

efficacious as conventional ADC in xenograft models and are tolerated at higher doses in relevant preclinical models. THIOMABTM antibodies have been engineered for drug attachment at different locations of the antibody (*e.g.*, specific amino acid positions (*i.e.*, sites) within the light chain-Fab, heavy chain-Fab and heavy chain-Fc). The *in vitro* & *in vivo* stability, efficacy and PK properties of THIOMABTM antibodies provide a unique advantage over conventional ADCs due to their homogeneity and site-specific conjugation to cytotoxic drugs.

[0010] SUMMARY

[0011] This application includes disclosure of novel, isolated cysteine engineered antibodies comprising a free cysteine amino acid in the heavy chain or light chain (THIOMABTM antibodies).

[0012] Disclosed is a process to prepare the isolated cysteine engineered antibodies (THIOMABTM antibodies) by mutagenizing a nucleic acid sequence of a parent antibody by replacing one or more amino acid residues by cysteine to encode the cysteine engineered antibody; expressing the cysteine engineered antibody; and isolating the cysteine engineered antibody.

[0013] Also disclosed are conjugates of isolated cysteine engineered antibodies (THIOMABTM antibodies) wherein the antibody is covalently attached to a capture label, a detection label, a drug moiety, or a solid support.

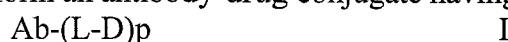
[0013a] In one aspect, there is provided a cysteine engineered antibody comprising an A140C engineered cysteine amino acid in the heavy chain according to EU numbering, and a K149C engineered cysteine amino acid in the light chain according to Kabat numbering.

[0014] In certain embodiments, the disclosure is a cysteine engineered antibody comprising a cysteine mutation selected from the cysteine mutations identified in any of **Tables 1-4** and preferably in either **Table 1** or **2**. In specific embodiments, the cysteine mutation is a free cysteine amino acid. In certain embodiments, the cysteine mutation in the heavy chain selected from the cysteine mutations identified in **Tables 2** or **3**. In certain embodiments, the cysteine mutation in the in the light chain selected from the cysteine mutations identified in **Tables 1** or **4**. In certain embodiments, the cysteine mutation is selected from the group consisting of HC-I195C, HC-S420C, HC-Y432C, and LC-G64C (according to Kabat numbering). In certain embodiments, the cysteine mutation is selected from the group consisting of HC-Y432C and LC-G64C (according to Kabat numbering). In certain embodiments, the cysteine mutation is a heavy chain mutation and is selected from the group consisting of Y33C, G162C, V184C, I195C, S420C, Y432C, and Q434C (according to Kabat numbering). In certain embodiments, the cysteine mutation is a heavy chain mutation and is selected from the group consisting of R19C, E46C, T57C, Y59C,

selected from ^3H , ^{11}C , ^{14}C , ^{18}F , ^{32}P , ^{35}S , ^{64}Cu , ^{68}Ga , ^{86}Y , ^{89}Zr , ^{99}Tc , ^{111}In , ^{123}I , ^{124}I , ^{125}I , ^{131}I , ^{133}Xe , ^{177}Lu , ^{211}At , and ^{213}Bi . In specific embodiments the antibody is covalently attached to a detection label by a chelating ligand. In specific embodiments the chelating ligand is selected from DOTA, DOTP, DOTMA, DTPA and TETA.

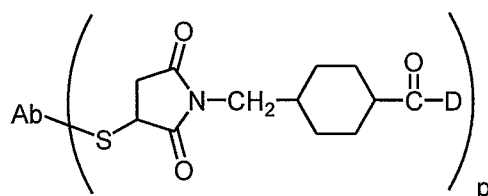
[0022a] Disclosed is an antibody drug conjugate, wherein the antibody drug conjugate is selected from one of the antibody drug conjugates recited in Table 18 or Table 19.

[0022b] In one aspect, there is provided an antibody-drug conjugate comprising the cysteine engineered antibody as described herein, wherein the antibody is covalently attached to a drug moiety to form an antibody-drug conjugate having Formula I:

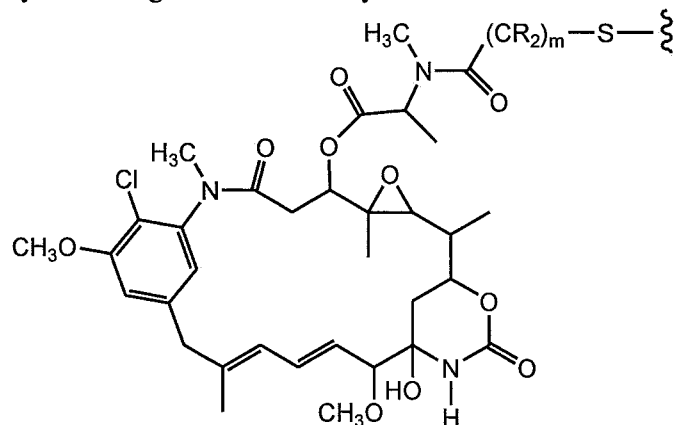


where Ab is the antibody, L is a linker, D is the drug moiety, and p is 1, 2, 3, or 4, and wherein the drug moiety is conjugated to an engineered cysteine amino acid as described herein.

[0023] In certain embodiments, a cysteine engineered antibody as described herein is covalently attached to a drug moiety selected from a maytansinoid, an auristatin, a dolastatin, a trichothecene, CC1065, a calicheamicin, enediyne antibiotics, a taxane, and an anthracycline to form an antibody-drug conjugate having Formula I (*i.e.*, $\text{Ab}-(\text{L}-\text{D})_p$) where Ab is the antibody, L is a linker, D is the drug moiety, and p is 1, 2, 3, or 4. In specific embodiments the antibody-drug conjugate has the structure:



[0024] In specific embodiments, the drug (D) of Formula I of that is conjugated to a cysteine engineered antibody as described herein is a maytansinoid having the structure:



[0025] wherein the wavy line indicates the covalent attachment of the sulfur atom of D to the linker; R is independently selected from H, methyl, ethyl, 1-propyl, 2-propyl, 1-butyl, 2-methyl-1-propyl, 2-butyl, 2-methyl-2-propyl, 1-pentyl, 2-pentyl, 3-pentyl, 2-methyl-2-butyl, 3-methyl-2-butyl, 3-methyl-1-butyl, 2-methyl-1-butyl, 1-hexyl, 2-hexyl, 3-hexyl, 2-methyl-2-pentyl, 3-methyl-2-pentyl, 4-methyl-2-pentyl, 3-methyl-3-pentyl, 2-methyl-3-pentyl, 2,3-dimethyl-2-butyl, and 3,3-dimethyl-2-butyl; and m is 1, 2, or 3.

