

DUAL-CLEAVAGE ESTER LINKERS FOR ANTIBODY-DRUG CONJUGATES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 63/138,255, filed January 15, 2021, the disclosure of which is incorporated herein by reference.

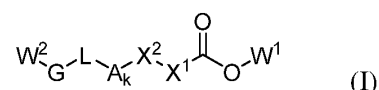
INTRODUCTION

[0002] Antibody-drug conjugates (ADCs) have emerged over the past two decades as a new class of targeted-delivery therapies. A typical ADC (FIG. 1) comprises an antibody-based targeting element connected to a highly potent pharmaceutical agent (payload) via a chemical linker. The molar ratio of targeting element (e.g., antibody) to attached payload can vary, and is referred to as the drug-to-antibody ratio (DAR).

SUMMARY

[0003] The present disclosure provides antibody-drug conjugate structures, where the antibody-drug conjugate includes a cleavable linker containing an ester group that links the antibody to the drug. The disclosure also encompasses compounds and methods for production of such conjugates. In addition, the disclosure also encompasses pharmaceutical compositions and methods of using the conjugates.

[0004] Aspects of the present disclosure include a conjugate of formula (I):



wherein:

W¹ is a drug;

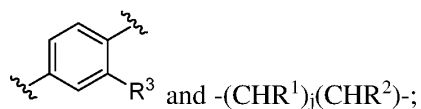
W² is a polypeptide;

A is an amino acid residue, where k is 0 or an integer from 1 to 5;

L is a linker;

G is a conjugation moiety;

X¹ is selected from:



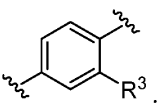
R^1 is selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, substituted heterocyclyl and R^3 , wherein R^1 is optionally substituted with R^3 ;

j is 0 or an integer from 1 to 5;

R^2 is R^3 ; or R^2 is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl, wherein R^2 substituted with R^3 ;

X^2 is $-\text{NH}-$ or $-\text{C}(\text{O})-$;

each R^3 is independently a glycoside or glycoside derivative.

[0005] In some embodiments, X^1 is .

[0006] In some embodiments, X^1 is $-(\text{CHR}^1)_j(\text{CHR}^2)-$.

[0007] In some embodiments, j is 0.

[0008] In some embodiments, R^2 is alkyl.

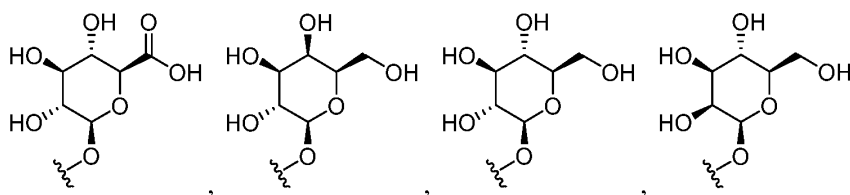
[0009] In some embodiments, R^2 is aryl.

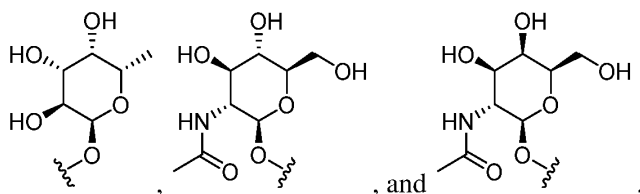
[0010] In some embodiments, j is 1.

[0011] In some embodiments, R^1 is hydrogen.

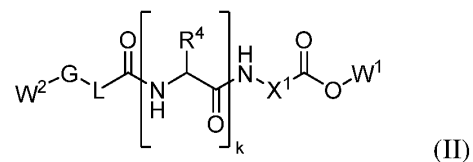
[0012] In some embodiments, R^1 is substituted with R^3 .

[0013] In some embodiments, each R^3 is independently selected from:



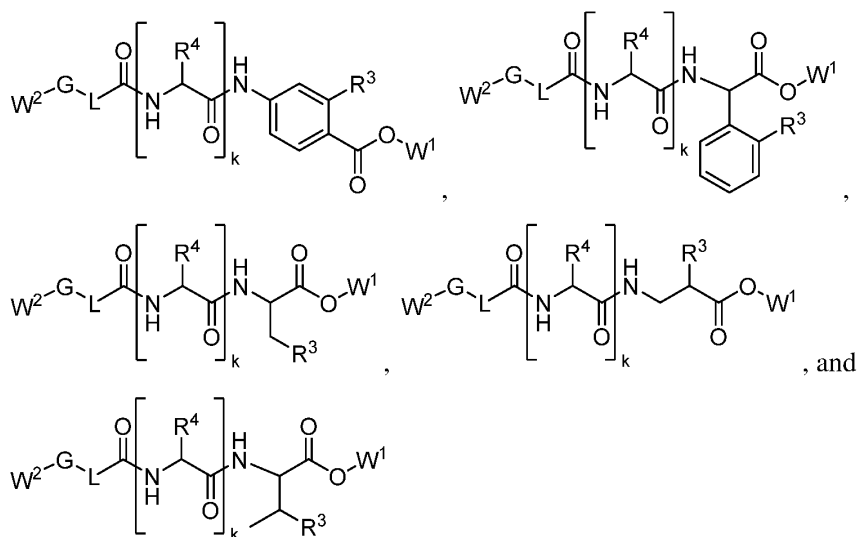


[0014] In some embodiments, the conjugate is of formula (II):

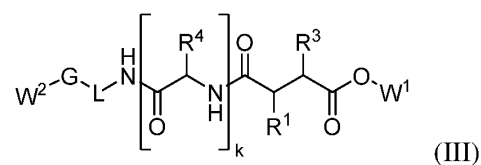


wherein R^4 is an amino acid side chain.

[0015] In some embodiments, the conjugate is selected from:

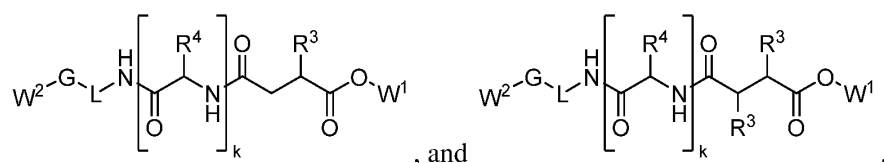


[0016] In some embodiments, the conjugate is of formula (III):



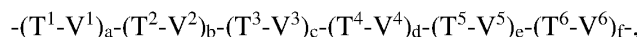
wherein R^4 is an amino acid side chain.

[0017] In some embodiments, the conjugate is selected from:



[0018] In some embodiments, k is 2.

[0019] In some embodiments, L comprises:



wherein

a, b, c, d, e and f are each independently 0 or 1;

T^1 , T^2 , T^3 , T^4 , T^5 and T^6 are each independently selected from a covalent bond, $(\text{C}_1\text{-C}_{12})$ alkyl, substituted $(\text{C}_1\text{-C}_{12})$ alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl, $(\text{EDA})_w$, $(\text{PEG})_n$, $(\text{AA})_p$, $-(\text{CR}^{13}\text{OH})_m$ -, 4-amino-piperidine (4AP), meta-amino-benzyloxy (MABO), meta-amino-benzyloxycarbonyl (MABC), para-amino-benzyloxy (PABO), para-amino-benzyloxycarbonyl (PABC), para-aminobenzyl (PAB), para-amino-benzylamino (PABA), an acetal, a hydrazine, a disulfide, and an ester, wherein EDA is an ethylene diamine moiety, PEG is a polyethylene glycol, and AA is an amino acid residue or an amino acid analog, wherein each w is an integer from 1 to 20, each n is an integer from 1 to 30, each p is an integer from 1 to 20, and each m is an integer from 1 to 12;

V^1 , V^2 , V^3 , V^4 , V^5 and V^6 are each independently selected from the group consisting of a covalent bond, $-\text{CO}-$, $-\text{NR}^{15}-$, $-\text{NR}^{15}(\text{CH}_2)_q-$, $-\text{NR}^{15}(\text{C}_6\text{H}_4)-$, $-\text{CONR}^{15}-$, $-\text{NR}^{15}\text{CO}-$, $-\text{C}(\text{O})\text{O}-$, $-\text{OC}(\text{O})-$, $-\text{O}-$, $-\text{S}-$, $-\text{S}(\text{O})-$, $-\text{SO}_2-$, $-\text{SO}_2\text{NR}^{15}-$, $-\text{NR}^{15}\text{SO}_2-$ and $-\text{P}(\text{O})\text{OH}-$, wherein each q is an integer from 1 to 6;

each R^{13} is independently selected from hydrogen, an alkyl, a substituted alkyl, an aryl, and a substituted aryl; and

each R^{15} is independently selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, carboxyl, carboxyl ester, acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl.

