation of a medicament for treating a disease or a disorder.

58. The method of any one of claims 55 to 57, wherein the disease or disorder is a cancer.