[0026] In specific embodiments, the drug (D) of Formula I of that is conjugated to a cysteine engineered antibody as described herein is selected from the structures:

selected from the group consisting of a maleimide, an iodoacetamide, and a pyridyl disulfide. In specific embodiments, the linker (L) is a pyridyl disulfide. In specific embodiments, the linker (L) is a pyridyl disulfide and the drug (D) is MMAE.

[0031a] In one aspect, there is provided a method of preparing an antibody-drug conjugate comprising reacting at least one cysteine of a cysteine engineered antibody (Ab) with a linker-drug intermediate to form an antibody-drug conjugate having Formula I:



wherein Ab is any cysteine engineered antibody as described herein, L is a linker, D is a drug moiety, and p is 1, 2, 3, or 4.

[0032] In certain embodiments, this disclosure comprises a method of preparing an antibody-drug conjugate comprising reacting at least one free cysteine of a cysteine engineered antibody (Ab) with a linker-drug (L-D) reagent to form an antibody-drug conjugate having Formula (*i.e.*, $\text{Ab}-(\text{L}-\text{D})_p$) wherein Ab is the cysteine engineered antibody, L is a linker, D is a drug moiety, and p is 1, 2, 3, or 4; and wherein the cysteine engineered antibody comprises one or more free cysteine amino acids, wherein at least one free cysteine amino acid is selected from the cysteine mutations identified in any of **Tables 1 or 2** or alternatively is selected from an alternate stable cysteine mutation identified in **Tables 3 or 4**. In certain embodiments, this disclosure comprises a method of preparing an antibody-drug conjugate comprising reacting at least one free cysteine of a cysteine engineered antibody (Ab) with a linker-drug (L-D) reagent to form an antibody-drug conjugate having Formula (*i.e.*, $\text{Ab}-(\text{L}-\text{D})_p$) wherein Ab is the cysteine engineered antibody, L is a linker, D is a drug moiety, and p is 1, 2, 3, or 4; and wherein the cysteine engineered antibody comprises one or more free cysteine amino acids, wherein at least one free cysteine amino acid is preferably selected from the cysteine mutations identified in **Tables 1 and 2** or alternatively is selected from an alternate stable cysteine mutation identified in **Tables 3 or 4**.

[0033] In specific embodiments, the methods can be used to make a cysteine engineered antibody, wherein the in the cysteine mutation is in the heavy chain and is selected from the cysteine mutations identified in **Tables 2 or 3**. In specific embodiments, the methods can be used to make a cysteine engineered antibody, wherein the in the cysteine mutation is in the light chain and is selected from the cysteine mutations identified in **Tables 1 and 4**. In a preferred embodiment, the methods can be used to make a cysteine engineered antibody, wherein the in the cysteine mutation is selected from the cysteine mutations identified in **Figure 21**. In other embodiments, the methods can be used to make a cysteine engineered antibody, wherein the in the cysteine mutation is selected from the cysteine mutations identified in **Table 5**. In certain embodiments, the methods can be used to make a cysteine engineered antibody, wherein the in the cysteine mutation is selected from the group consisting of HC-I195C, HC-S420C, HC-Y432C, and LC-G64C (according to Kabat

CLAIMS

1. A cysteine engineered antibody comprising an A140C engineered cysteine amino acid in the heavy chain according to EU numbering, and a K149C engineered cysteine amino acid in the light chain according to Kabat numbering.

2. The cysteine engineered antibody of claim 1, wherein the cysteine engineered antibody comprises the sequences:

HC-A140C	TSGGT <u>C</u> ALGCL	SEQ ID NO.:6 in the heavy chain,
and		
LC-K149C	AKVQW <u>C</u> VDNAL	SEQ ID NO.:4 in the light chain.

3. The cysteine engineered antibody of claim 1 or claim 2 prepared by a process comprising:

- (i) mutagenizing a nucleic acid sequence of a parent antibody by replacing one or more amino acid residues by cysteine to encode the cysteine engineered antibody;
- (ii) expressing the cysteine engineered antibody; and
- (iii) isolating the cysteine engineered antibody.

4. The cysteine engineered antibody of claim 3 wherein the mutagenizing comprises site directed mutagenesis.

5. The cysteine engineered antibody of claim 3 wherein the cysteine is a free cysteine and wherein the process further comprises:

- (i) reacting the free cysteine of the cysteine engineered antibody with a thiol-reactive affinity reagent to generate an affinity labelled, cysteine engineered antibody comprising the affinity reagent covalently attached to the free cysteine; and
- (ii) measuring the binding of the affinity labelled, cysteine engineered antibody to a capture media.

6. The cysteine engineered antibody of claim 5 wherein the thiol-reactive reagent comprises a maleimide moiety.

7. The cysteine engineered antibody of claim 5 wherein the thiol-reactive reagent comprises a biotin moiety and the capture media comprises streptavidin.

8. The cysteine engineered antibody of any of claims 1-7, wherein the cysteine engineered antibody is selected from a monoclonal antibody, an antibody fragment, a

bispecific antibody, a chimeric antibody, a human antibody, and a humanized antibody.

9. The cysteine engineered antibody of any of claims 1-8, wherein the antibody fragment is a Fab fragment.

10. The cysteine engineered antibody of any of claims 1-9, wherein the cysteine engineered antibody is an anti-HER2 antibody.

11. The cysteine engineered antibody of claim 10, wherein the anti-HER2 antibody is trastuzumab.

12. The cysteine engineered antibody of any of claims 1-9, wherein the cysteine engineered antibody is an anti-Ly6E antibody.

