

CLAIMS

1. A biparatopic antibody or antigen binding fragment thereof that specifically binds a human folate receptor 1 (FR α), wherein the antibody or antigen-binding fragment comprises:
 - (a) a first FR α -binding domain that comprises a first variable heavy chain (VH) and a first variable light chain (VL) and that bind to a first epitope of FR α ; and
 - (b) a second FR α -binding domain that comprises a second VH and a second VL and that binds to a second epitope of FR α .
2. The biparatopic antibody or antigen binding fragment thereof of claim 1, wherein the first FR α -binding domain specifically binds to the same FR α epitope as an antibody comprising a VH amino acid sequence selected from the group consisting of SEQ ID NOs:24, 25, 26, and 57, and a VL amino acid sequence selected from the group consisting of SEQ ID NOs:19, 20, and 21.
3. The biparatopic antibody or antigen binding fragment thereof of claim 1, wherein the first FR α -binding domain competitively inhibits binding to the same FR α epitope as an antibody comprising a VH amino acid sequence selected from the group consisting of SEQ ID NOs:24, 25, 26, and 57, and a VL amino acid sequence selected from the group consisting of SEQ ID NOs:19, 20, and 21.
4. The biparatopic antibody or antigen binding fragment thereof of any one of claims 1-3, wherein the second FR α -binding domain specifically binds to the same FR α epitope as an antibody comprising a VH amino acid sequence of SEQ ID NO:22 or 23 and a VL amino acid sequence of SEQ ID NO:17 or 18.
5. The biparatopic antibody or antigen binding fragment thereof of any one of claims 1-3, wherein the second FR α -binding domain competitively inhibits binding to the same FR α epitope as an antibody comprising a VH amino acid sequence of SEQ ID NO:22 or 23 and a VL amino acid sequence of SEQ ID NO:17 or 18.

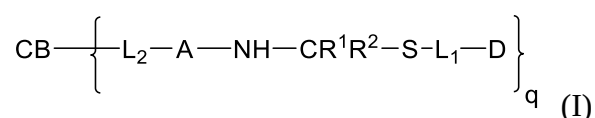
6. The biparatopic antibody or antigen binding fragment thereof of any one of claims 1-5, wherein the first VH comprises VH CDR1-3 comprising the amino acid sequences of (a) SEQ ID NOs: 10-12 or (b) SEQ ID NOs: 15, 16, and 12, respectively and the first VL comprises VL CDR1-3 comprising the amino acid sequences of SEQ ID NOs: 4-6, respectively.
7. The biparatopic antibody or antigen binding fragment thereof of any one of claims 1-6, wherein the first VH comprises the amino acid sequence selected from the group consisting of SEQ ID NOs: 24, 25, 26, and 57, and/or wherein the first VL comprises the amino acid sequence selected from the group consisting of SEQ ID NOs: 19, 20, and 21.
8. The biparatopic antibody or antigen binding fragment thereof of any one of claims 1-7, wherein the second VH comprises VH CDR1-3 comprising the amino acid sequences of (a) SEQ ID NOs: 7-9 or (b) SEQ ID NOs: 13, 14, and 9, respectively and the second VL comprises VL CDR1-3 comprising the amino acid sequences of SEQ ID NOs: 1-3, respectively.
9. The biparatopic antibody or antigen binding fragment thereof of any one of claims 1-8, wherein the second VH comprises the amino acid sequence of SEQ ID NO: 22 or 23 and/or wherein the second VL comprises the amino acid sequence of SEQ ID NO: 17 or 18.
10. The biparatopic antibody or antigen-binding fragment thereof of any one of claims 1-9, wherein the first VH and VL pair and/or the second VH and VL pair is murine, non-human, humanized, chimeric, resurfaced, or human.
11. The biparatopic antibody or antigen-binding fragment thereof of any one of claims 1-10, wherein said antibody or antigen-binding fragment thereof binds to human FR α but not FOLR2 or FOLR3.
12. The biparatopic antibody or antigen-binding fragment thereof of any one of claims 1-11, wherein the first FR α -binding domain is an single-chain variable fragment (scFv).

13. The biparatopic antibody or antigen-binding fragment thereof of claim 12, wherein the scFv of the first FR α binding domain has a peptide orientation of VH-linker-VL.
14. The biparatopic antibody or antigen-binding fragment thereof of claim 12, wherein the scFv of the first FR α binding domain has a peptide orientation of VL-linker-VH.
15. The biparatopic antibody or antigen-binding fragment thereof of any one of claims 1-11, wherein the second FR α -binding domain is an single-chain variable fragment (scFv).
16. The biparatopic antibody or antigen-binding fragment thereof of claim 15, wherein the scFv of the second FR α binding domain has a peptide orientation of VH-linker-VL.
17. The biparatopic antibody or antigen-binding fragment thereof of claim 15, wherein the scFv of the second FR α binding domain has a peptide orientation of VL-linker-VH.
18. The biparatopic antibody or antigen binding fragment thereof of any one of claims 13-17, wherein the linker is glycine-serine linker.
19. The biparatopic antibody or antigen binding fragment thereof of any one of claims 1-18, wherein the second FR α -binding domain comprises an amino acid sequence selected from SEQ ID NOs: 27-29.
20. The biparatopic antibody or antigen binding fragment thereof of any one of claims 1-18, wherein the first FR α -binding domain comprises an amino acid sequence selected from SEQ ID NOs: 30-32.
21. The biparatopic antibody or antigen binding fragment thereof of any one of claims 1-20, comprising the amino acid sequence of (i) SEQ ID NOs:33 and 34, (ii) SEQ ID NOs: 35 and 36, (iii) SEQ ID NOs: 37 and 38, or (iv) SEQ ID NOs: 39 and 40.

22. The biparatopic antibody or antigen binding fragment thereof of any one of claims 1-20, comprising the amino acid sequences of SEQ ID NOs: 41-43.
23. The biparatopic antibody or antigen binding fragment thereof of any one of claims 1-20, comprising the amino acid sequences of SEQ ID NOs: 44-46.
24. The biparatopic antibody or antigen binding fragment thereof of any one of claims 1-23, wherein the biparatopic antibody or antigen binding fragment thereof is a tetravalent biparatopic antibody or antigen binding fragment thereof.
25. The biparatopic antibody or antigen binding fragment thereof of any one of claims 1-23, wherein the biparatopic antibody or antigen binding fragment thereof is a bivalent biparatopic antibody or antigen binding fragment thereof.
26. The biparatopic antibody or antigen binding fragment thereof of any one of claims 1-25, wherein the bispecific antibody or antigen-binding fragment thereof comprises FR α -binding domains selected from the group consisting of tandem scFvs, a diabody, a triabody, a tetrabody, and a knob-in-hole structure.
27. The biparatopic antibody or antigen binding fragment thereof of any one of claims 1-20, and 22-26, which has a knob-in-hole (KIH) structure.
28. The biparatopic antibody or antigen binding fragment thereof of claim 27, wherein the FR α binding domain comprising SEQ ID NOs: 1-3 and 7-9 is on the knob side of the KIH structure.
29. The biparatopic antibody or antigen binding fragment thereof of claim 27, wherein the FR α binding domain comprising SEQ ID NOs: 1-3 and 7-9 is on the hole side of the KIH structure.

30. The biparatopic antibody or antigen binding fragment thereof of any one of claims 27-29, wherein the FR α binding domain comprising SEQ ID NOs: 4-6 and 10-12 is on the knob side of the KIH structure.
31. The biparatopic antibody or antigen binding fragment thereof of any one of claims 27-29, wherein the FR α binding domain comprising SEQ ID NOs: 4-6 and 10-12 is on the hole side of the KIH structure.
32. The biparatopic antibody or antigen-binding fragment thereof of any one of claims 1-31, which comprises a full length antibody.
33. The biparatopic antibody or antigen-binding fragment thereof of any one of claims 1-32, wherein the first FR α -binding domain is a full length antibody.
34. The biparatopic antibody or antigen-binding fragment thereof of any one of claims 1-33, wherein the second FR α -binding domain is a full length antibody.
35. The biparatopic antibody or antigen-binding fragment thereof of any one of claims 1-31, which comprises an antigen-binding fragment.
36. The biparatopic antibody or antigen-binding fragment thereof of any one of claims 1-35, wherein the first FR α -binding domain is an antigen-binding fragment.
37. The biparatopic antibody or antigen-binding fragment thereof of any one of claims 1-36, wherein the second FR α -binding domain is an antigen-binding fragment.
38. A biparatopic antibody or antigen binding fragment thereof comprising the amino acid sequences of SEQ ID NOs: 41-43.
39. A combination of isolated nucleic acid molecules encoding the biparatopic antibody or antigen binding fragment thereof of claims 1-38.