[0020] In some embodiments, the conjugate includes a linker L wherein:

T^1 is $(\text{C}_1\text{-C}_{12})$ alkyl and V^1 is $-\text{CO}-$;

T^2 is an amino acid analog and V^2 is $-\text{NH}-$;

T^3 is $(\text{PEG})_n$ and V^3 is $-\text{CO}-$; and

d to f are each 0; or

wherein:

T^1 is $(\text{C}_1\text{-C}_{12})$ alkyl and V^1 is $-\text{CO}-$;

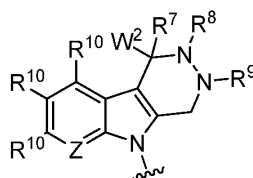
T^2 is an amino acid analog and V^2 is $-\text{NH}-$;

T^3 is $(\text{PEG})_n$ and V^3 is $-\text{CONH}-$;

T^4 is $(\text{PEG})_n$ and V^4 is $-\text{CO}-$;

e and f are each 0.

[0021] In some embodiments, G is:



wherein:

Z is CR^{10} or N,

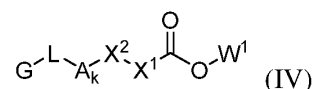
R^7 is selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl;

R^8 and R^9 are each independently selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, amino, substituted amino, carboxyl, carboxyl ester, acyl, acyloxy, acyl amino, amino acyl, alkylamide, substituted alkylamide, sulfonyl, thioalkoxy, substituted thioalkoxy, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl, or R^8 and R^9 are optionally cyclically linked to form a 5 or 6-membered heterocyclyl;

each R^{10} is independently selected from hydrogen, halogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, amino, substituted amino, carboxyl, carboxyl ester, acyl, acyloxy, acyl amino, amino acyl, alkylamide, substituted alkylamide, sulfonyl, thioalkoxy, substituted thioalkoxy, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl.

[0022] In some embodiments, G is selected from an acetal, a hydrazone, an oxime, a sulfide, a disulfide, a triazole, an ester, and an amide.

[0023] Aspects of the present disclosure include a compound of formula (IV):



wherein:

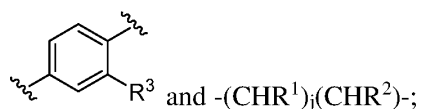
W^1 is a drug;

A is an amino acid residue, where k is 0 or an integer from 1 to 5;

L is a linker;

G is a conjugation moiety;

X^1 is selected from:



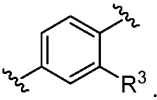
R^1 is selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, substituted heterocyclyl and R^3 , wherein R^1 is optionally substituted with R^3 ;

j is 0 or an integer from 1 to 5;

R^2 is R^3 ; or R^2 is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl, wherein R^2 is substituted with R^3 ;

X^2 is $-NH-$ or $-C(O)-$;

each R^3 is independently a glycoside or glycoside derivative.

[0024] In some embodiments, X^1 is .

[0025] In some embodiments, X^1 is $-(CHR^1)_j(CHR^2)-$.

[0026] In some embodiments, j is 0.

[0027] In some embodiments, R^2 is alkyl.

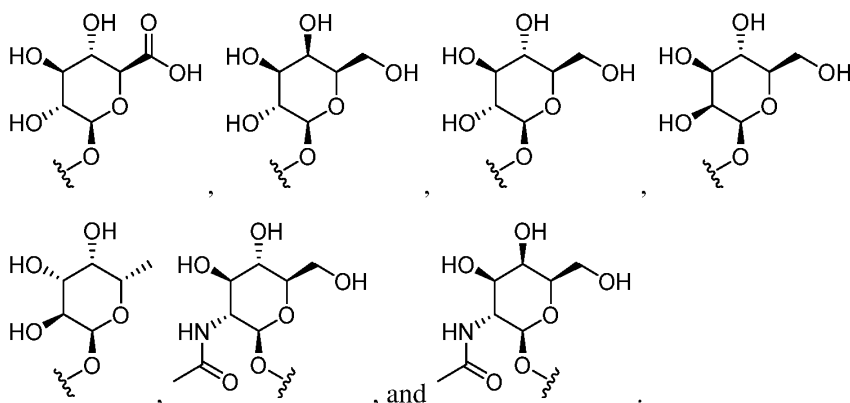
[0028] In some embodiments, R^2 is aryl.

[0029] In some embodiments, j is 1.

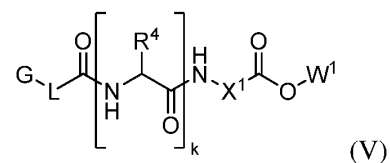
[0030] In some embodiments, R¹ is hydrogen.

[0031] In some embodiments, R¹ is substituted with R³.

[0032] In some embodiments, each R³ is independently selected from:

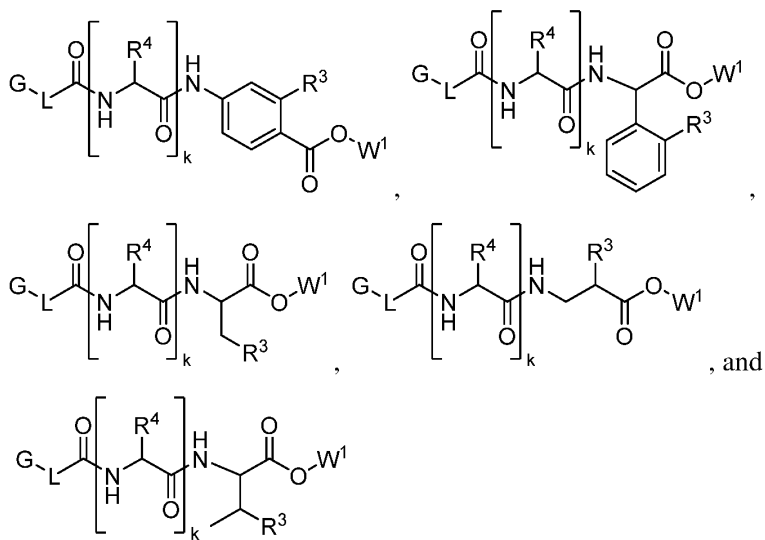


[0033] In some embodiments, the compound is of formula (V):

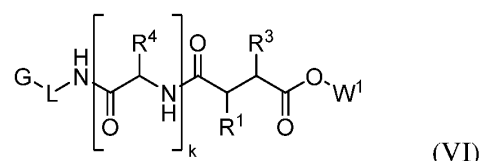


wherein R⁴ is an amino acid side chain.

[0034] In some embodiments, the compound is selected from:

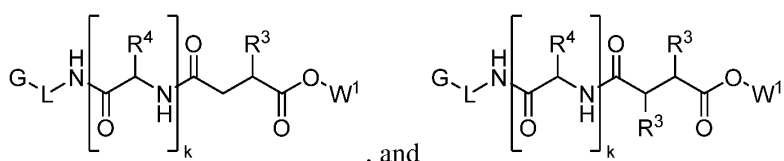


[0035] In some embodiments, the compound is of formula (VI):



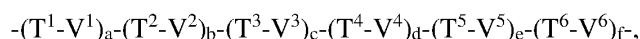
wherein R⁴ is an amino acid side chain.

[0036] In some embodiments, the compound is selected from:



[0037] In some embodiments, k is 2.