13. The cysteine engineered antibody of claim 12, wherein the anti-Ly6E antibody comprises

(i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 179, and an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 180; an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 181; an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 176; an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 177; and an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 178; or

(ii) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 175 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 174.

14. The cysteine engineered antibody of any of claims 1-9, wherein the cysteine engineered antibody is an anti-CD79b antibody.

15. The cysteine engineered antibody of claim 14, wherein the anti-CD79b antibody comprises

(i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 186, and an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 187; an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 188; an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 189; an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 190; and an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 191; or

(ii) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 184 and a light chain variable region comprising the amino acid sequence of SEQ ID

NO: 185.

16. The cysteine engineered antibody of any of claims 1-9, wherein the cysteine engineered antibody is an anti-MUC16 antibody.

17. The cysteine engineered antibody of claim 16, wherein the anti-MUC16 antibody comprises

(i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 152, and an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 153; an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 154; an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 150; an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 151; and an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 152; or

(ii) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 156 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 157.

18. The cysteine engineered antibody of any of claims 1-9, wherein the cysteine engineered antibody is an anti-STEAP1 antibody.

19. The cysteine engineered antibody of claim 18, wherein the anti-STEAP1 antibody comprises

(i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 157, and an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 158; an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 159; an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 160; an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 161; and an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 162; or

(ii) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 163 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 164.

20. The cysteine engineered antibody of any of claims 1-9, wherein the cysteine engineered antibody is an anti-NaPi2b antibody.

21. The cysteine engineered antibody of claim 20, wherein the anti-NaPi2b antibody comprises

(i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 165, and an

HVR-H2 comprising the amino acid sequence of SEQ ID NO: 167; an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 168; an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 169; an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 170; and an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 171; or

(ii) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 172 and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 173.

22. The cysteine engineered antibody of any of claims 1-9, wherein the cysteine engineered antibody is an anti-CD22 antibody.

23. The cysteine engineered antibody of claim 22, wherein the anti-CD22 antibody comprises

(i) an HVR-H1 comprising the amino acid sequence of SEQ ID NO: 192, and an HVR-H2 comprising the amino acid sequence of SEQ ID NO: 193; an HVR-H3 comprising the amino acid sequence of SEQ ID NO: 194; an HVR-L1 comprising the amino acid sequence of SEQ ID NO: 195; an HVR-L2 comprising the amino acid sequence of SEQ ID NO: 196; and an HVR-L3 comprising the amino acid sequence of SEQ ID NO: 197

24. The cysteine engineered antibody of any of claims 1-9, wherein the cysteine engineered antibody binds to one or more of receptors (1)-(53):

- (1) BMPR1B (bone morphogenetic protein receptor-type IB);
- (2) E16 (LAT1, SLC7A5);
- (3) STEAP1 (six transmembrane epithelial antigen of prostate);
- (4) 0772P (CA125, MUC16);
- (5) MPF (MPF, MSLN, SMR, megakaryocyte potentiating factor, mesothelin);
- (6) Napi3b (NaPi2b, NAPI-3B, NPTIIB, SLC34A2, solute carrier family 34 (sodium phosphate), member 2, type II sodium-dependent phosphate transporter 3b);
- (7) Sema 5b (FLJ10372, KIAA1445, Mm.42015, SEMA5B, SEMAG, Semaphorin 5b Hlog, sema domain, seven thrombospondin repeats (type 1 and type 1-like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5B);
- (8) PSCA hlg (2700050C12Rik, C530008O16Rik, RIKEN cDNA 2700050C12, RIKEN cDNA 2700050C12 gene);
- (9) ETBR (Endothelin type B receptor);
- (10) MSG783 (RNF124, hypothetical protein FLJ20315);

- (11) STEAP2 (HGNC_8639, IPCA-1, PCANAP1, STAMP1, STEAP2, STMP, prostate cancer associated gene 1, prostate cancer associated protein 1, six transmembrane epithelial antigen of prostate 2, six transmembrane prostate protein);
- (12) TrpM4 (BR22450, FLJ20041, TRPM4, TRPM4B, transient receptor potential cation channel, subfamily M, member 4);
- (13) CRIPTO (CR, CR1, CRGF, CRIPTO, TDGF1, teratocarcinoma-derived growth factor);
- (14) CD21 (CR2 (Complement receptor 2) or C3DR (C3d/Epstein Barr virus receptor) or Hs.73792);
- (15) CD79b (CD79B, CD79 β , Ig β (immunoglobulin-associated beta), B29);
- (16) FcRH2 (IFGP4, IRTA4, SPAP1A (SH2 domain containing phosphatase anchor protein 1a), SPAP1B, SPAP1C);
- (17) HER2;
- (18) NCA;
- (19) MDP;
- (20) IL20R α ;
- (21) Brevican;
- (22) EphB2R;
- (23) ASLG659;
- (24) PSCA;
- (25) GEDA;
- (26) BAFF-R (B cell -activating factor receptor, BLyS receptor 3, BR3);
- (27) CD22 (B-cell receptor CD22-B isoform);
- (28) CD79a (CD79A, CD79 α , immunoglobulin-associated alpha, a B cell-specific protein);
- (29) CXCR5 (Burkitt's lymphoma receptor 1, a G protein-coupled receptor);
- (30) HLA-DOB (Beta subunit of MHC class II molecule (Ia antigen);
- (31) P2X5 (Purinergic receptor P2X ligand-gated ion channel 5);
- (32) CD72 (B-cell differentiation antigen CD72, Lyb-2);
- (33) LY64 (Lymphocyte antigen 64 (RP105), type I membrane protein of the leucine rich repeat (LRR) family);
- (34) FcRH1 (Fc receptor-like protein 1);
- (35) IRTA2 (Immunoglobulin superfamily receptor translocation associated 2);