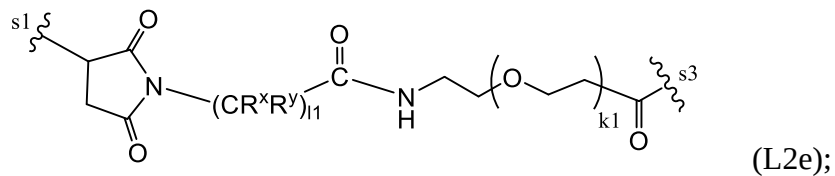
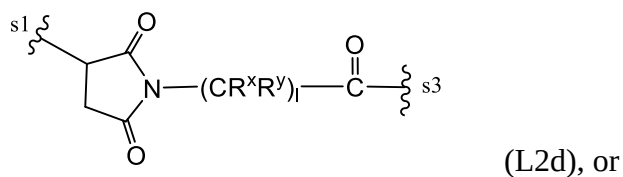
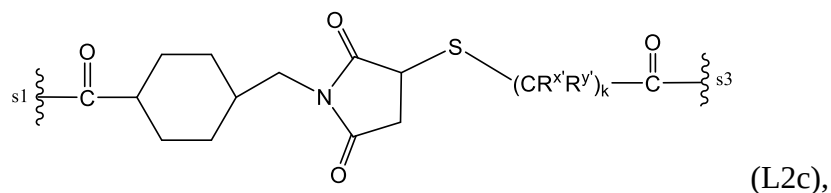
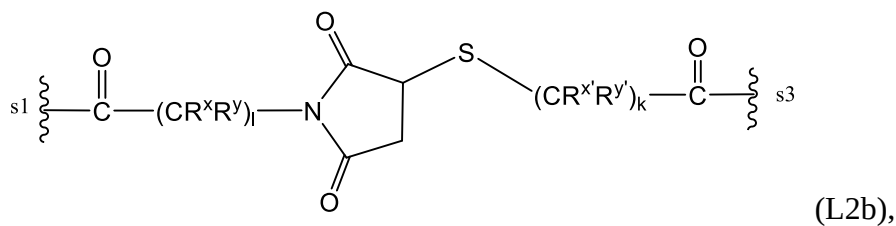
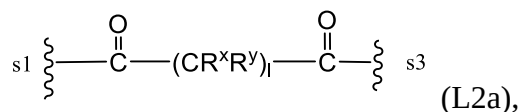
40. An isolated vector comprising one of the nucleic acid molecules of claim 39.
41. A host cell comprising the combination of isolated nucleic acid molecules of claim 39, or the isolated vector of claim 40.
42. The host cell of claim 41, which is selected from the group consisting of *E. coli*, *Pseudomonas*, *Bacillus*, *Streptomyces*, yeast, CHO, YB/20, NS0, PER-C6, HEK-293T, NIH-3T3, HeLa, BHK, Hep G2, SP2/0, R1.1, B-W, L-M, COS 1, COS 7, BSC1, BSC40, BMT10 cell, plant cell, insect cell, and human cell in tissue culture.
43. A pharmaceutical composition comprising the biparatopic antibody or antigen of any one of claims 1-38, the combination of nucleic acid molecule of claim 39, the vector of claim 40, or the host cell of claim 41 or 42, and a pharmaceutically acceptable carrier or excipient.
44. A pharmaceutical composition comprising the biparatopic antibody of any one of claims 1-38, and a pharmaceutically carrier or excipient.
45. A method of making the biparatopic antibody of any one of claims 1-23 comprising (a) culturing a cell expressing said antibody; and (b) isolating the antibody from said cultured cell.
46. The method of claim 45, wherein said cell is a eukaryotic cell.
47. An immunoconjugate represented by the following formula:



or a pharmaceutically acceptable salt thereof, wherein:

CB is the biparatopic antibody or an antigen-binding fragment thereof of any one of claims 1-38;

L₂ is represented by one of the following formula:



wherein:

R^x , R^y , $\text{R}^{x'}$ and $\text{R}^{y'}$, for each occurrence, are independently H, -OH, halogen, -O-(C_{1-4} alkyl), $-\text{SO}_3\text{H}$, $-\text{NR}_{40}\text{R}_{41}\text{R}_{42}^+$, or a C_{1-4} alkyl optionally substituted with -OH, halogen, SO_3H or $\text{NR}_{40}\text{R}_{41}\text{R}_{42}^+$, wherein R_{40} , R_{41} and R_{42} are each independently H or a C_{1-4} alkyl;

l and k are each independently an integer from 1 to 10;

$l1$ is an integer from 2 to 5;

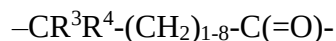
$k1$ is an integer from 1 to 5; and

$s1$ indicates the site connected to the cell-binding agent CB and $s3$ indicates the site connected to the A group;

A is an amino acid residue or a peptide comprising 2 to 20 amino acid residues;

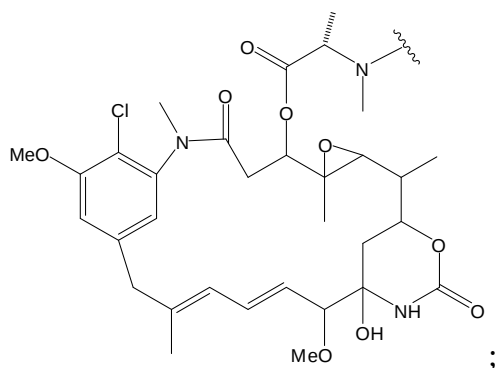
R^1 and R^2 are each independently H or a C_{1-3} alkyl;

L_1 is represented by the following formula:



wherein R^3 and R^4 are each independently H or Me, and the $-C(=O)-$ moiety in L_1 is connected to D;

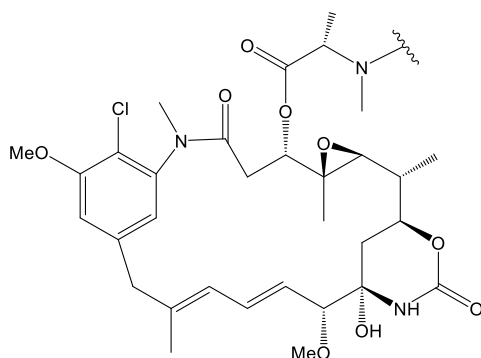
D is represented by the following formula:



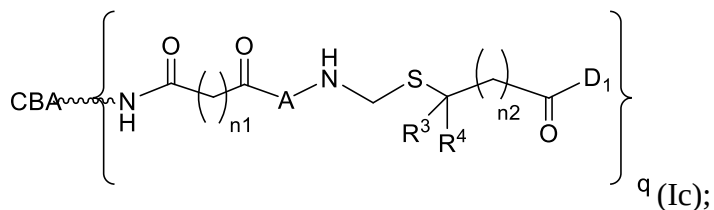
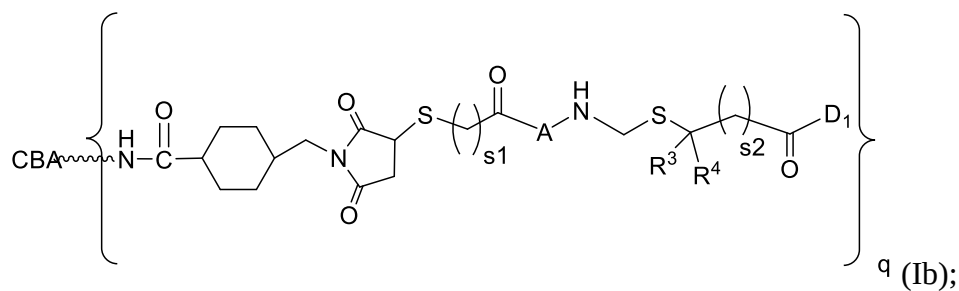
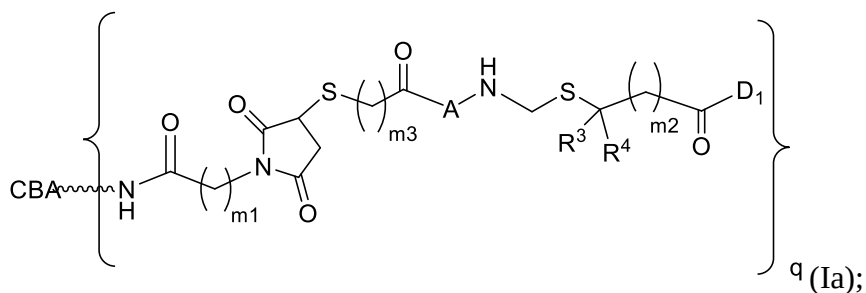
q is an integer from 1 to 20.

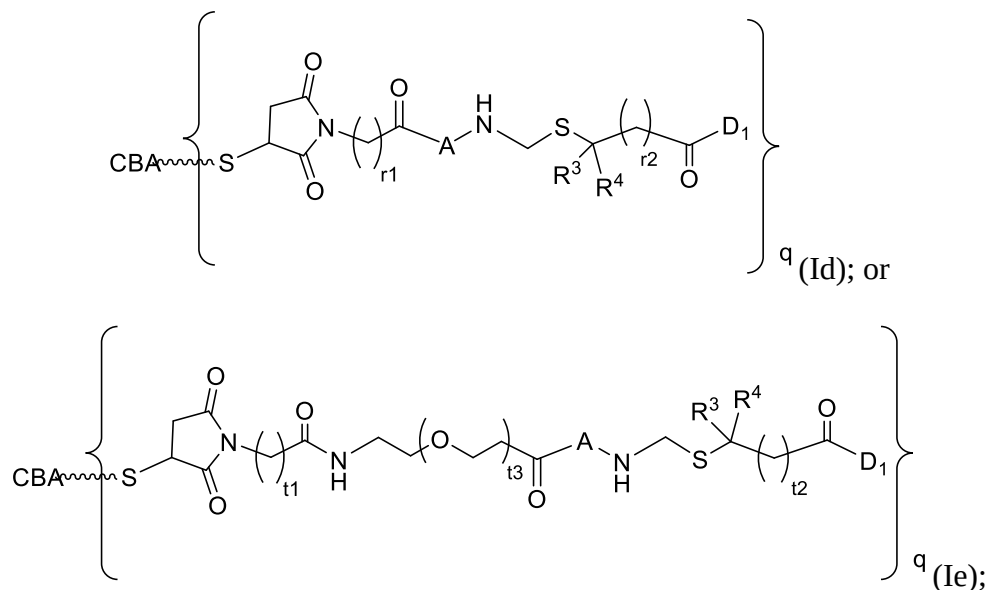
48. The immunoconjugate of claim 47, wherein R^x , R^y , $R^{x'}$ and $R^{y'}$ are all H; and l and k are each independently an integer an integer from 2 to 6.
49. The immunoconjugate of claim 47 or 48, wherein A is a peptide containing 2 to 5 amino acid residues.
50. The immunoconjugate of claim 49, wherein A is selected from the group consisting of Gly-Gly-Gly, Ala-Val, Val-Ala, D-Val-Ala, Val-Cit, D-Val-Cit, Val-Lys, Phe-Lys, Lys-Lys, Ala-Lys, Phe-Cit, Leu-Cit, Ile-Cit, Phe-Ala, Phe- N^9 -tosyl-Arg, Phe- N^9 -nitro-Arg, Phe-Phe-Lys, D-Phe-Phe-Lys, Gly-Phe-Lys, Leu-Ala-Leu, Ile-Ala-Leu, Val-Ala-Val, Ala-Ala-Ala, D-Ala-Ala-Ala, Ala-D-Ala-Ala, Ala-Ala-D-Ala, Ala-Leu-Ala-Leu (SEQ ID NO:54), β -Ala-Leu-Ala-Leu (SEQ ID NO:55), Gly-Phe-Leu-Gly (SEQ ID NO:56), Val-Arg, Arg-Arg, Val-D-Cit, Val-D-Lys, Val-D-Arg, D-Val-Cit, D-Val-Lys, D-Val-Arg, D-Val-D-Cit, D-Val-D-Lys, D-Val-D-Arg, D-Arg-D-Arg, Ala-Ala, Ala-D-Ala, D-Ala-Ala, D-Ala-D-Ala, Ala-Met, Gln-Val, Asn-Ala, Gln-Phe, Gln-Ala, D-Ala-Pro, and D-Ala-tBu-Gly, wherein the first amino acid in each peptide is connected to L_2 group and the last amino acid in each peptide is connected to $-NH-CR^1R^2-S-L_1-D$.