[0038] In some embodiments, L comprises:



wherein

a, b, c, d, e and f are each independently 0 or 1;

T¹, T², T³, T⁴, T⁵ and T⁶ are each independently selected from a covalent bond, (C₁-C₁₂)alkyl, substituted (C₁-C₁₂)alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl, (EDA)_w, (PEG)_n, (AA)_p, -(CR¹³OH)_m-, 4-amino-piperidine (4AP), meta-amino-benzyloxy (MABO), meta-amino-benzyloxycarbonyl (MABC), para-amino-benzyloxy (PABO), para-amino-benzyloxycarbonyl (PABC), para-aminobenzyl (PAB), para-amino-benzylamino (PABA), an acetal, a hydrazine, a disulfide, and an ester, wherein EDA is an ethylene diamine moiety, PEG is a polyethylene glycol, and AA is an amino acid residue or an amino acid analog, wherein each w is an integer from 1 to 20, each n is an integer from 1 to 30, each p is an integer from 1 to 20, and each m is an integer from 1 to 12;

V¹, V², V³, V⁴, V⁵ and V⁶ are each independently selected from the group consisting of a covalent bond, -CO-, -NR¹⁵-, -NR¹⁵(CH₂)_q-, -NR¹⁵(C₆H₄)-, -CONR¹⁵-, -NR¹⁵CO-, -C(O)O-, -OC(O)-, -O-, -S-, -S(O)-, -SO₂-, -SO₂NR¹⁵-, -NR¹⁵SO₂- and -P(O)OH-, wherein each q is an integer from 1 to 6;

each R¹³ is independently selected from hydrogen, an alkyl, a substituted alkyl, an aryl, and a substituted aryl; and

each R^{15} is independently selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, carboxyl, carboxyl ester, acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl.

[0039] In some embodiments, the compound includes a linker L wherein:

T^1 is (C_1-C_{12}) alkyl and V^1 is $-CO-$;

T^2 is an amino acid analog and V^2 is $-NH-$;

T^3 is $(PEG)_n$ and V^3 is $-CO-$; and

d to f are each 0; or

wherein:

T^1 is (C_1-C_{12}) alkyl and V^1 is $-CO-$;

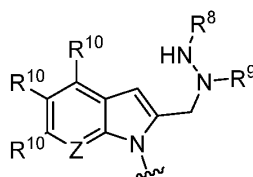
T^2 is an amino acid analog and V^2 is $-NH-$;

T^3 is $(PEG)_n$ and V^3 is $-CONH-$;

T^4 is $(PEG)_n$ and V^4 is $-CO-$;

e and f are each 0.

[0040] In some embodiments, G is:



wherein:

Z is CR^{10} or N,

R^8 and R^9 are each independently selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, amino, substituted amino, carboxyl, carboxyl ester, acyl, acyloxy, acyl amino, amino acyl, alkylamide, substituted alkylamide, sulfonyl, thioalkoxy, substituted thioalkoxy, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl, or R^8 and R^9 are optionally cyclically linked to form a 5 or 6-membered heterocyclyl;

each R^{10} is independently selected from hydrogen, halogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, amino,

substituted amino, carboxyl, carboxyl ester, acyl, acyloxy, acyl amino, amino acyl, alkylamide, substituted alkylamide, sulfonyl, thioalkoxy, substituted thioalkoxy, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl.

[0041] In some embodiments, G is selected from an acetal, an NHS-ester, a PFP-ester, a TFP-ester, an isocyanate, an isothiocyanate, an acyl halide, a maleimide, an iodoacetamide, a phosphine, an alkyne, an azide, a hydrazine, an alkoxyamine, a disulfide, an ester, and an amide.

[0042] Aspects of the present disclosure include a pharmaceutical composition comprising a conjugate as described herein, and a pharmaceutically-acceptable excipient.

[0043] Aspects of the present disclosure include a method of administering a conjugate to a subject, where the method includes administering to a subject a conjugate as described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0044] FIG. 1 shows a schematic drawing of the main components of an antibody-drug conjugate (ADC), according to embodiments of the present disclosure.

[0045] FIG. 2 shows linker strategies for ADCs. (FIG. 2, panel A) Carbamate linkage for amine-containing payloads; (FIG. 2, panel B) Carboxylic ester linker for hydroxy group-containing payloads.

[0046] FIG. 3 shows dual-cleavage design of ester linker with an adjacent monosaccharide, according to embodiments of the present disclosure.

[0047] FIG. 4 shows examples of structural design for dual-cleavage carboxylic ester linkers for ADCs, according to embodiments of the present disclosure.

[0048] FIG. 5 shows a schematic of the HIPS ligation for the synthesis of ADCs. Antibodies carrying aldehyde moieties are reacted with a Hydrazino-iso-Pictet-Spengler (HIPS) linker and payload to generate a site-specifically conjugated ADC with a stable azacarboline linkage.

[0049] FIG. 6 shows a graph of Compound **21** CH1-3/CT-tagged sacituzumab conjugate yields a DAR of 3.41 as determined by hydrophobic interaction column (HIC).

[0050] FIG. 7 shows a graph of Compound **21** CH1-3/CT-tagged sacituzumab conjugate is 98.1% monomeric as determined by analytical size exclusion chromatography (SEC).

[0051] FIG. 8 shows a graph of Compound **21** CH1-3/CT-tagged polatuzumab conjugate yields a DAR of 3.67 as determined by HIC.

[0052] FIG. 9 shows a graph of Compound **21** CH1-3/CT-tagged polatuzumab conjugate is 97.4% monomeric as determined by analytical SEC.

[0053] FIG. 10 shows a graph of Compound **52** CT-tagged anti-FITC conjugate yields a DAR of 1.61 as determined by HIC.

[0054] FIG. 11 shows a graph of Compound **52** CT-tagged anti-FITC conjugate is 98.1% monomeric as determined by analytical SEC.

[0055] FIG. 12 shows a graph of Compound **52** CT-tagged trastuzumab conjugate yields a DAR of 1.56 as determined by HIC.

[0056] FIG. 13 shows a graph of Compound **52** CT-tagged trastuzumab conjugate is 95.6% monomeric as determined by analytical SEC.

[0057] FIG. 14 shows a graph of Compound **52** CT-tagged sacituzumab conjugate yields a DAR of 1.02 as determined by HIC.

[0058] FIG. 15 shows a graph of Compound **52** CT-tagged sacituzumab conjugate is 98.5% monomeric as determined by analytical SEC.

[0059] FIG. 16 shows a graph of Compound **49** CT-tagged anti-FITC conjugate yields a DAR of 1.09 as determined by HIC.

[0060] FIG. 17 shows a graph of Compound **49** CT-tagged anti-FITC conjugate is 90.0% monomeric as determined by analytical SEC.

[0061] FIG. 18 shows a graph of Compound **49** CT-tagged trastuzumab conjugate yields a DAR of 1.09 as determined by HIC.

[0062] FIG. 19 shows a graph of Compound **49** CT-tagged trastuzumab conjugate is 90.0% monomeric as determined by analytical SEC.

[0063] FIG. 20 shows a graph of Compound **49** CT-tagged sacituzumab conjugate yields a DAR of 0.80 as determined by HIC.

[0064] FIG. 21 shows a graph of Compound **49** CT-tagged sacituzumab conjugate is 93.3% monomeric as determined by analytical SEC.

[0065] FIG. 22 shows a graph of *in vitro* potency of HER2 targeted or isotype control ADCs carrying (**21**) or CL2A-SN38 as compared to (**28**) against SK-BR-3 cells.

[0066] FIG. 23 shows a graph of *in vitro* potency of TROP-2 targeted or isotype control ADCs carrying (21) or CL2A-SN38 as compared to (28) against MDA-MB-468 cells.

[0067] FIG. 24 shows a graph of *in vitro* potency of TROP-2 targeted or isotype control ADCs carrying (21) as compared to (28) or (12) against MDA-MB-468 cells.

[0068] FIG. 25 shows a graph of *in vitro* potency of TROP-2 targeted or isotype control ADCs carrying (49) as compared to (41) against MDA-MB-468 cells.

[0069] FIG. 26 shows a graph of *in vitro* potency of HER2 targeted or isotype control ADCs carrying (21) as compared to (28) or (12) against SK-BR-3 cells.

[0070] FIG. 27 shows a graph of *in vitro* potency of HER2 targeted or isotype control ADCs carrying (49) as compared to (41) against SK-BR-3 cells.