- (36) TENB2 (putative transmembrane proteoglycan);
- (37) PMEL17 (silver homolog; SILV; D12S53E; PMEL17; SI; SIL);
- (38) TMEFF1 (transmembrane protein with EGF-like and two follistatin-like domains 1; Tomoregulin-1);
- (39) GDNF-Ra1 (GDNF family receptor alpha 1; GFRA1; GDNFR; GDNFRA; RETL1; TRNR1; RET1L; GDNFR-alpha1; GFR-ALPHA-1);
- (40) Ly6E (lymphocyte antigen 6 complex, locus E; Ly67,RIG-E,SCA-2,TSA-1)
- (41) TMEM46 (shisa homolog 2);
- (42) Ly6G6D (lymphocyte antigen 6 complex, locus G6D; Ly6-D, MEGT1);
- (43) LGR5 (leucine-rich repeat-containing G protein-coupled receptor 5; GPR49, GPR67);
- (44) RET (ret proto-oncogene; MEN2A; HSCR1; MEN2B; MTC1; PTC; CDHF12; Hs.168114; RET51; RET-ELE1);
- (45) LY6K (lymphocyte antigen 6 complex, locus K; LY6K; HSJ001348; FLJ35226);
- (46) GPR19 (G protein-coupled receptor 19; Mm.4787);
- (47) GPR54 (KISS1 receptor; KISS1R; GPR54; HOT7T175; AXOR12);
- (48) ASPHD1 (aspartate beta-hydroxylase domain containing 1; LOC253982);
- (49) Tyrosinase (TYR; OCAIA; OCA1A; tyrosinase; SHEP3);
- (50) TMEM118 (ring finger protein, transmembrane 2; RNFT2; FLJ14627);
- (51) GPR172A (G protein-coupled receptor 172A; GPCR41; FLJ11856; D15Ert747e);
- (52) CD33; and
- (53) CLL-1 (CLEC12A, MICL, and DCAL2).

25. The cysteine engineered antibody of any of claims 1-24, wherein the antibody is covalently attached to a capture label, a detection label, a drug moiety, or a solid support.

26. The cysteine engineered antibody of claim 25, wherein the antibody is covalently attached to a biotin capture label.

27. The cysteine engineered antibody of claim 25, wherein the antibody is covalently attached to a fluorescent dye detection label.

28. The cysteine engineered antibody of claim 27, wherein the fluorescent dye is selected from a fluorescein type, a rhodamine type, dansyl, Lissamine, a cyanine, a phycoerythrin, Texas Red, and an analog thereof.

29. The cysteine engineered antibody of claim 25, wherein the antibody is covalently attached to a radionuclide detection label selected from ^3H , ^{11}C , ^{14}C , ^{18}F , ^{32}P , ^{35}S , ^{64}Cu , ^{68}Ga , ^{86}Y , ^{89}Zr , ^{99}Tc , ^{111}In , ^{123}I , ^{124}I , ^{125}I , ^{131}I , ^{133}Xe , ^{177}Lu , ^{211}At , and ^{213}Bi .

30. The cysteine engineered antibody of claim 25, wherein the antibody is covalently attached to a detection label by a chelating ligand.

31. The cysteine engineered antibody of claim 30 wherein the chelating ligand is selected from DOTA, DOTP, DOTMA, DTPA and TETA.

32. An antibody-drug conjugate comprising the cysteine engineered antibody of claim any of claims 1-24, wherein the antibody is covalently attached to a drug moiety to form an antibody-drug conjugate having Formula I:



where Ab is the antibody, L is a linker, D is the drug moiety, and p is 1, 2, 3, or 4, and wherein the drug moiety is conjugated to an engineered cysteine amino acid of claims 1 or 2.

33. The antibody-drug conjugate of claim 32, wherein L comprises a group selected from 6-maleimidocaproyl (MC), maleimidopropanoyl (MP), valine-citrulline (val-cit (-vc)), alanine-phenylalanine (ala-phe), and p-aminobenzyloxycarbonyl (PAB).

34. The antibody-drug conjugate of claim 32 prepared from a linker reagent selected from N-Succinimidyl 4-(2-pyridylthio) pentanoate (SPP), N-succinimidyl 4-(N-maleimidomethyl) cyclohexane-1 carboxylate (SMCC), 4-(2-Pyridyldithio)butyric acid-N-hydroxysuccinimide ester (SPDB), and N-Succinimidyl (4-iodo-acetyl) aminobenzoate (SIAB).

35. The antibody-drug conjugate of claim 32, prepared from a linker reagent comprising maleimide, iodoacetamide, bromoacetamide or disulfide.

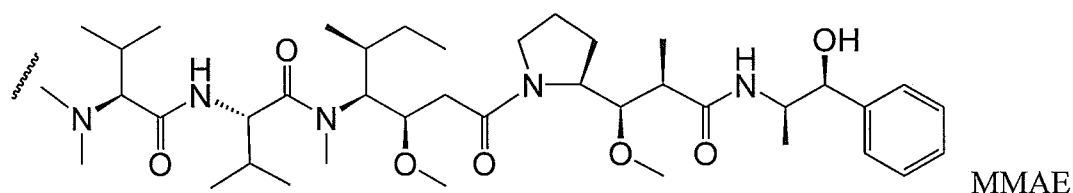
36. The antibody-drug conjugate of claim 32, wherein L forms a disulfide linker.