51. The immunoconjugate of any one of claims 47-50, wherein R^1 and R^2 are both H.
52. The immunoconjugate of any one of claims 47-51, wherein L_1 is $-(CH_2)_{4-6}-C(=O)-$.
53. The immunoconjugate of any one of claims 47-52, wherein D is represented by the following formula:



54. The immunoconjugate of any one of claims 47-53, wherein the immunoconjugate is represented by the following formula:





or a pharmaceutically acceptable salt thereof, wherein:

$\text{CBA} \sim \text{N} - \text{H}$ is the biparatopic antibody or an antigen-binding fragment thereof of any one of claims 1-38 connected to the L_2 group through a Lys amine group;

$\text{CBA} \sim \text{S} -$ is the biparatopic antibody or an antigen-binding fragment thereof of any one of claims 1-38 connected to the L_2 group through a Cys thiol group;

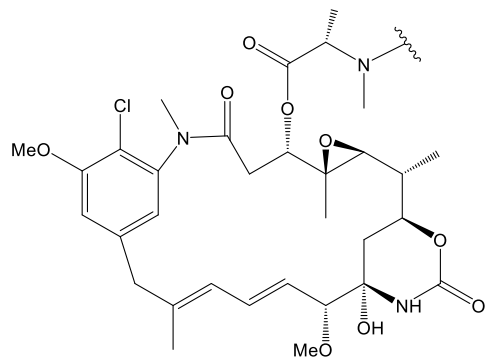
R^3 and R^4 are each independently H or Me;

$m1, m3, n1, r1, s1$ and $t1$ are each independently an integer from 1 to 6;

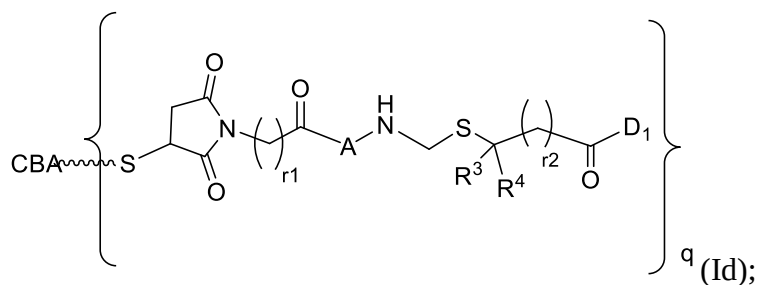
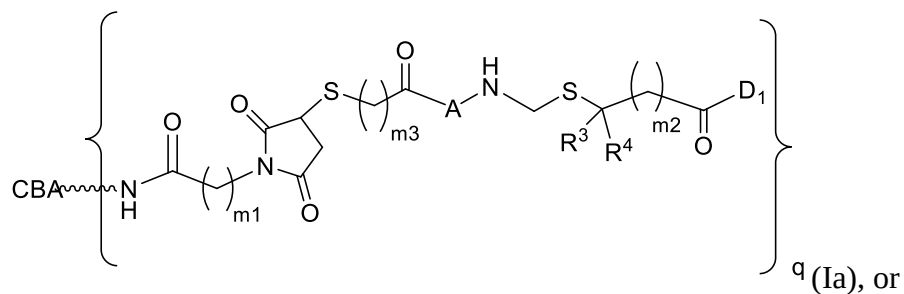
$m2, n2, r2, s2$ and $t2$ are each independently an integer from 1 to 7;

$t3$ is an integer from 1 to 12;

D_1 is represented by the following formula:



55. The immunoconjugate of claim 54, wherein the immunoconjugate is represented by the following formula:



wherein:

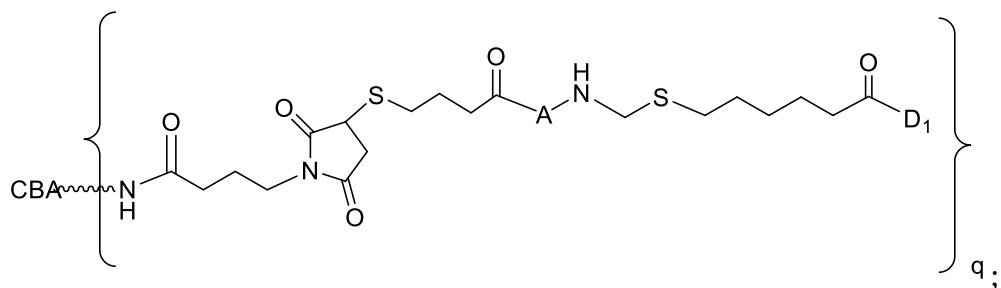
m1 and m3 are each independently an integer from 2 to 4;

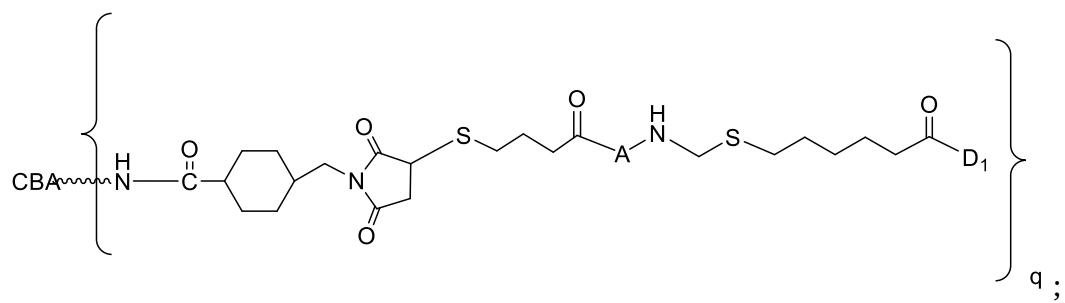
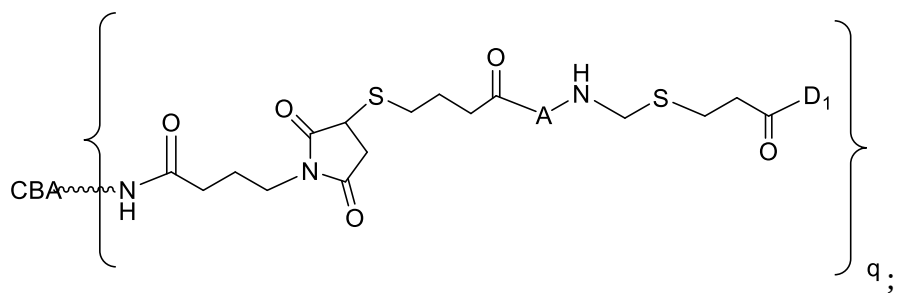
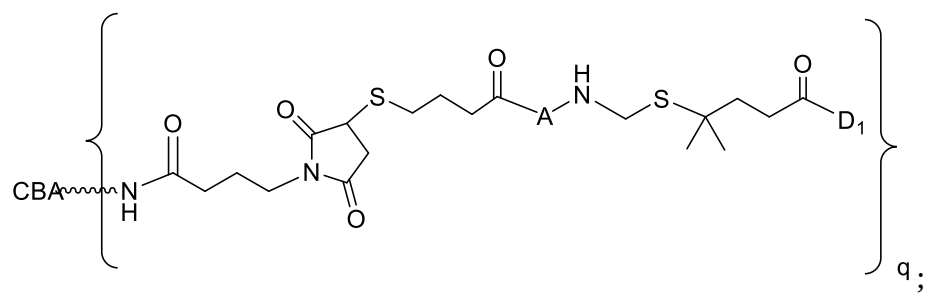
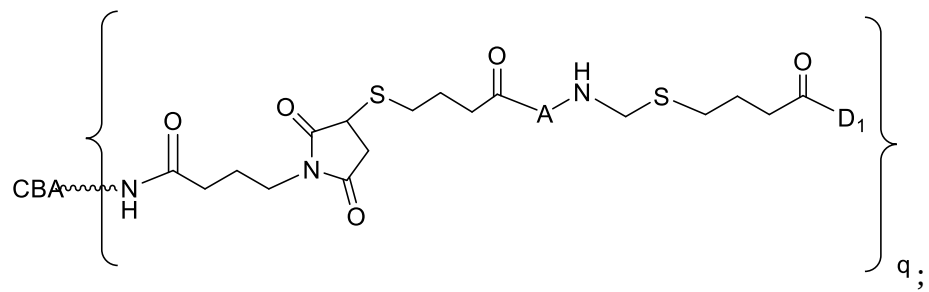
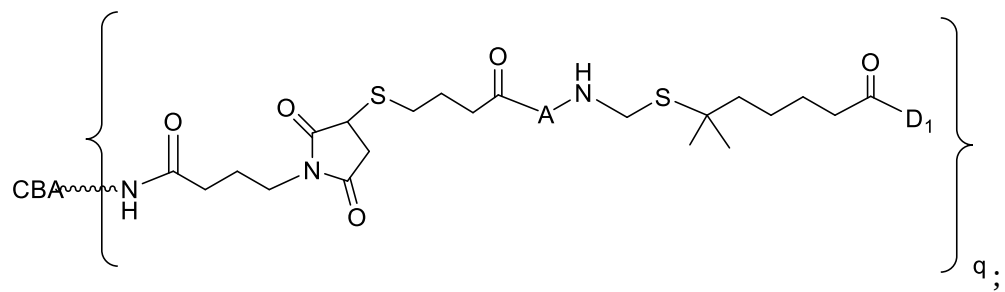
m2 is an integer from 2 to 5;