[0071] FIG. 28 shows a graph of *in vitro* potency of HER2 targeted or isotype control ADCs carrying (49) as compared to (41) against NCI-N87 cells.

[0072] FIG. 29 shows a graph of *in vitro* potency of HER2 targeted or isotype control ADCs carrying (21) as compared to (28) or (12) against NCI-N87 cells.

[0073] FIG. 30 shows a graph of *in vitro* potency of HER2 targeted or isotype control ADCs carrying (52) as compared to (41) against SK-BR-3 cells.

[0074] FIG. 31 shows a graph of *in vitro* potency of TROP-2 targeted or isotype control ADCs carrying (52) as compared to (41) against MDA-MB-468 cells.

DEFINITIONS

[0075] The following terms have the following meanings unless otherwise indicated. Any undefined terms have their art recognized meanings.

[0076] “Alkyl” refers to monovalent saturated aliphatic hydrocarbyl groups having from 1 to 10 carbon atoms and such as 1 to 6 carbon atoms, or 1 to 5, or 1 to 4, or 1 to 3 carbon atoms. This term includes, by way of example, linear and branched hydrocarbyl groups such as methyl (CH₃-), ethyl (CH₃CH₂-), n-propyl (CH₃CH₂CH₂-), isopropyl ((CH₃)₂CH-), n-butyl (CH₃CH₂CH₂CH₂-), isobutyl ((CH₃)₂CHCH₂-), sec-butyl ((CH₃)(CH₃CH₂)CH-), t-butyl ((CH₃)₃C-), n-pentyl (CH₃CH₂CH₂CH₂CH₂-), and neopentyl ((CH₃)₃CCH₂-).

[0077] The term “substituted alkyl” refers to an alkyl group as defined herein wherein one or more carbon atoms in the alkyl chain (except the C₁ carbon atom) have been optionally replaced with a heteroatom such as -O-, -N-, -S-, -S(O)_n- (where n is 0 to 2), -NR- (where R is hydrogen

or alkyl) and having from 1 to 5 substituents selected from the group consisting of alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, oxo, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocycloxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclyl, heterocycloxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-aryl, -SO-heteroaryl, -SO₂-alkyl, -SO₂-aryl, -SO₂-heteroaryl, and -NR^aR^b, wherein R' and R'' may be the same or different and are chosen from hydrogen, optionally substituted alkyl, cycloalkyl, alkenyl, cycloalkenyl, alkynyl, aryl, heteroaryl and heterocyclic.

[0078] “Alkylene” refers to divalent aliphatic hydrocarbyl groups preferably having from 1 to 6 and more preferably 1 to 3 carbon atoms that are either straight-chained or branched, and which are optionally interrupted with one or more groups selected from -O-, -NR¹⁰-, -NR¹⁰C(O)-, -C(O)NR¹⁰- and the like. This term includes, by way of example, methylene (-CH₂-), ethylene (-CH₂CH₂-), n-propylene (-CH₂CH₂CH₂-), iso-propylene (-CH₂CH(CH₃)-), (-C(CH₃)₂CH₂CH₂-), (-C(CH₃)₂CH₂C(O)-), (-C(CH₃)₂CH₂C(O)NH-), (-CH(CH₃)CH₂-), and the like.

[0079] “Substituted alkylene” refers to an alkylene group having from 1 to 3 hydrogens replaced with substituents as described for carbons in the definition of “substituted” below.

[0080] The term “alkane” refers to alkyl group and alkylene group, as defined herein.

[0081] The term “alkylaminoalkyl”, “alkylaminoalkenyl” and “alkylaminoalkynyl” refers to the groups R'NHR''- where R' is alkyl group as defined herein and R'' is alkylene, alkenylene or alkynylene group as defined herein.

[0082] The term “alkaryl” or “aralkyl” refers to the groups -alkylene-aryl and -substituted alkylene-aryl where alkylene, substituted alkylene and aryl are defined herein.

[0083] “Alkoxy” refers to the group -O-alkyl, wherein alkyl is as defined herein. Alkoxy includes, by way of example, methoxy, ethoxy, n-propoxy, isopropoxy, n-butoxy, t-butoxy, sec-butoxy, n-pentoxy, and the like. The term “alkoxy” also refers to the groups alkenyl-O-, cycloalkyl-O-, cycloalkenyl-O-, and alkynyl-O-, where alkenyl, cycloalkyl, cycloalkenyl, and alkynyl are as defined herein.

[0084] The term “substituted alkoxy” refers to the groups substituted alkyl-O-, substituted alkenyl-O-, substituted cycloalkyl-O-, substituted cycloalkenyl-O-, and substituted alkynyl-O-

where substituted alkyl, substituted alkenyl, substituted cycloalkyl, substituted cycloalkenyl and substituted alkynyl are as defined herein.

[0085] The term “alkoxyamino” refers to the group –NH-alkoxy, wherein alkoxy is defined herein.

[0086] The term “haloalkoxy” refers to the groups alkyl-O- wherein one or more hydrogen atoms on the alkyl group have been substituted with a halo group and include, by way of examples, groups such as trifluoromethoxy, and the like.

[0087] The term “haloalkyl” refers to a substituted alkyl group as described above, wherein one or more hydrogen atoms on the alkyl group have been substituted with a halo group. Examples of such groups include, without limitation, fluoroalkyl groups, such as trifluoromethyl, difluoromethyl, trifluoroethyl and the like.

[0088] The term “alkylalkoxy” refers to the groups -alkylene-O-alkyl, alkylene-O-substituted alkyl, substituted alkylene-O-alkyl, and substituted alkylene-O-substituted alkyl wherein alkyl, substituted alkyl, alkylene and substituted alkylene are as defined herein.

[0089] The term “alkylthioalkoxy” refers to the group -alkylene-S-alkyl, alkylene-S-substituted alkyl, substituted alkylene-S-alkyl and substituted alkylene-S-substituted alkyl wherein alkyl, substituted alkyl, alkylene and substituted alkylene are as defined herein.

[0090] “Alkenyl” refers to straight chain or branched hydrocarbyl groups having from 2 to 6 carbon atoms and preferably 2 to 4 carbon atoms and having at least 1 and preferably from 1 to 2 sites of double bond unsaturation. This term includes, by way of example, bi-vinyl, allyl, and but-3-en-1-yl. Included within this term are the cis and trans isomers or mixtures of these isomers.

[0091] The term “substituted alkenyl” refers to an alkenyl group as defined herein having from 1 to 5 substituents, or from 1 to 3 substituents, selected from alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, oxo, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocycloxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclyl, heterocycloxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO₂-alkyl, -SO₂-substituted alkyl, -SO₂-aryl and -SO₂-heteroaryl.

[0092] “Alkynyl” refers to straight or branched monovalent hydrocarbyl groups having from 2 to 6 carbon atoms and preferably 2 to 3 carbon atoms and having at least 1 and preferably from 1 to 2 sites of triple bond unsaturation. Examples of such alkynyl groups include acetylenyl ($-\text{C}\equiv\text{CH}$), and propargyl ($-\text{CH}_2\text{C}\equiv\text{CH}$).

[0093] The term “substituted alkynyl” refers to an alkynyl group as defined herein having from 1 to 5 substituents, or from 1 to 3 substituents, selected from alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, oxo, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocycloxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclyl, heterocycloxy, hydroxyamino, alkoxyamino, nitro, $-\text{SO}$ -alkyl, $-\text{SO}$ -substituted alkyl, $-\text{SO}$ -aryl, $-\text{SO}$ -heteroaryl, $-\text{SO}_2$ -alkyl, $-\text{SO}_2$ -substituted alkyl, $-\text{SO}_2$ -aryl, and $-\text{SO}_2$ -heteroaryl.

[0094] “Alkynyloxy” refers to the group $-\text{O}$ -alkynyl, wherein alkynyl is as defined herein. Alkynyloxy includes, by way of example, ethynyloxy, propynyloxy, and the like.