37. The antibody-drug conjugate of claim 35, wherein the linker reagent comprises a pyridyl disulfide (PDS).

38. The antibody-drug conjugate of claim 32, wherein L comprises valine-citrulline (val-cit (-vc)).

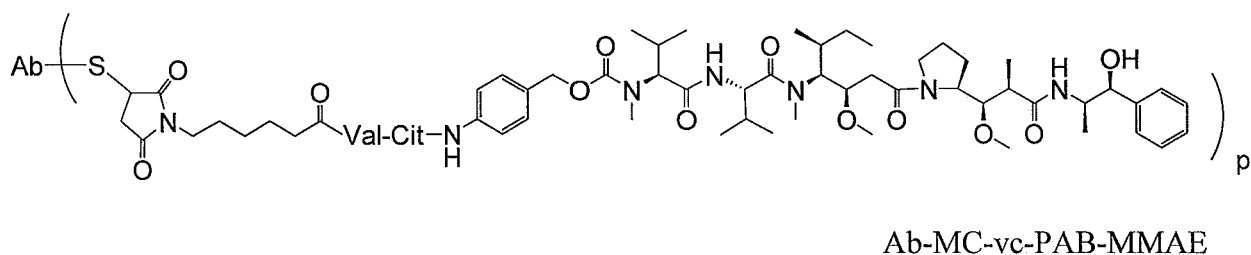
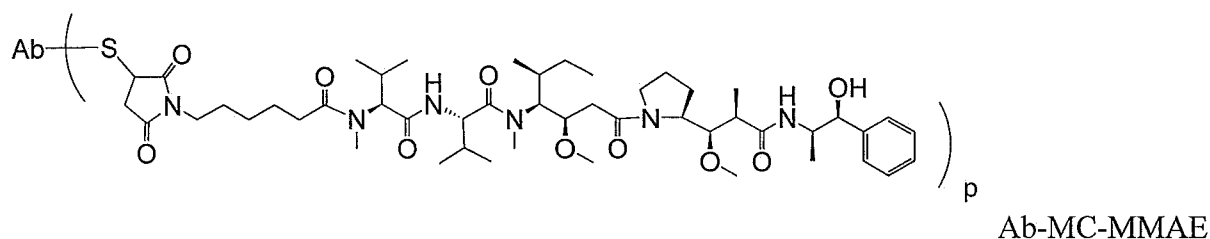
39. The antibody-drug conjugate of any of claims 32-38, wherein the drug moiety (D) is a maytansinoid, an auristatin, a dolastatin, a trichothecene, CC1065, a calicheamicin, enediyne antibiotics, a taxane, a pyrrolobenzodiazepine (PBD) dimer, a 1-(Chloromethyl)-2,3-dihydro-1H-benzo[e]indole (CBI) dimer, a CBI-PBD heterodimer, or an anthracycline.

40. The antibody-drug conjugate of claim 39 wherein D is a monomethylauristatin drug moiety MMAE having the structure:



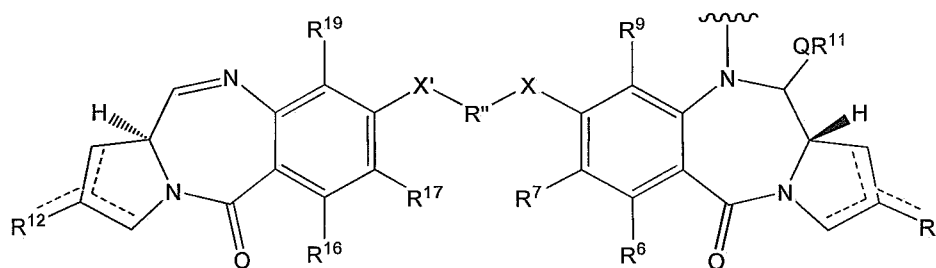
wherein the wavy line indicates the covalent attachment site to the linker.

41. The antibody-drug conjugate of claim 39 wherein the antibody-drug conjugate is selected from the structures:



where Val is valine; Cit is citrulline; and p is 1, 2, 3, or 4.

42. The antibody drug conjugate of claim 39 wherein D is a PBD dimer drug having the structure:



and salts and solvates thereof, wherein:

the wavy line indicates the covalent attachment site to the linker;

the dotted lines indicate the optional presence of a double bond between C1 and C2 or C2 and C3;

R^2 is independently selected from H, OH, =O, =CH₂, CN, R, OR, =CH-R^D, =C(R^D)₂, O-SO₂-R, CO₂R and COR, and optionally further selected from halo or dihalo, wherein R^D is independently selected from R, CO₂R, COR, CHO, CO₂H, and halo;

R^6 and R^9 are independently selected from H, R, OH, OR, SH, SR, NH₂, NHR, NRR', NO₂, Me₃Sn and halo;

R^7 is independently selected from H, R, OH, OR, SH, SR, NH₂, NHR, NRR', NO₂, Me₃Sn and halo;

Q is independently selected from O, S and NH;

R^{11} is either H, or R or, where Q is O, SO₃M, where M is a metal cation;

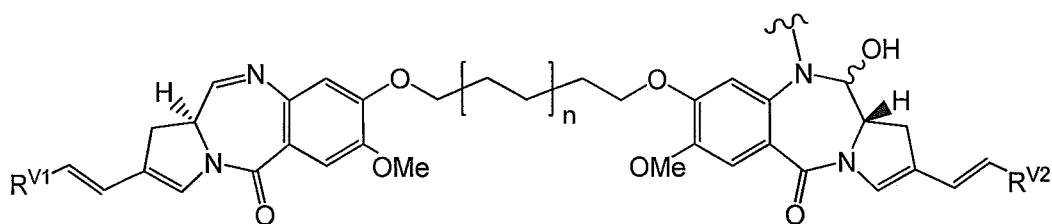
R and R' are each independently selected from optionally substituted C₁₋₈ alkyl, C₁₋₁₂ alkyl, C₃₋₈ heterocyclyl, C₃₋₂₀ heterocycle, and C₅₋₂₀ aryl groups, and optionally in relation to the group NRR', R and R' together with the nitrogen atom to which they are attached form an optionally substituted 4-, 5-, 6- or 7-membered heterocyclic ring;

R^{12} , R^{16} , R^{19} and R^{17} are as defined for R^2 , R^6 , R^9 and R^7 respectively;

R'' is a C₃₋₁₂ alkylene group, which chain may be interrupted by one or more heteroatoms, *e.g.* O, S, N(H), NMe and/or aromatic rings, *e.g.* benzene or pyridine, which rings are optionally substituted; and