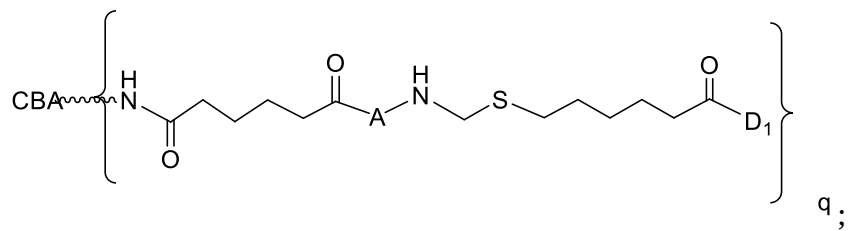
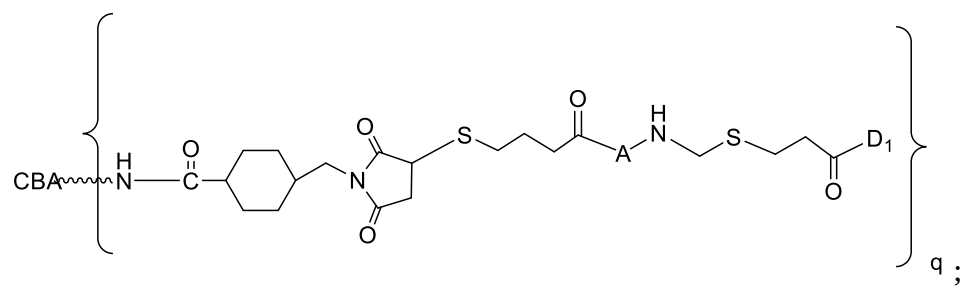
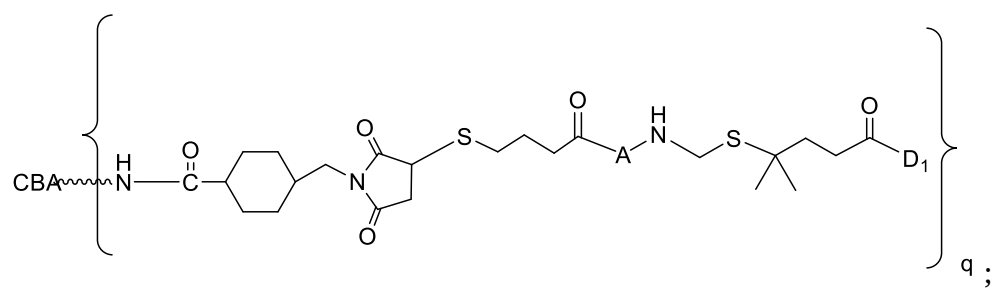
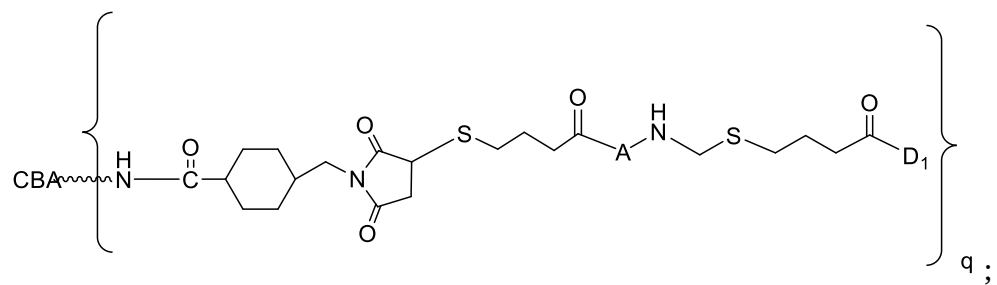
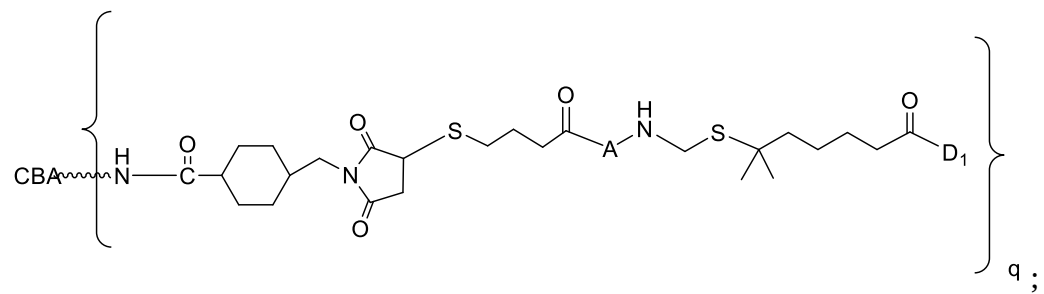
r1 is an integer from 2 to 6; and

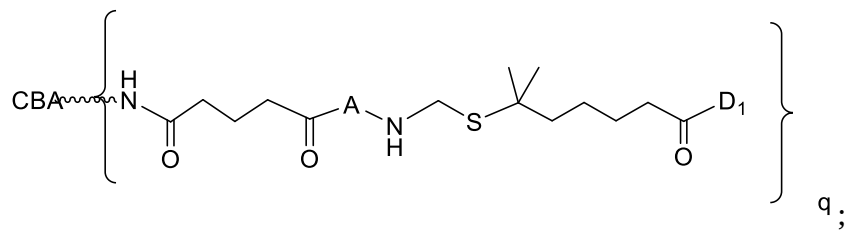
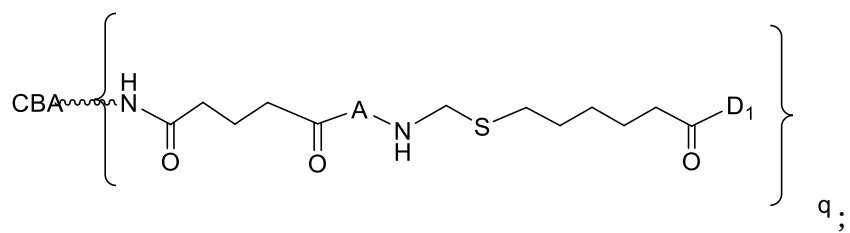
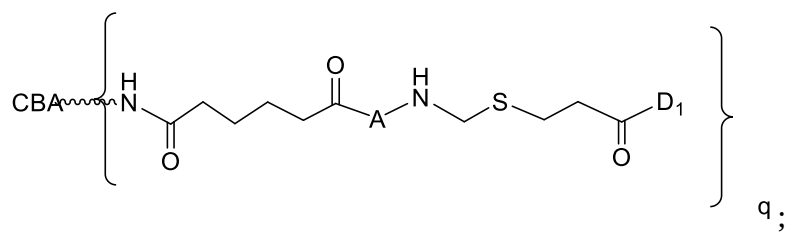
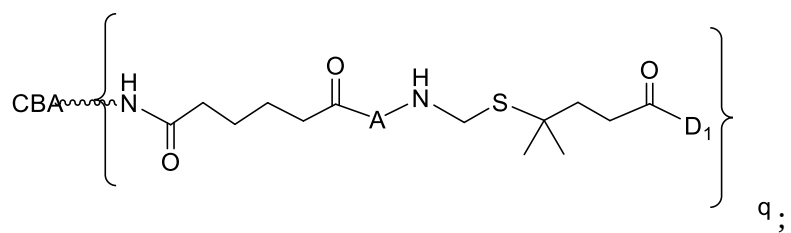
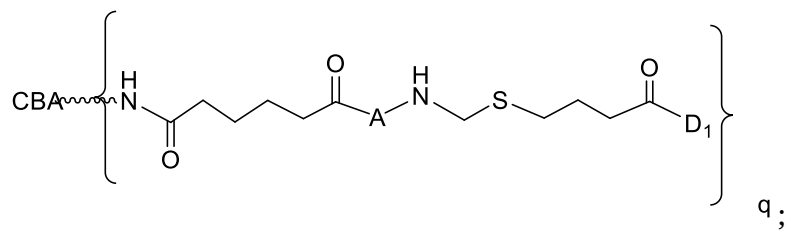
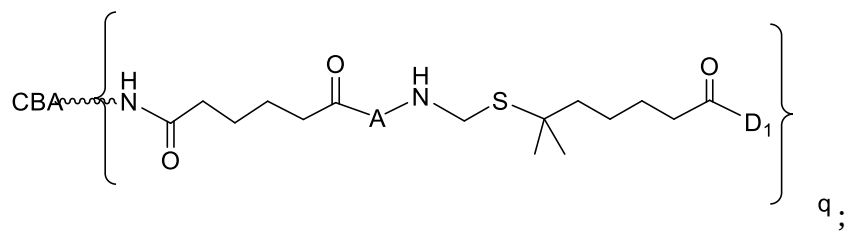
r2 is an integer from 2 to 5.