[0095] “Acyl” refers to the groups $\text{H}-\text{C}(\text{O})-$, alkyl- $\text{C}(\text{O})-$, substituted alkyl- $\text{C}(\text{O})-$, alkenyl- $\text{C}(\text{O})-$, substituted alkenyl- $\text{C}(\text{O})-$, alkynyl- $\text{C}(\text{O})-$, substituted alkynyl- $\text{C}(\text{O})-$, cycloalkyl- $\text{C}(\text{O})-$, substituted cycloalkyl- $\text{C}(\text{O})-$, cycloalkenyl- $\text{C}(\text{O})-$, substituted cycloalkenyl- $\text{C}(\text{O})-$, aryl- $\text{C}(\text{O})-$, substituted aryl- $\text{C}(\text{O})-$, heteroaryl- $\text{C}(\text{O})-$, substituted heteroaryl- $\text{C}(\text{O})-$, heterocyclyl- $\text{C}(\text{O})-$, and substituted heterocyclyl- $\text{C}(\text{O})-$, wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic, and substituted heterocyclic are as defined herein. For example, acyl includes the “acetyl” group $\text{CH}_3\text{C}(\text{O})-$.

[0096] “Acylamino” refers to the groups $-\text{NR}^{20}\text{C}(\text{O})\text{alkyl}$, $-\text{NR}^{20}\text{C}(\text{O})\text{substituted alkyl}$, $\text{N R}^{20}\text{C}(\text{O})\text{cycloalkyl}$, $-\text{NR}^{20}\text{C}(\text{O})\text{substituted cycloalkyl}$, $-\text{NR}^{20}\text{C}(\text{O})\text{cycloalkenyl}$, $-\text{NR}^{20}\text{C}(\text{O})\text{substituted cycloalkenyl}$, $-\text{NR}^{20}\text{C}(\text{O})\text{alkenyl}$, $-\text{NR}^{20}\text{C}(\text{O})\text{substituted alkenyl}$, $-\text{NR}^{20}\text{C}(\text{O})\text{alkynyl}$, $-\text{NR}^{20}\text{C}(\text{O})\text{substituted alkynyl}$, $-\text{NR}^{20}\text{C}(\text{O})\text{aryl}$, $-\text{NR}^{20}\text{C}(\text{O})\text{substituted aryl}$, $-\text{NR}^{20}\text{C}(\text{O})\text{heteroaryl}$, $-\text{NR}^{20}\text{C}(\text{O})\text{substituted heteroaryl}$, $-\text{NR}^{20}\text{C}(\text{O})\text{heterocyclic}$, and $-\text{NR}^{20}\text{C}(\text{O})\text{substituted heterocyclic}$, wherein R^{20} is hydrogen or alkyl and wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl,

substituted alkynyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic, and substituted heterocyclic are as defined herein.

[0097] “Aminocarbonyl” or the term “aminoacyl” refers to the group $-C(O)NR^{21}R^{22}$, wherein R^{21} and R^{22} independently are selected from the group consisting of hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, heteroaryl, substituted heteroaryl, heterocyclic, and substituted heterocyclic and where R^{21} and R^{22} are optionally joined together with the nitrogen bound thereto to form a heterocyclic or substituted heterocyclic group, and wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic, and substituted heterocyclic are as defined herein.

[0098] “Aminocarbonylamino” refers to the group $-NR^{21}C(O)NR^{22}R^{23}$ where R^{21} , R^{22} , and R^{23} are independently selected from hydrogen, alkyl, aryl or cycloalkyl, or where two R groups are joined to form a heterocyclyl group.

[0099] The term “alkoxycarbonylamino” refers to the group $-NRC(O)OR$ where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclyl wherein alkyl, substituted alkyl, aryl, heteroaryl, and heterocyclyl are as defined herein.

[00100] The term “acyloxy” refers to the groups alkyl-C(O)O-, substituted alkyl-C(O)O-, cycloalkyl-C(O)O-, substituted cycloalkyl-C(O)O-, aryl-C(O)O-, heteroaryl-C(O)O-, and heterocyclyl-C(O)O- wherein alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, aryl, heteroaryl, and heterocyclyl are as defined herein.

[00101] “Aminosulfonyl” refers to the group $-SO_2NR^{21}R^{22}$, wherein R^{21} and R^{22} independently are selected from the group consisting of hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, heteroaryl, substituted heteroaryl, heterocyclic, substituted heterocyclic and where R^{21} and R^{22} are optionally joined together with the nitrogen bound thereto to form a heterocyclic or substituted heterocyclic group and alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl,

substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic and substituted heterocyclic are as defined herein.

[00102] “Sulfonylamino” refers to the group $-\text{NR}^{21}\text{SO}_2\text{R}^{22}$, wherein R^{21} and R^{22} independently are selected from the group consisting of hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, heteroaryl, substituted heteroaryl, heterocyclic, and substituted heterocyclic and where R^{21} and R^{22} are optionally joined together with the atoms bound thereto to form a heterocyclic or substituted heterocyclic group, and wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic, and substituted heterocyclic are as defined herein.

[00103] “Aryl” or “Ar” refers to a monovalent aromatic carbocyclic group of from 6 to 18 carbon atoms having a single ring (such as is present in a phenyl group) or a ring system having multiple condensed rings (examples of such aromatic ring systems include naphthyl, anthryl and indanyl) which condensed rings may or may not be aromatic, provided that the point of attachment is through an atom of an aromatic ring. This term includes, by way of example, phenyl and naphthyl. Unless otherwise constrained by the definition for the aryl substituent, such aryl groups can optionally be substituted with from 1 to 5 substituents, or from 1 to 3 substituents, selected from acyloxy, hydroxy, thiol, acyl, alkyl, alkoxy, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, substituted alkyl, substituted alkoxy, substituted alkenyl, substituted alkynyl, substituted cycloalkyl, substituted cycloalkenyl, amino, substituted amino, aminoacyl, acylamino, alkaryl, aryl, aryloxy, azido, carboxyl, carboxylalkyl, cyano, halogen, nitro, heteroaryl, heteroaryloxy, heterocyclyl, heterocycloxy, aminoacyloxy, oxyacylamino, thioalkoxy, substituted thioalkoxy, thioaryloxy, thioheteroaryloxy, $-\text{SO}-$ alkyl, $-\text{SO}-$ substituted alkyl, $-\text{SO}-$ aryl, $-\text{SO}-$ heteroaryl, $-\text{SO}_2-$ alkyl, $-\text{SO}_2-$ substituted alkyl, $-\text{SO}_2-$ aryl, $-\text{SO}_2-$ heteroaryl and trihalomethyl.

[00104] “Aryloxy” refers to the group $-\text{O}-$ aryl, wherein aryl is as defined herein, including, by way of example, phenoxy, naphthoxy, and the like, including optionally substituted aryl groups as also defined herein.

[00105] “Amino” refers to the group $-\text{NH}_2$.

[00106] The term “substituted amino” refers to the group -NRR where each R is independently selected from the group consisting of hydrogen, alkyl, substituted alkyl, cycloalkyl, substituted cycloalkyl, alkenyl, substituted alkenyl, cycloalkenyl, substituted cycloalkenyl, alkynyl, substituted alkynyl, aryl, heteroaryl, and heterocyclyl provided that at least one R is not hydrogen.

[00107] The term “azido” refers to the group -N₃.

[00108] “Carboxyl,” “carboxy” or “carboxylate” refers to -CO₂H or salts thereof.

[00109] “Carboxyl ester” or “carboxy ester” or the terms “carboxyalkyl” or “carboxylalkyl” refers to the groups -C(O)O-alkyl, -C(O)O-substituted alkyl, -C(O)O-alkenyl, -C(O)O-substituted alkenyl, -C(O)O-alkynyl, -C(O)O-substituted alkynyl, -C(O)O-aryl, -C(O)O-substituted aryl, -C(O)O-cycloalkyl, -C(O)O-substituted cycloalkyl, -C(O)O-cycloalkenyl, -C(O)O-substituted cycloalkenyl, -C(O)O-heteroaryl, -C(O)O-substituted heteroaryl, -C(O)O-heterocyclic, and -C(O)O-substituted heterocyclic, wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic, and substituted heterocyclic are as defined herein.