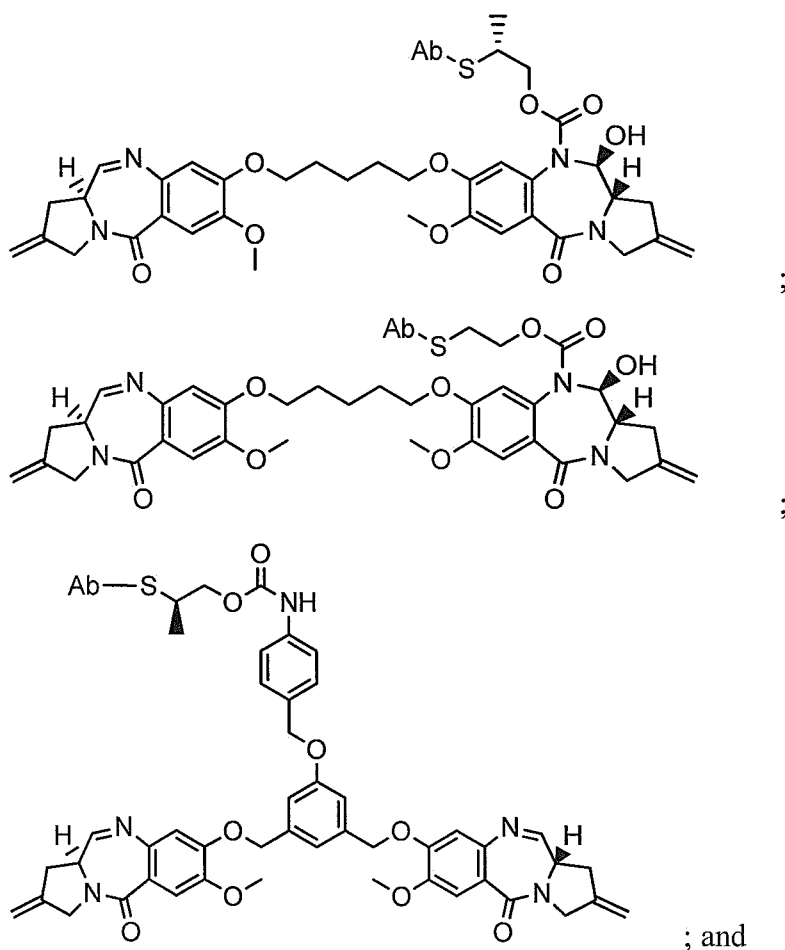
X and X' are independently selected from O, S and N(H).

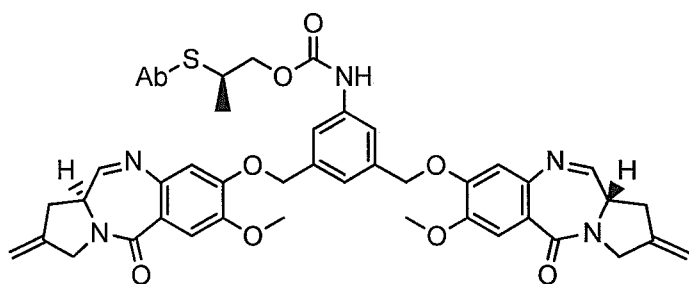
43. The antibody drug conjugate of claim 42 wherein the structure of the PBD dimer is:



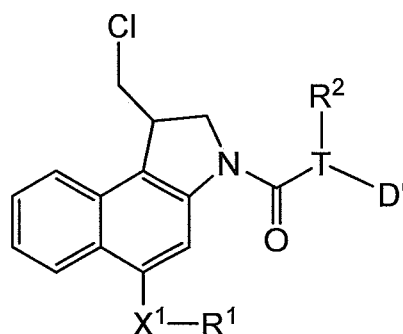
including salts and solvates thereof, wherein the wavy line indicates the covalent attachment site to the linker; the wavy line connected to the OH indicates the S or R configuration; R^{V1} and R^{V2} are independently selected from H, methyl, ethyl and phenyl (which phenyl may be optionally substituted with fluoro, particularly in the 4 position) and C_{5-6} heterocyclyl; wherein R^{V1} and R^{V2} may be the same or different; and n is 0 or 1.

44. The antibody drug conjugate of claim 32 selected from:





45. The antibody drug conjugate of claim 39 wherein D is a CBI dimer drug having the structure:



where

R^1 is selected from H, $P(O)_3H_2$, $C(O)NR^aR^b$, or a bond to a linker (L);

R^2 is selected from H, $P(O)_3H_2$, $C(O)NR^aR^b$, or a bond to a linker (L);

R^a and R^b are independently selected from H and C_1-C_6 alkyl optionally substituted with one or more F, or R^a and R^b form a five or six membered heterocyclyl group;

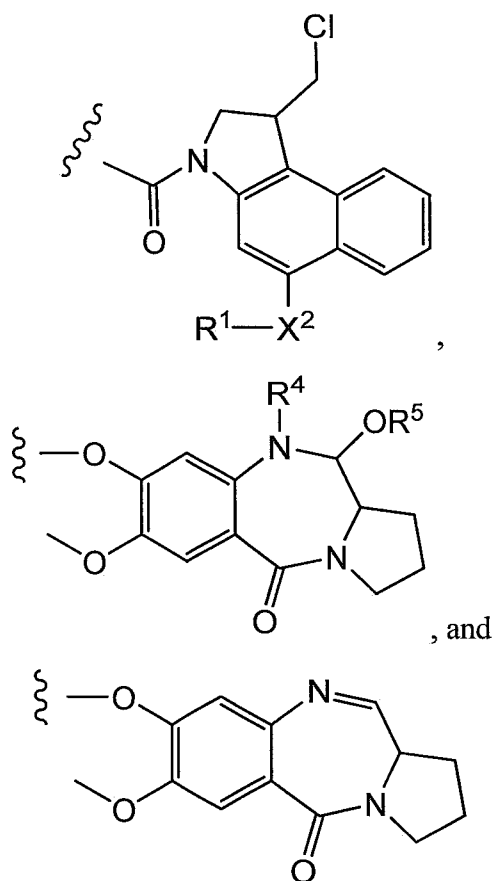
T is a tether group selected from C_3-C_{12} alkylene, Y, $(C_1-C_6 \text{ alkylene})-Y-(C_1-C_6 \text{ alkylene})$, $(C_1-C_6 \text{ alkylene})-Y-(C_1-C_6 \text{ alkylene})-Y-(C_1-C_6 \text{ alkylene})$, $(C_2-C_6 \text{ alkenylene})-Y-(C_2-C_6 \text{ alkenylene})$, and $(C_2-C_6 \text{ alkynylene})-Y-(C_2-C_6 \text{ alkynylene})$;

where Y is independently selected from O, S, NR^1 , aryl, and heteroaryl;

where alkylene, alkenylene, aryl, and heteroaryl are independently and optionally substituted with F, OH, $O(C_1-C_6 \text{ alkyl})$, NH_2 , $NHCH_3$, $N(CH_3)_2$, $OP(O)_3H_2$, and $C_1-C_6 \text{ alkyl}$, where alkyl is optionally substituted with one or more F;

or alkylene, alkenylene, aryl, and heteroaryl are independently and optionally substituted with a bond to L;