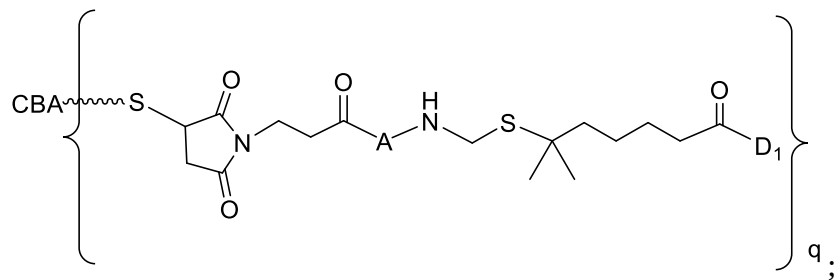
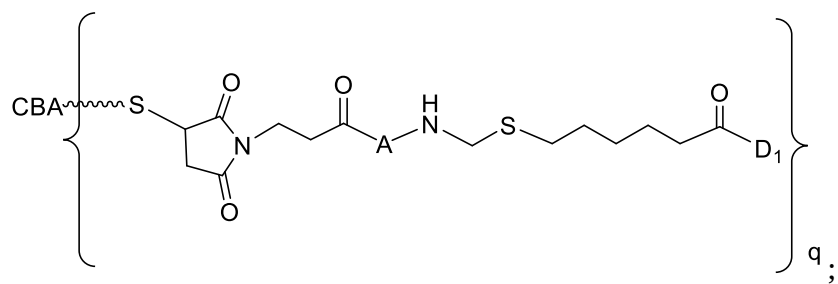
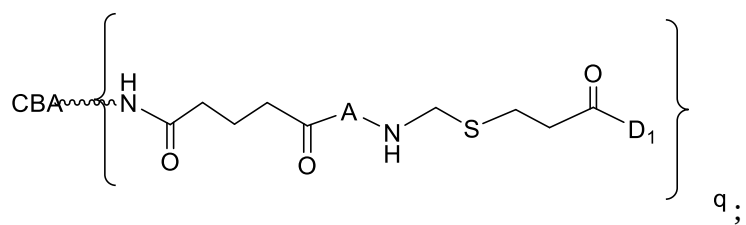
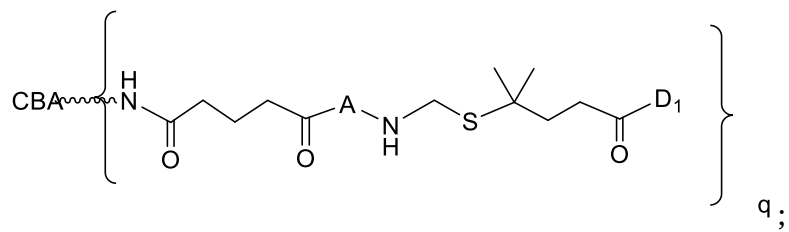
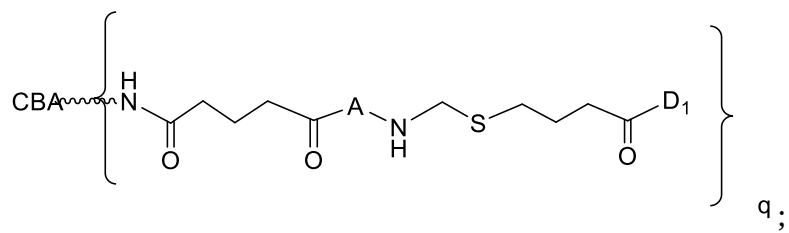
56. The immunoconjugate of claim 54 or 55, wherein A is Ala-Ala-Ala, Ala-D-Ala-Ala, Ala-Ala, D-Ala-Ala, Val-Ala, D-Val-Ala, D-Ala-Pro, or D-Ala-tBu-Gly.
57. The immunoconjugate of claim 54, wherein the immunoconjugate is represented by the following formula:

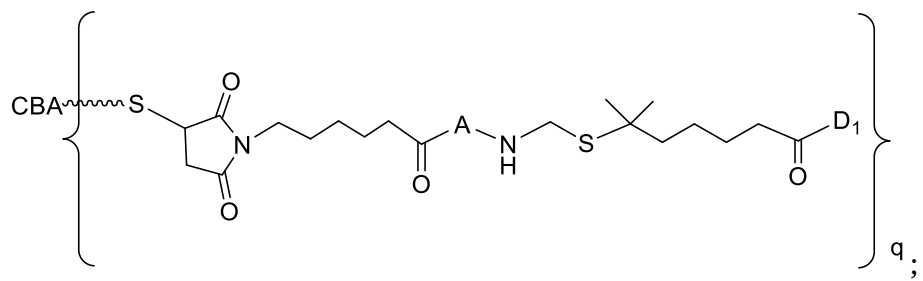
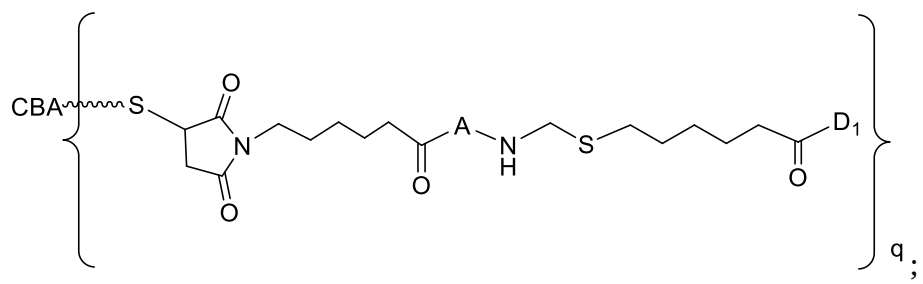
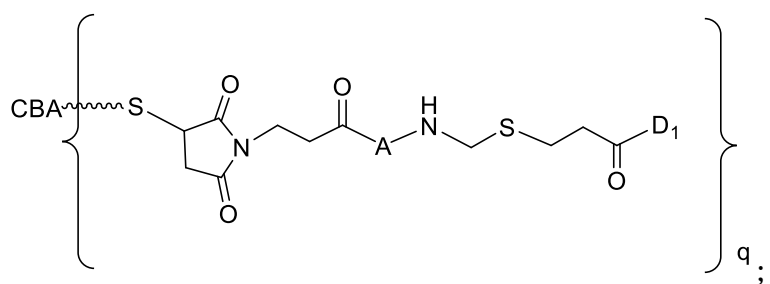
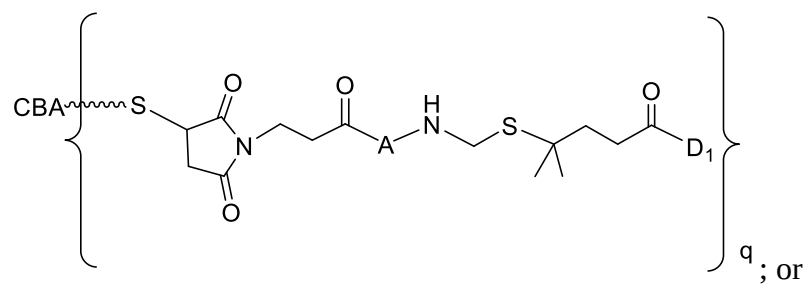
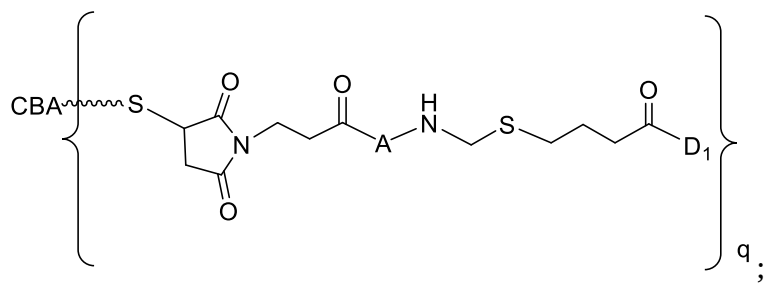