[00110] “(Carboxyl ester)oxy” or “carbonate” refers to the groups -O-C(O)O-alkyl, -O-C(O)O-substituted alkyl, -O-C(O)O-alkenyl, -O-C(O)O-substituted alkenyl, -O-C(O)O-alkynyl, -O-C(O)O-substituted alkynyl, -O-C(O)O-aryl, -O-C(O)O-substituted aryl, -O-C(O)O-cycloalkyl, -O-C(O)O-substituted cycloalkyl, -O-C(O)O-cycloalkenyl, -O-C(O)O-substituted cycloalkenyl, -O-C(O)O-heteroaryl, -O-C(O)O-substituted heteroaryl, -O-C(O)O-heterocyclic, and -O-C(O)O-substituted heterocyclic, wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic, and substituted heterocyclic are as defined herein.

[00111] “Cyano” or “nitrile” refers to the group -CN.

[00112] “Cycloalkyl” refers to cyclic alkyl groups of from 3 to 10 carbon atoms having single or multiple cyclic rings including fused, bridged, and spiro ring systems. Examples of suitable cycloalkyl groups include, for instance, adamantyl, cyclopropyl, cyclobutyl, cyclopentyl, cyclooctyl and the like. Such cycloalkyl groups include, by way of example, single ring

structures such as cyclopropyl, cyclobutyl, cyclopentyl, cyclooctyl, and the like, or multiple ring structures such as adamantanyl, and the like.

[00113] The term “substituted cycloalkyl” refers to cycloalkyl groups having from 1 to 5 substituents, or from 1 to 3 substituents, selected from alkyl, substituted alkyl, alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, oxo, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclyl, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO₂-alkyl, -SO₂-substituted alkyl, -SO₂-aryl and -SO₂-heteroaryl.

[00114] “Cycloalkenyl” refers to non-aromatic cyclic alkyl groups of from 3 to 10 carbon atoms having single or multiple rings and having at least one double bond and preferably from 1 to 2 double bonds.

[00115] The term “substituted cycloalkenyl” refers to cycloalkenyl groups having from 1 to 5 substituents, or from 1 to 3 substituents, selected from alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, keto, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocyclooxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclyl, heterocyclooxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO₂-alkyl, -SO₂-substituted alkyl, -SO₂-aryl and -SO₂-heteroaryl.

[00116] “Cycloalkynyl” refers to non-aromatic cycloalkyl groups of from 5 to 10 carbon atoms having single or multiple rings and having at least one triple bond.

[00117] “Cycloalkoxy” refers to -O-cycloalkyl.

[00118] “Cycloalkenyloxy” refers to -O-cycloalkenyl.

[00119] “Halo” or “halogen” refers to fluoro, chloro, bromo, and iodo.

[00120] “Hydroxy” or “hydroxyl” refers to the group -OH.

[00121] “Heteroaryl” refers to an aromatic group of from 1 to 15 carbon atoms, such as from 1 to 10 carbon atoms and 1 to 10 heteroatoms selected from the group consisting of oxygen, nitrogen, and sulfur within the ring. Such heteroaryl groups can have a single ring (such as,

pyridinyl, imidazolyl or furyl) or multiple condensed rings in a ring system (for example as in groups such as, indoliziny, quinolinyl, benzofuran, benzimidazolyl or benzothienyl), wherein at least one ring within the ring system is aromatic. To satisfy valence requirements, any heteroatoms in such heteroaryl rings may or may not be bonded to H or a substituent group, e.g., an alkyl group or other substituent as described herein. In certain embodiments, the nitrogen and/or sulfur ring atom(s) of the heteroaryl group are optionally oxidized to provide for the N-oxide (N→O), sulfinyl, or sulfonyl moieties. This term includes, by way of example, pyridinyl, pyrrolyl, indolyl, thiophenyl, and furanyl. Unless otherwise constrained by the definition for the heteroaryl substituent, such heteroaryl groups can be optionally substituted with 1 to 5 substituents, or from 1 to 3 substituents, selected from acyloxy, hydroxy, thiol, acyl, alkyl, alkoxy, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, substituted alkyl, substituted alkoxy, substituted alkenyl, substituted alkynyl, substituted cycloalkyl, substituted cycloalkenyl, amino, substituted amino, aminoacyl, acylamino, alkaryl, aryl, aryloxy, azido, carboxyl, carboxylalkyl, cyano, halogen, nitro, heteroaryl, heteroaryloxy, heterocyclyl, heterocycloxy, aminoacyloxy, oxyacylamino, thioalkoxy, substituted thioalkoxy, thioaryloxy, thioheteroaryloxy, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO₂-alkyl, -SO₂-substituted alkyl, -SO₂-aryl and -SO₂-heteroaryl, and trihalomethyl.

[00122] The term “heteroaralkyl” refers to the groups -alkylene-heteroaryl where alkylene and heteroaryl are defined herein. This term includes, by way of example, pyridylmethyl, pyridylethyl, indolylmethyl, and the like.

[00123] “Heteroaryloxy” refers to -O-heteroaryl.

[00124] “Heterocycle,” “heterocyclic,” “heterocycloalkyl,” and “heterocyclyl” refer to a saturated or unsaturated group having a single ring or multiple condensed rings, including fused bridged and spiro ring systems, and having from 3 to 20 ring atoms, including 1 to 10 hetero atoms. These ring atoms are selected from nitrogen, sulfur, or oxygen, where, in fused ring systems, one or more of the rings can be cycloalkyl, aryl, or heteroaryl, provided that the point of attachment is through the non-aromatic ring. In certain embodiments, the nitrogen and/or sulfur atom(s) of the heterocyclic group are optionally oxidized to provide for the N-oxide, -S(O)-, or -SO₂- moieties. To satisfy valence requirements, any heteroatoms in such heterocyclic rings may or may not be bonded to one or more H or one or more substituent group(s), e.g., an alkyl group or other substituent as described herein.

[00125] Examples of heterocycles and heteroaryls include, but are not limited to, azetidine, pyrrole, imidazole, pyrazole, pyridine, pyrazine, pyrimidine, pyridazine, indolizine, isoindole, indole, dihydroindole, indazole, purine, quinolizine, isoquinoline, quinoline, phthalazine, naphthylpyridine, quinoxaline, quinazoline, cinnoline, pteridine, carbazole, carboline, phenanthridine, acridine, phenanthroline, isothiazole, phenazine, isoxazole, phenoxazine, phenothiazine, imidazolidine, imidazoline, piperidine, piperazine, indoline, phthalimide, 1,2,3,4-tetrahydroisoquinoline, 4,5,6,7-tetrahydrobenzo[b]thiophene, thiazole, thiazolidine, thiophene, benzo[b]thiophene, morpholinyl, thiomorpholinyl (also referred to as thiamorpholinyl), 1,1-dioxothiomorpholinyl, piperidinyl, pyrrolidine, tetrahydrofuranyl, and the like.

[00126] Unless otherwise constrained by the definition for the heterocyclic substituent, such heterocyclic groups can be optionally substituted with 1 to 5, or from 1 to 3 substituents, selected from alkoxy, substituted alkoxy, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, acyl, acylamino, acyloxy, amino, substituted amino, aminoacyl, aminoacyloxy, oxyaminoacyl, azido, cyano, halogen, hydroxyl, oxo, thioketo, carboxyl, carboxylalkyl, thioaryloxy, thioheteroaryloxy, thioheterocycloxy, thiol, thioalkoxy, substituted thioalkoxy, aryl, aryloxy, heteroaryl, heteroaryloxy, heterocyclyl, heterocycloxy, hydroxyamino, alkoxyamino, nitro, -SO-alkyl, -SO-substituted alkyl, -SO-aryl, -SO-heteroaryl, -SO₂-alkyl, -SO₂-substituted alkyl, -SO₂-aryl, -SO₂-heteroaryl, and fused heterocycle.

[00127] “Heterocyclyloxy” refers to the group -O-heterocyclyl.

[00128] The term “heterocyclylthio” refers to the group heterocyclic-S-.

[00129] The term “heterocyclene” refers to the diradical group formed from a heterocycle, as defined herein.

[00130] The term “hydroxyamino” refers to the group -NHOH.