D' is a drug moiety selected from:



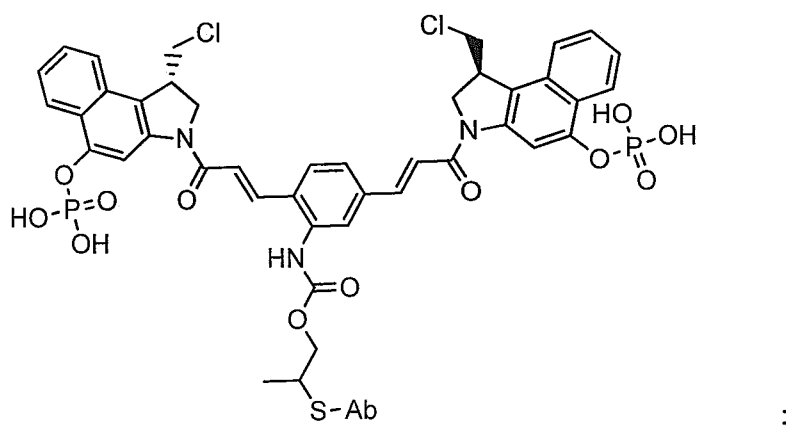
where the wavy line indicates the site of attachment to T;

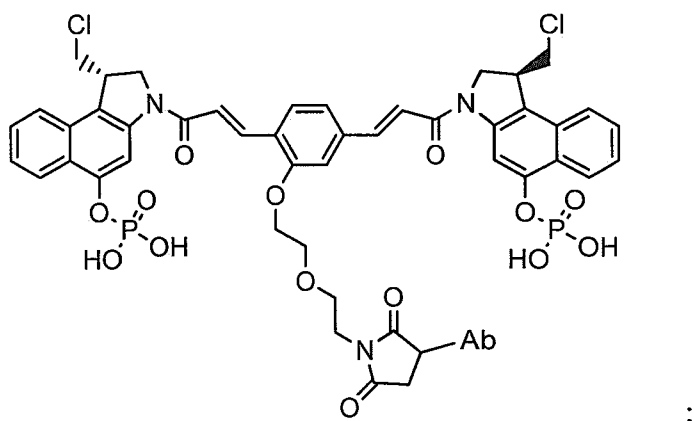
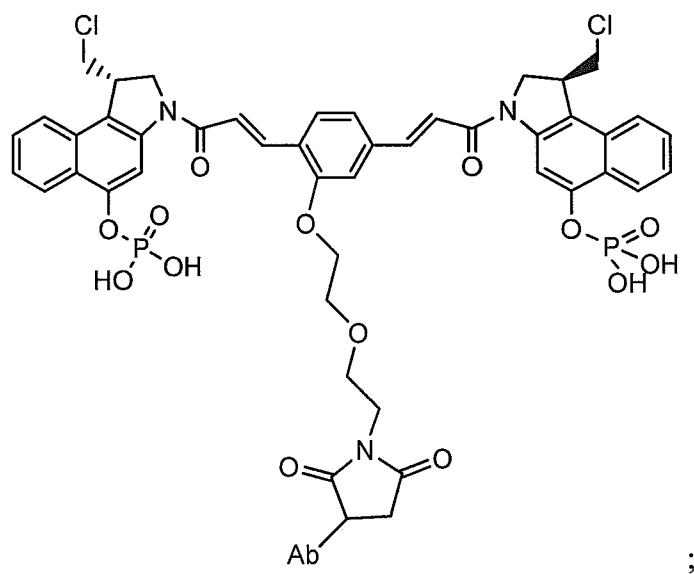
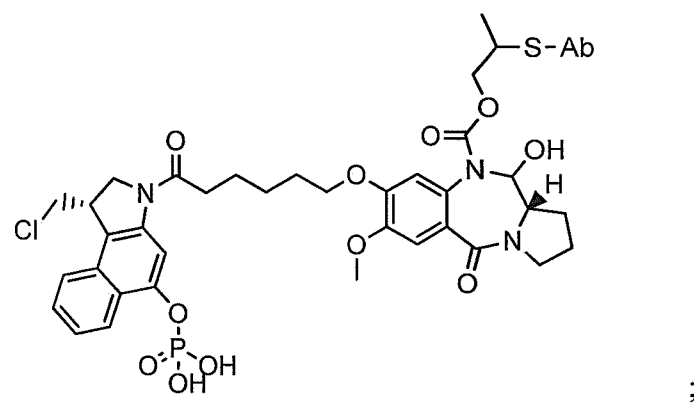
X^1 and X^2 are independently selected from O and NR^3 , where R^3 is selected from H and C_1 – C_6 alkyl optionally substituted with one or more F;