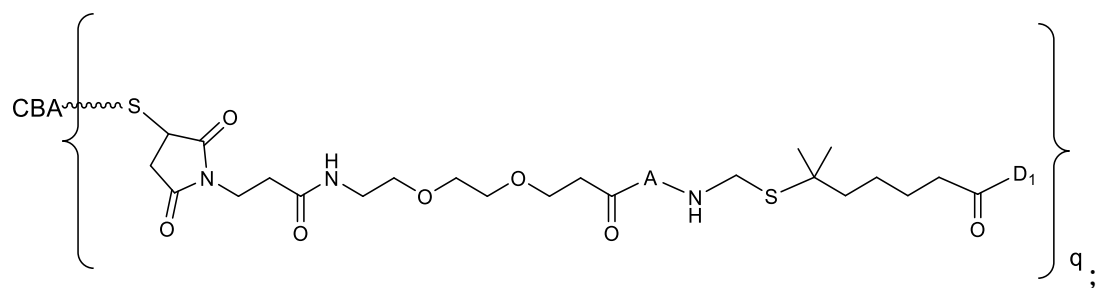
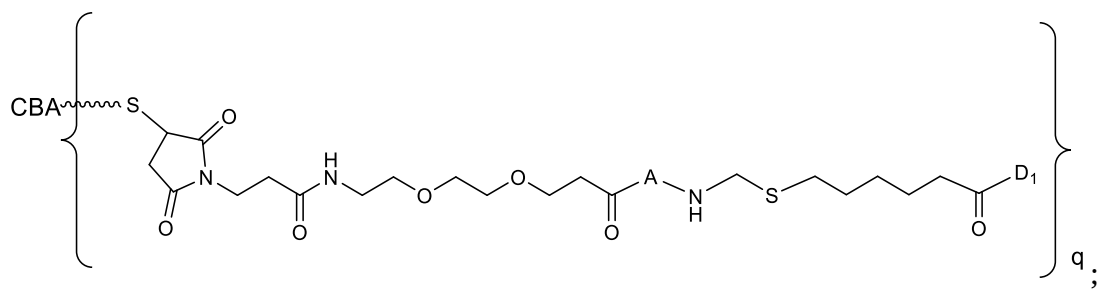
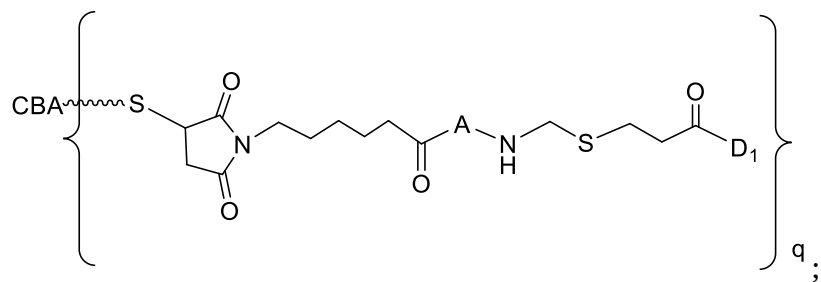
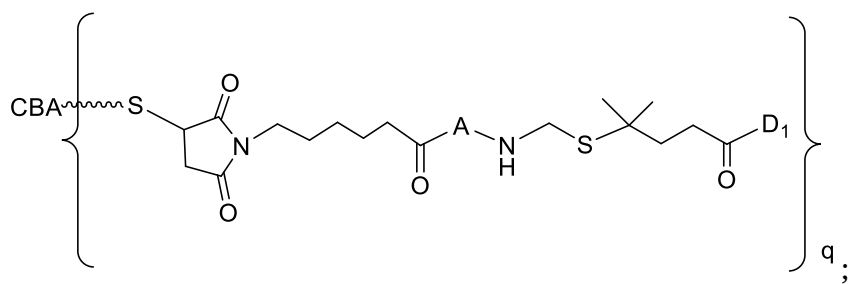
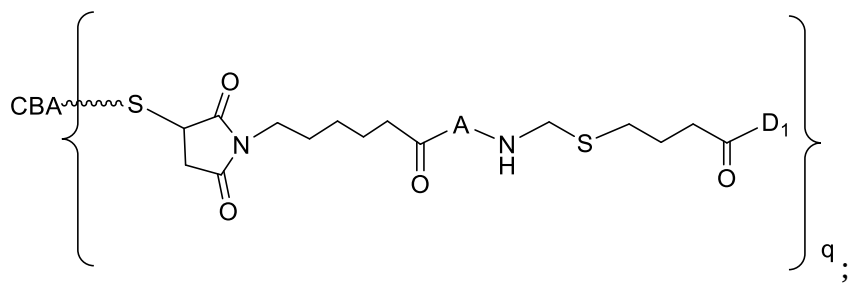


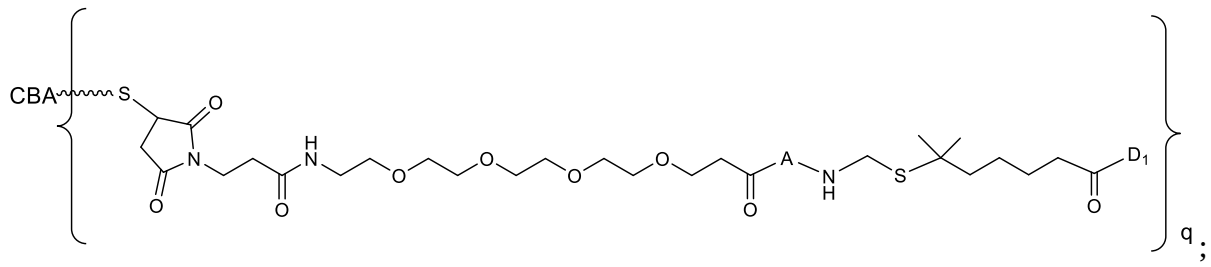
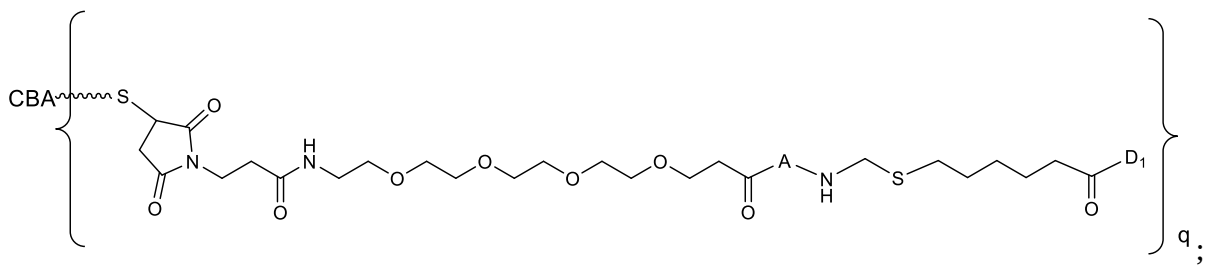
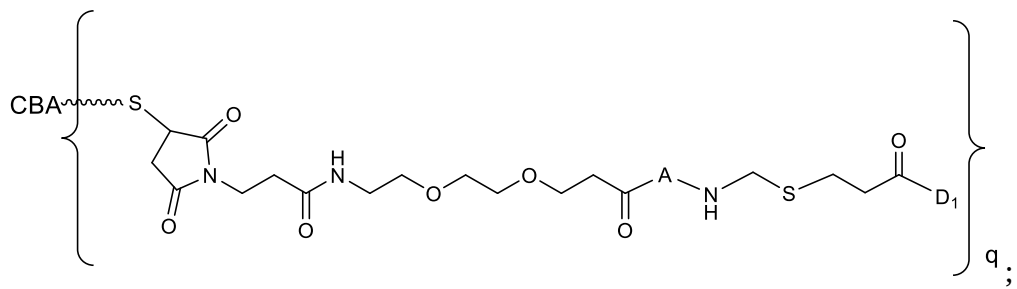
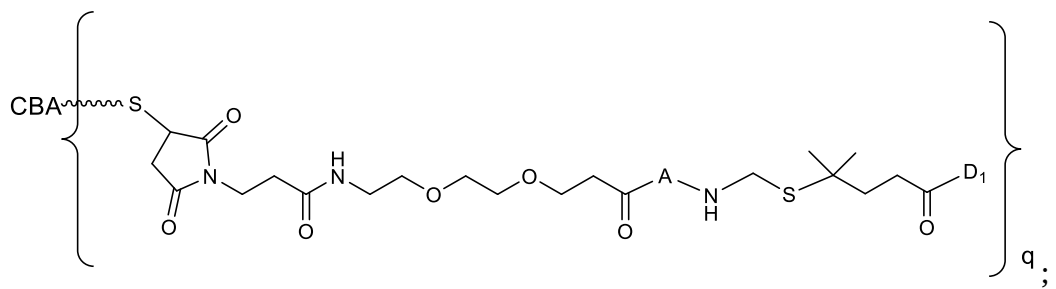
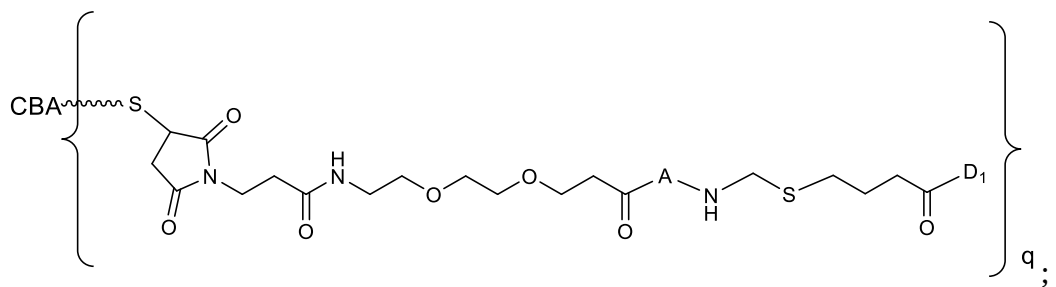