[00131] “Nitro” refers to the group -NO₂.

[00132] “Oxo” refers to the atom (=O).

[00133] “Sulfonyl” refers to the group -SO₂-alkyl, -SO₂-substituted alkyl, -SO₂-alkenyl, -SO₂-substituted alkenyl, -SO₂-cycloalkyl, -SO₂-substituted cycloalkyl, -SO₂-cycloalkenyl, -SO₂-substituted cycloalkenyl, -SO₂-aryl, -SO₂-substituted aryl, -SO₂-heteroaryl, -SO₂-substituted heteroaryl, -SO₂-heterocyclic, and -SO₂-substituted heterocyclic, wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl,

heterocyclic, and substituted heterocyclic are as defined herein. Sulfonyl includes, by way of example, methyl-SO₂-, phenyl-SO₂-, and 4-methylphenyl-SO₂-.

[00134] “Sulfonyloxy” refers to the group -OSO₂-alkyl, -OSO₂-substituted alkyl, -OSO₂-alkenyl, -OSO₂-substituted alkenyl, -OSO₂-cycloalkyl, -OSO₂-substituted cycloalkyl, -OSO₂-cycloalkenyl, -OSO₂-substituted cycloalkenyl, -OSO₂-aryl, -OSO₂-substituted aryl, -OSO₂-heteroaryl, -OSO₂-substituted heteroaryl, -OSO₂-heterocyclic, and -OSO₂-substituted heterocyclic, wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic, and substituted heterocyclic are as defined herein.

[00135] “Sulfate” or “sulfate ester” refers the group -O-SO₂-OH, -O-SO₂-O-alkyl, -O-SO₂-O-substituted alkyl, -O-SO₂-O-alkenyl, -O-SO₂-O-substituted alkenyl, -O-SO₂-O-cycloalkyl, -O-SO₂-O-substituted cycloalkyl, -O-SO₂-O-cycloalkenyl, -O-SO₂-O-substituted cycloalkenyl, -O-SO₂-O-aryl, -O-SO₂-O-substituted aryl, -O-SO₂-O-heteroaryl, -O-SO₂-O-substituted heteroaryl, -O-SO₂-O-heterocyclic, and -O-SO₂-O-substituted heterocyclic, wherein alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, cycloalkyl, substituted cycloalkyl, cycloalkenyl, substituted cycloalkenyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, heterocyclic, and substituted heterocyclic are as defined herein.

[00136] The term “aminocarbonyloxy” refers to the group -OC(O)NRR where each R is independently hydrogen, alkyl, substituted alkyl, aryl, heteroaryl, or heterocyclic wherein alkyl, substituted alkyl, aryl, heteroaryl and heterocyclic are as defined herein.

[00137] “Thiol” refers to the group -SH.

[00138] “Thioxo” or the term “thioketo” refers to the atom (=S).

[00139] “Alkylthio” or the term “thioalkoxy” refers to the group -S-alkyl, wherein alkyl is as defined herein. In certain embodiments, sulfur may be oxidized to -S(O)-. The sulfoxide may exist as one or more stereoisomers.

[00140] The term “substituted thioalkoxy” refers to the group -S-substituted alkyl.

[00141] The term “thioaryloxy” refers to the group aryl-S- wherein the aryl group is as defined herein including optionally substituted aryl groups also defined herein.

[00142] The term “thioheteroaryloxy” refers to the group heteroaryl-S- wherein the heteroaryl group is as defined herein including optionally substituted aryl groups as also defined herein.

[00143] The term “thioheterocyclooxy” refers to the group heterocyclyl-S- wherein the heterocyclyl group is as defined herein including optionally substituted heterocyclyl groups as also defined herein.

[00144] In addition to the disclosure herein, the term “substituted,” when used to modify a specified group or radical, can also mean that one or more hydrogen atoms of the specified group or radical are each, independently of one another, replaced with the same or different substituent groups as defined below.

[00145] In addition to the groups disclosed with respect to the individual terms herein, substituent groups for substituting for one or more hydrogens (any two hydrogens on a single carbon can be replaced with =O, =NR⁷⁰, =N-OR⁷⁰, =N₂ or =S) on saturated carbon atoms in the specified group or radical are, unless otherwise specified, -R⁶⁰, halo, =O, -OR⁷⁰, -SR⁷⁰, -NR⁸⁰R⁸⁰, trihalomethyl, -CN, -OCN, -SCN, -NO, -NO₂, =N₂, -N₃, -SO₂R⁷⁰, -SO₂O⁻M⁺, -SO₂OR⁷⁰, -OSO₂R⁷⁰, -OSO₂O⁻M⁺, -OSO₂OR⁷⁰, -P(O)(O⁻)₂(M⁺)₂, -P(O)(OR⁷⁰)O⁻M⁺, -P(O)(OR⁷⁰)₂, -C(O)R⁷⁰, -C(S)R⁷⁰, -C(NR⁷⁰)R⁷⁰, -C(O)O⁻M⁺, -C(O)OR⁷⁰, -C(S)OR⁷⁰, -C(O)NR⁸⁰R⁸⁰, -C(NR⁷⁰)NR⁸⁰R⁸⁰, -OC(O)R⁷⁰, -OC(S)R⁷⁰, -OC(O)O⁻M⁺, -OC(O)OR⁷⁰, -OC(S)OR⁷⁰, -NR⁷⁰C(O)R⁷⁰, -NR⁷⁰C(S)R⁷⁰, -NR⁷⁰CO₂⁻M⁺, -NR⁷⁰CO₂R⁷⁰, -NR⁷⁰C(S)OR⁷⁰, -NR⁷⁰C(O)NR⁸⁰R⁸⁰, -NR⁷⁰C(NR⁷⁰)R⁷⁰ and -NR⁷⁰C(NR⁷⁰)NR⁸⁰R⁸⁰, where R⁶⁰ is selected from the group consisting of optionally substituted alkyl, cycloalkyl, heteroalkyl, heterocycloalkylalkyl, cycloalkylalkyl, aryl, arylalkyl, heteroaryl and heteroarylalkyl, each R⁷⁰ is independently hydrogen or R⁶⁰; each R⁸⁰ is independently R⁷⁰ or alternatively, two R⁸⁰'s, taken together with the nitrogen atom to which they are bonded, form a 5-, 6- or 7-membered heterocycloalkyl which may optionally include from 1 to 4 of the same or different additional heteroatoms selected from the group consisting of O, N and S, of which N may have -H or C₁-C₃ alkyl substitution; and each M⁺ is a counter ion with a net single positive charge. Each M⁺ may independently be, for example, an alkali ion, such as K⁺, Na⁺, Li⁺; an ammonium ion, such as ⁺N(R⁶⁰)₄; or an alkaline earth ion, such as [Ca²⁺]_{0.5}, [Mg²⁺]_{0.5}, or [Ba²⁺]_{0.5} (“subscript 0.5 means that one of the counter ions for such divalent alkali earth ions can be an ionized form of a compound of the invention and the other a typical counter ion such as chloride, or two ionized compounds disclosed herein can serve as counter ions for such divalent alkali earth ions, or a doubly ionized compound of the invention can serve as the counter ion for such divalent alkali earth ions). As specific examples, -NR⁸⁰R⁸⁰ is meant to

include -NH₂, -NH-alkyl, *N*-pyrrolidinyl, *N*-piperazinyl, 4*N*-methyl-piperazin-1-yl and *N*-morpholinyl.