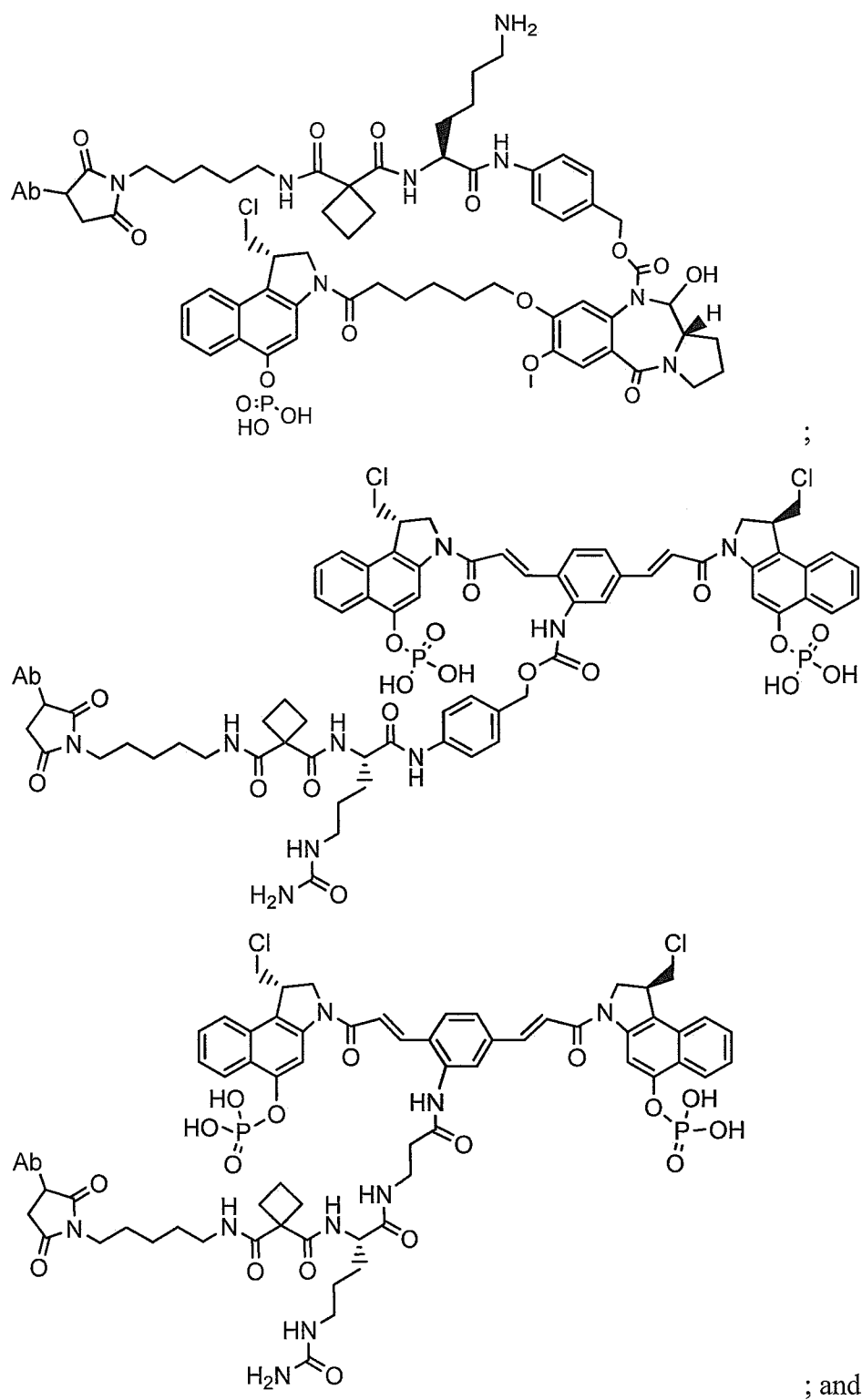
R^4 is H, CO_2R , or a bond to a linker (L), where R is C_1 – C_6 alkyl or benzyl; and

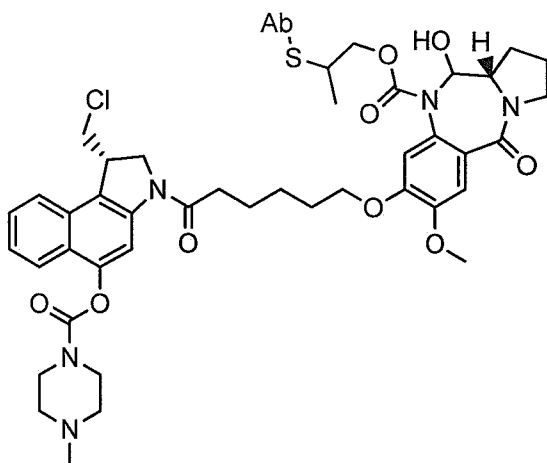
R^5 is H or C_1 – C_6 alkyl.

46. The antibody drug conjugate of claim 45 selected from:









47. A method of preparing an antibody-drug conjugate comprising reacting at least one cysteine of a cysteine engineered antibody (Ab) with a linker-drug intermediate to form an antibody-drug conjugate having Formula I:

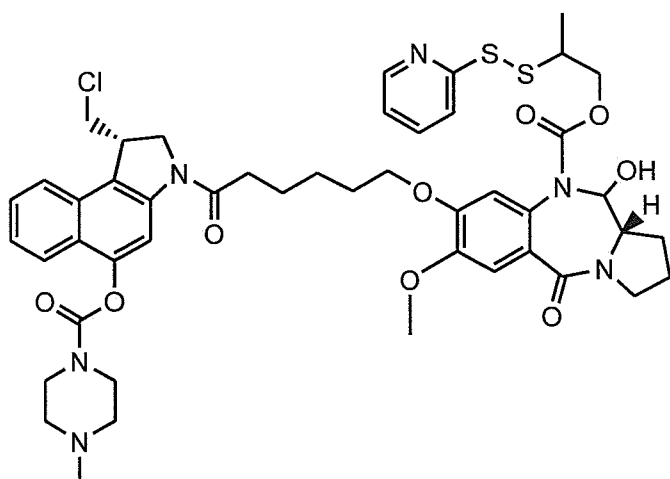


wherein Ab is the cysteine engineered antibody of any of claims 1-24, L is a linker, D is a drug moiety, and p is 1, 2, 3, or 4.

48. The antibody drug conjugate of claim 32 wherein L comprises a pyridyl disulfide (PDS), and D is selected from the group consisting of a CBI-PBD heterodimer, cryptophycin, a taxoid, and tubulysin M.

49. The antibody drug conjugate of claim 32 wherein L comprises a -vc linker, and D is selected from the group consisting of a CBI-PBD heterodimer, cryptophycin, a taxoid, and tubulysin M.

50. The antibody drug conjugate of claim 48 wherein the D is the CBI-PBD heterodimer:



51. The antibody drug conjugate of claim 32 wherein L comprises a pyridyl disulfide (PDS).

52. A pharmaceutical composition comprising a cysteine engineered antibody or an antibody drug conjugate of any of claims 1-51.