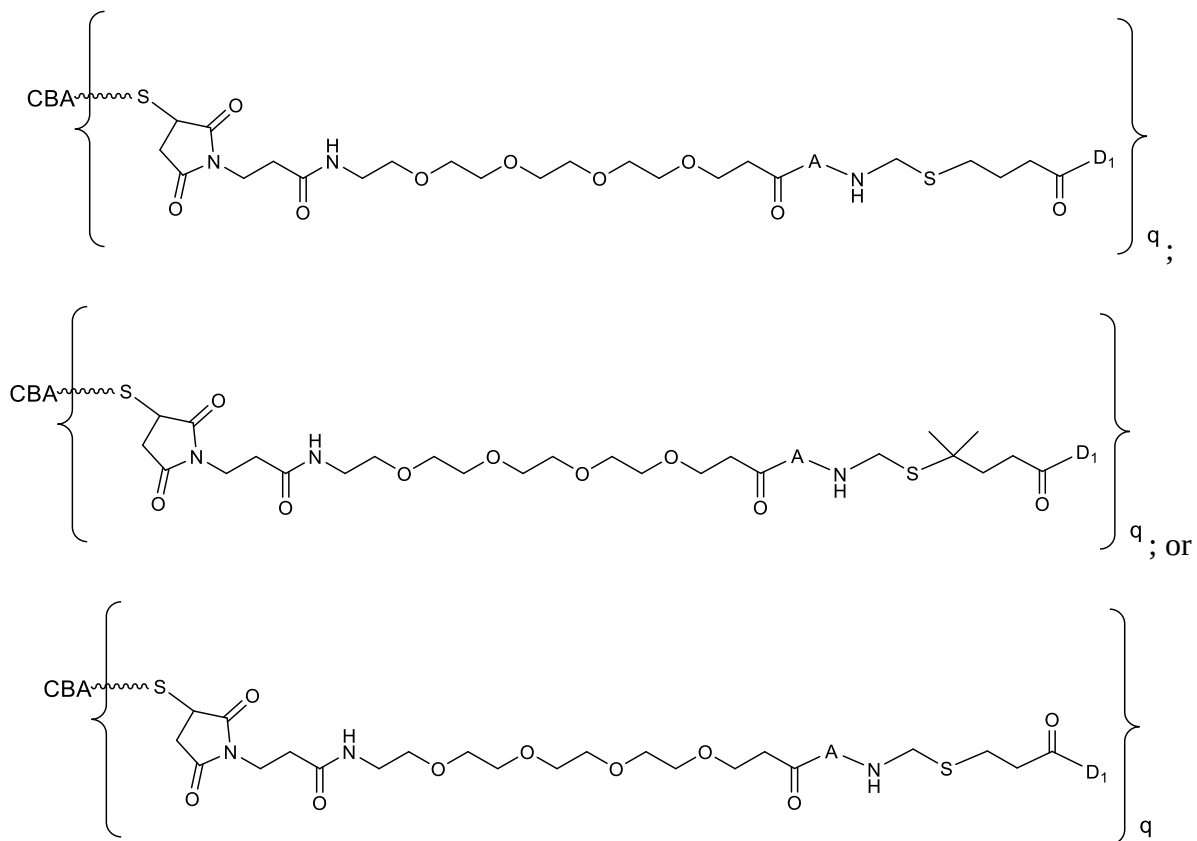








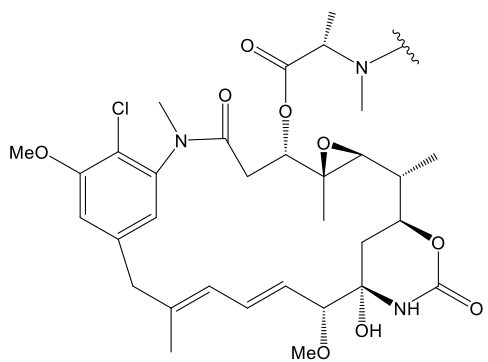




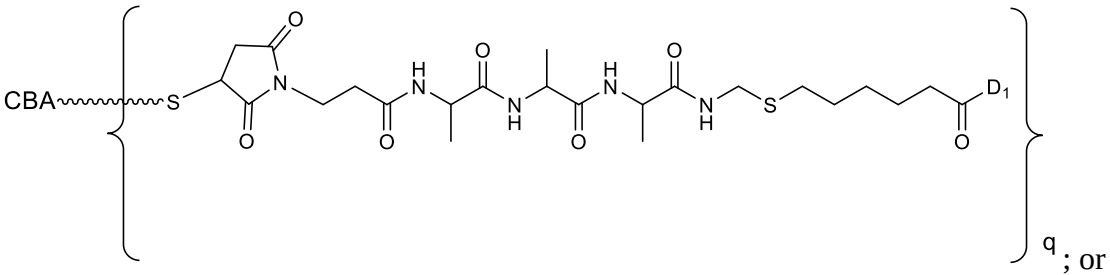
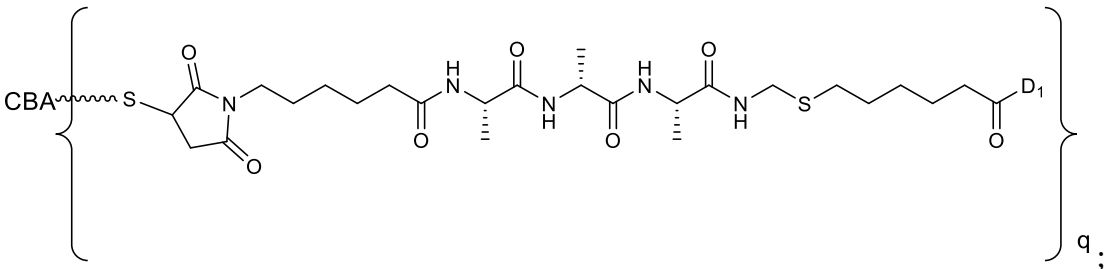
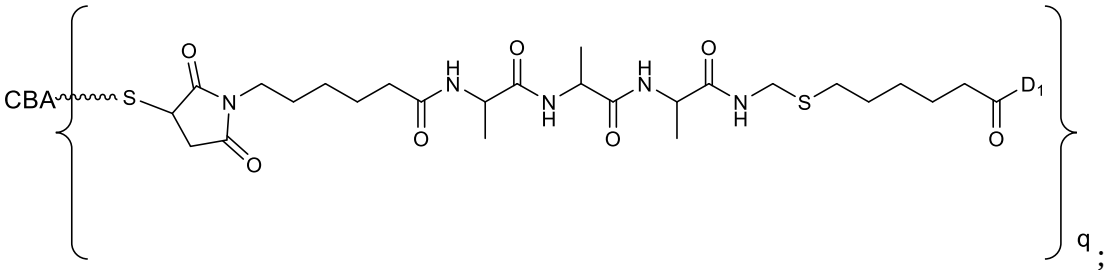
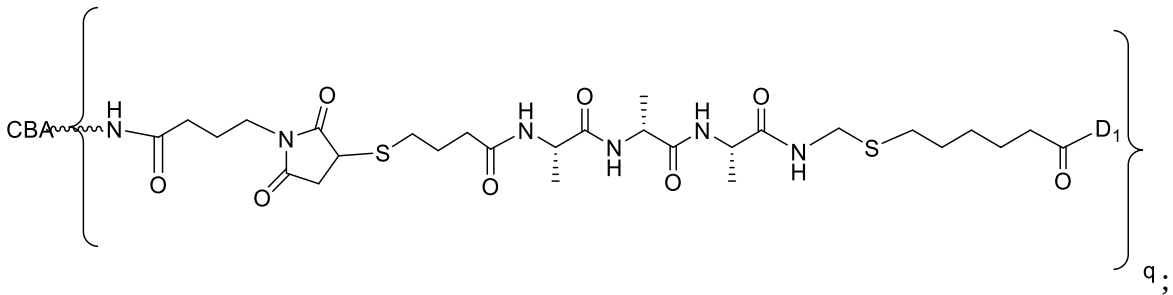
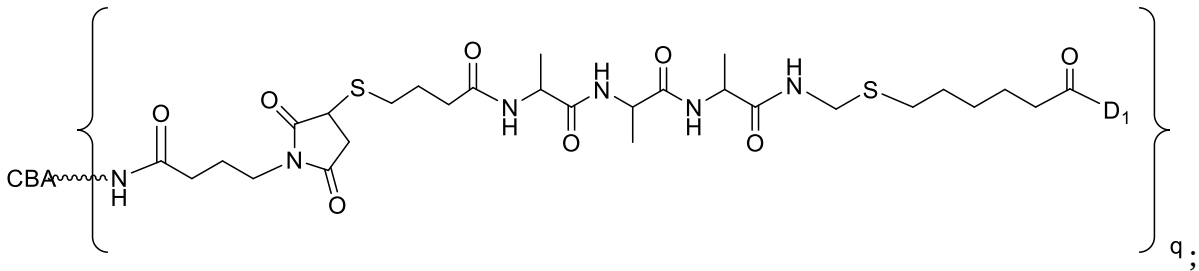
or a pharmaceutically acceptable salt thereof, wherein:

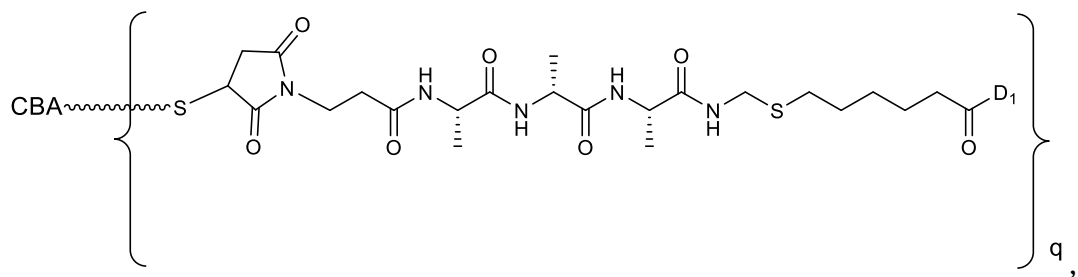
A is Ala-Ala-Ala, Ala-D-Ala-Ala, Ala-Ala, D-Ala-Ala, Val-Ala, D-Val-Ala, D-Ala-Pro, or D-Ala-tBu-Gly, and

D₁ is represented by the following formula:

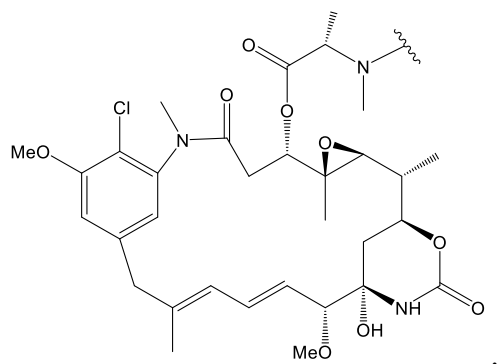


58. The immunoconjugate of claim 57, wherein the immunoconjugate is represented by the following formula:

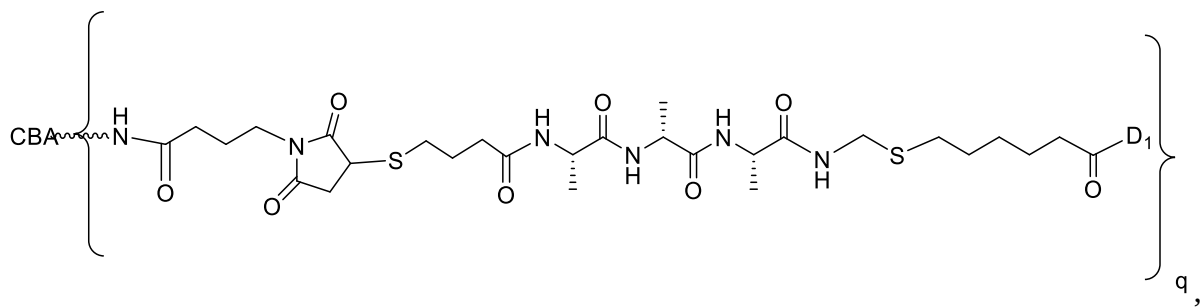




wherein D₁ is represented by the following formula:



59. The immunoconjugate of claim 47, wherein the immunoconjugate is represented by the following formula:

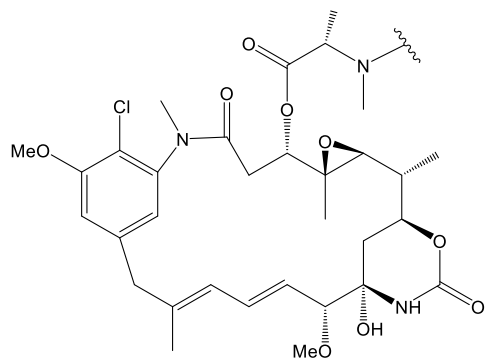


wherein:

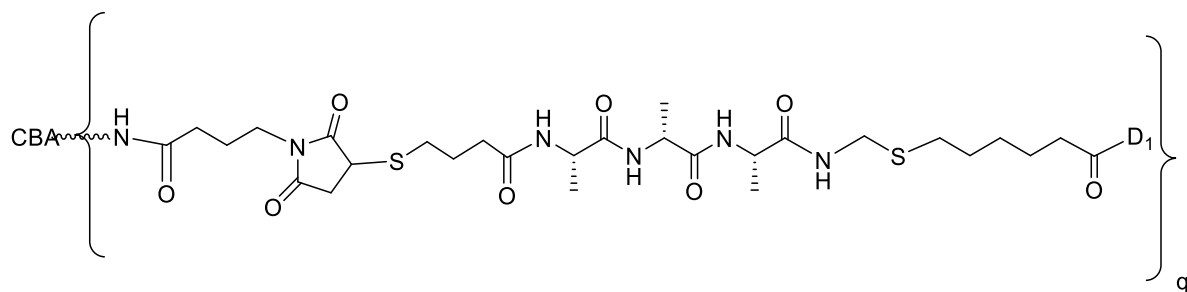
CBA is the biparatopic antibody or an antigen-binding fragment thereof of any one of claims 1-38;

q is an integer from 1 to 10;

D₁ is represented by the following formula:



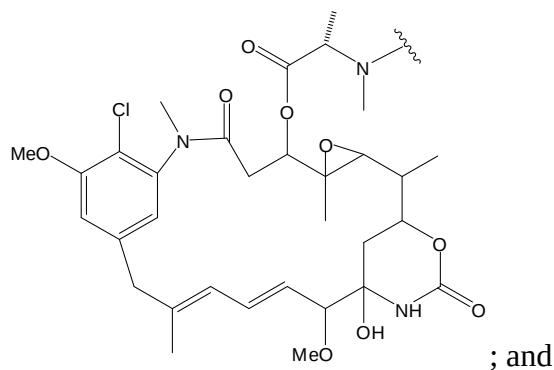
60. An immunoconjugate represented by the following formula:



or a pharmaceutically acceptable salt thereof, wherein:

CBA is the biparatopic antibody or an antigen-binding fragment comprising the amino acid sequences of SEQ ID NOs: 41-43;

D₁ is represented by the following formula:



q is an integer from 1 to 10.

61. An immunoconjugate having the formula (A) - (L) - (C), wherein:

(A) is a biparatopic antibody or antigen binding fragment of any one of claims 1-38;

(L) is a linker; and

(C) is a cytotoxic agent. wherein said linker (L) links (A) to (C).

62. The immunoconjugate of claim 61, wherein said linker is selected from the group consisting of a cleavable linker, a non-cleavable linker, a hydrophilic linker, and a dicarboxylic acid based linker.

63. The immunoconjugate of claim 62, wherein said linker is selected from the group consisting of N-(γ maleimidobutyloxy)sulfosuccinimide ester (sulfo-GMBS or sGMBS), γ maleimidobutyric acid N-succinimidyl ester (GMBS), N-succinimidyl 4-(2-pyridyldithio)-2-sulfobutanoate (sulfo-SPDB); N-succinimidyl 4-(2-pyridyldithio)pentanoate (SPP) or N-succinimidyl 4-(2-pyridyldithio)-2-sulfopentanoate (sulfo-SPP); N-succinimidyl 4-(2-pyridyldithio)butanoate (SPDB), N-succinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate (SMCC); N-sulfosuccinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate (sulfoSMCC); N-succinimidyl-4-(iodoacetyl)-aminobenzoate (SIAB); and N-succinimidyl-[(N-maleimidopropionamido)-tetraethyleneglycol] ester (NHS-PEG4-maleimide).

64. The immunoconjugate of any one of claims 61-63, wherein the linker is sulfo-GMBS.

65. The immunoconjugate of any one of claims 61-63, wherein the linker is GMBS.

66. The immunoconjugate of any one of claims 61-63, wherein the linker is sulfo-SPDB.

67. The immunoconjugate of any one of claims 61-66, wherein said cytotoxic agent is selected from the group consisting of a maytansinoid, maytansinoid analog, benzodiazepine, taxoid, CC-1065, CC-1065 analog, duocarmycin, duocarmycin analog, calicheamicin, dolastatin, dolastatin analog, auristatin, tomaymycin derivative, and leptomycin derivative or a prodrug of the agent.

68. The immunoconjugate of any one of claims 61-67, wherein said cytotoxic agent is a maytansinoid.
69. The immunoconjugate of claim 68, wherein said maytansinoid is DM4.
70. The immunoconjugate of claim 68, wherein said maytansinoid is DM21.
71. The immunoconjugate of any one of claims 61-70, further comprising a second (C).
72. The immunoconjugate of claim 71, further comprising a third (C).
73. The immunoconjugate of claim 72, further comprising a fourth (C).
74. A composition comprising at least one immunoconjugate of any one of claims 61-73, wherein the immunoconjugates comprise an average of 3-4 C per A.
75. A pharmaceutical composition comprising the immunoconjugate of any one of claims 47-74 and a pharmaceutically acceptable carrier.
76. The pharmaceutical composition of claim 75, wherein the pharmaceutical composition comprises an average of 2 to 5 drugs per antibody or antigen-binding fragment thereof.
77. The pharmaceutical composition of claim 75, wherein the pharmaceutical composition comprises an average of 3 to 4 drugs per antibody or antigen-binding fragment thereof.
78. The antibody or antigen binding fragment thereof of claims 1-38, the immunoconjugate of any one of claims 47-73, or the composition of any one of claims 43, 44, or 74-77 for use in the treatment of cancer.

79. The antibody, antigen binding fragment, immunoconjugate or composition for use of claim 78, wherein the cancer is ovarian cancer, uterine cancer, peritoneal cancer, fallopian tube cancer, endometrial cancer, lung cancer, or brain cancer.
80. The antibody, antigen binding fragment, immunoconjugate or composition for use of claim 78, wherein the cancer is ovarian cancer.
81. The antibody, antigen binding fragment, immunoconjugate or composition for use of claim 80, wherein the ovarian cancer is platinum-resistant epithelial ovarian cancer.
82. The antibody, antigen binding fragment, immunoconjugate or composition for use of claim 80, wherein the ovarian cancer is relapsed epithelial ovarian cancer.
83. The antibody, antigen binding fragment, immunoconjugate or composition for use of claim 80, wherein the ovarian cancer is platinum-refractory epithelial ovarian cancer.
84. The antibody, antigen binding fragment, immunoconjugate or composition for use of claim 78, wherein the cancer is uterine cancer.
85. The antibody, antigen binding fragment, immunoconjugate or composition for use of claim 78, wherein the cancer is peritoneal cancer.
86. The antibody, antigen binding fragment, immunoconjugate or composition for use of claim 78, wherein the cancer is fallopian tube cancer.
87. The antibody, antigen binding fragment, immunoconjugate or composition for use of claim 78, wherein the cancer is endometrial cancer.
88. The antibody, antigen binding fragment, immunoconjugate or composition for use of claim 78, wherein the cancer is lung cancer.
89. The antibody, antigen binding fragment, immunoconjugate or composition for use of claim 78, wherein the cancer is brain cancer.
90. The antibody, antigen binding fragment, immunoconjugate or composition for use of any one of claims 78-89, wherein the cancer is IMGN853-resistant.

91. The antibody, antigen binding fragment, immunoconjugate or composition for use of any one of claims claim 78-90, wherein the antibody, antigen binding fragment, immunoconjugate or composition is for use with a steroid.
92. Use of the antibody or antigen binding fragment thereof of any one of claims 1-38, the immunoconjugate of any one of claims 47-73, or the composition of any one of claims 43, 44, or 74-77 for in the manufacture of a medicament for the therapeutic treatment of cancer.
93. The use of claim 92, wherein the cancer is ovarian cancer, uterine cancer, peritoneal cancer, fallopian tube cancer, endometrial cancer, lung cancer, or brain cancer.
94. The use of claim 92, wherein the cancer is ovarian cancer.
95. The use of claim 94, wherein the ovarian cancer is platinum-resistant epithelial ovarian cancer.
96. The use of claim 94, wherein the ovarian cancer is relapsed epithelial ovarian cancer.
97. The use of claim 94, wherein the ovarian cancer is platinum-refractory epithelial ovarian cancer.
98. The use of claim 92, wherein the cancer is uterine cancer.
99. The use of claim 92, wherein the cancer is peritoneal cancer.
100. The use of claim 92, wherein the cancer is fallopian tube cancer.
101. The use of claim 92, wherein the cancer is endometrial cancer.
102. The use of claim 92, wherein the cancer is lung cancer.
103. The use of claim 92, wherein the cancer is brain cancer.
104. The use of any one of claims 92-103, wherein the cancer is IMGN853-resistant.
105. The use of any one of claims claim 92-104, wherein the medicament is for use with a steroid.