[00146] In addition to the disclosure herein, substituent groups for hydrogens on unsaturated carbon atoms in “substituted” alkene, alkyne, aryl and heteroaryl groups are, unless otherwise specified, -R⁶⁰, halo, -O⁻M⁺, -OR⁷⁰, -SR⁷⁰, -S⁻M⁺, -NR⁸⁰R⁸⁰, trihalomethyl, -CF₃, -CN, -OCN, -SCN, -NO, -NO₂, -N₃, -SO₂R⁷⁰, -SO₃⁻M⁺, -SO₃R⁷⁰, -OSO₂R⁷⁰, -OSO₃⁻M⁺, -OSO₃R⁷⁰, -PO₃⁻²(M⁺)₂, -P(O)(OR⁷⁰)O⁻M⁺, -P(O)(OR⁷⁰)₂, -C(O)R⁷⁰, -C(S)R⁷⁰, -C(NR⁷⁰)R⁷⁰, -CO₂⁻M⁺, -CO₂R⁷⁰, -C(S)OR⁷⁰, -C(O)NR⁸⁰R⁸⁰, -C(NR⁷⁰)NR⁸⁰R⁸⁰, -OC(O)R⁷⁰, -OC(S)R⁷⁰, -OCO₂⁻M⁺, -OCO₂R⁷⁰, -OC(S)OR⁷⁰, -NR⁷⁰C(O)R⁷⁰, -NR⁷⁰C(S)R⁷⁰, -NR⁷⁰CO₂⁻M⁺, -NR⁷⁰CO₂R⁷⁰, -NR⁷⁰C(S)OR⁷⁰, -NR⁷⁰C(O)NR⁸⁰R⁸⁰, -NR⁷⁰C(NR⁷⁰)R⁷⁰ and -NR⁷⁰C(NR⁷⁰)NR⁸⁰R⁸⁰, where R⁶⁰, R⁷⁰, R⁸⁰ and M⁺ are as previously defined, provided that in case of substituted alkene or alkyne, the substituents are not -O⁻M⁺, -OR⁷⁰, -SR⁷⁰, or -S⁻M⁺.

[00147] In addition to the groups disclosed with respect to the individual terms herein, substituent groups for hydrogens on nitrogen atoms in “substituted” heteroalkyl and cycloheteroalkyl groups are, unless otherwise specified, -R⁶⁰, -O⁻M⁺, -OR⁷⁰, -SR⁷⁰, -S⁻M⁺, -NR⁸⁰R⁸⁰, trihalomethyl, -CF₃, -CN, -NO, -NO₂, -S(O)₂R⁷⁰, -S(O)₂O⁻M⁺, -S(O)₂OR⁷⁰, -OS(O)₂R⁷⁰, -OS(O)₂O⁻M⁺, -OS(O)₂OR⁷⁰, -P(O)(O⁻)₂(M⁺)₂, -P(O)(OR⁷⁰)O⁻M⁺, -P(O)(OR⁷⁰)(OR⁷⁰), -C(O)R⁷⁰, -C(S)R⁷⁰, -C(NR⁷⁰)R⁷⁰, -C(O)OR⁷⁰, -C(S)OR⁷⁰, -C(O)NR⁸⁰R⁸⁰, -C(NR⁷⁰)NR⁸⁰R⁸⁰, -OC(O)R⁷⁰, -OC(S)R⁷⁰, -OC(O)OR⁷⁰, -OC(S)OR⁷⁰, -NR⁷⁰C(O)R⁷⁰, -NR⁷⁰C(S)R⁷⁰, -NR⁷⁰C(O)OR⁷⁰, -NR⁷⁰C(S)OR⁷⁰, -NR⁷⁰C(O)NR⁸⁰R⁸⁰, -NR⁷⁰C(NR⁷⁰)R⁷⁰ and -NR⁷⁰C(NR⁷⁰)NR⁸⁰R⁸⁰, where R⁶⁰, R⁷⁰, R⁸⁰ and M⁺ are as previously defined.

[00148] In addition to the disclosure herein, in a certain embodiment, a group that is substituted has 1, 2, 3, or 4 substituents, 1, 2, or 3 substituents, 1 or 2 substituents, or 1 substituent.

[00149] It is understood that in all substituted groups defined above, polymers arrived at by defining substituents with further substituents to themselves (e.g., substituted aryl having a substituted aryl group as a substituent which is itself substituted with a substituted aryl group, which is further substituted by a substituted aryl group, etc.) are not intended for inclusion herein. In such cases, the maximum number of such substitutions is three. For example, serial

substitutions of substituted aryl groups specifically contemplated herein are limited to substituted aryl-(substituted aryl)-substituted aryl.

[00150] Unless indicated otherwise, the nomenclature of substituents that are not explicitly defined herein are arrived at by naming the terminal portion of the functionality followed by the adjacent functionality toward the point of attachment. For example, the substituent “arylalkyloxycarbonyl” refers to the group (aryl)-(alkyl)-O-C(O)-.

[00151] As to any of the groups disclosed herein which contain one or more substituents, it is understood, of course, that such groups do not contain any substitution or substitution patterns which are sterically impractical and/or synthetically non-feasible. In addition, the subject compounds include all stereochemical isomers arising from the substitution of these compounds.

[00152] The term “pharmaceutically acceptable salt” means a salt which is acceptable for administration to a patient, such as a mammal (salts with counterions having acceptable mammalian safety for a given dosage regime). Such salts can be derived from pharmaceutically acceptable inorganic or organic bases and from pharmaceutically acceptable inorganic or organic acids. “Pharmaceutically acceptable salt” refers to pharmaceutically acceptable salts of a compound, which salts are derived from a variety of organic and inorganic counter ions well known in the art and include, by way of example only, sodium, potassium, calcium, magnesium, ammonium, tetraalkylammonium, and the like; and when the molecule contains a basic functionality, salts of organic or inorganic acids, such as hydrochloride, hydrobromide, formate, tartrate, besylate, mesylate, acetate, maleate, oxalate, and the like.

[00153] The term “salt thereof” means a compound formed when a proton of an acid is replaced by a cation, such as a metal cation or an organic cation and the like. Where applicable, the salt is a pharmaceutically acceptable salt, although this is not required for salts of intermediate compounds that are not intended for administration to a patient. By way of example, salts of the present compounds include those wherein the compound is protonated by an inorganic or organic acid to form a cation, with the conjugate base of the inorganic or organic acid as the anionic component of the salt.

[00154] “Solvate” refers to a complex formed by combination of solvent molecules with molecules or ions of the solute. The solvent can be an organic compound, an inorganic compound, or a mixture of both. Some examples of solvents include, but are not limited to,

methanol, *N,N*-dimethylformamide, tetrahydrofuran, dimethylsulfoxide, and water. When the solvent is water, the solvate formed is a hydrate.

[00155] “Stereoisomer” and “stereoisomers” refer to compounds that have same atomic connectivity but different atomic arrangement in space. Stereoisomers include cis-trans isomers, *E* and *Z* isomers, enantiomers, and diastereomers.

[00156] “Tautomer” refers to alternate forms of a molecule that differ only in electronic bonding of atoms and/or in the position of a proton, such as enol-keto and imine-enamine tautomers, or the tautomeric forms of heteroaryl groups containing a -N=C(H)-NH- ring atom arrangement, such as pyrazoles, imidazoles, benzimidazoles, triazoles, and tetrazoles. A person of ordinary skill in the art would recognize that other tautomeric ring atom arrangements are possible.

[00157] It will be appreciated that the term “or a salt or solvate or stereoisomer thereof” is intended to include all permutations of salts, solvates and stereoisomers, such as a solvate of a pharmaceutically acceptable salt of a stereoisomer of subject compound.

[00158] “Pharmaceutically effective amount” and “therapeutically effective amount” refer to an amount of a compound sufficient to treat a specified disorder or disease or one or more of its symptoms and/or to prevent the occurrence of the disease or disorder. In reference to tumorigenic proliferative disorders, a pharmaceutically or therapeutically effective amount comprises an amount sufficient to, among other things, cause the tumor to shrink or decrease the growth rate of the tumor.

[00159] “Patient” refers to human and non-human subjects, especially mammalian subjects.

[00160] The term “treating” or “treatment” as used herein means the treating or treatment of a disease or medical condition in a patient, such as a mammal (particularly a human) that includes: (a) preventing the disease or medical condition from occurring, such as, prophylactic treatment of a subject; (b) ameliorating the disease or medical condition, such as, eliminating or causing regression of the disease or medical condition in a patient; (c) suppressing the disease or medical condition, for example by, slowing or arresting the development of the disease or medical condition in a patient; or (d) alleviating a symptom of the disease or medical condition in a patient.

[00161] The terms “polypeptide,” “peptide,” and “protein” are used interchangeably herein to refer to a polymeric form of amino acids of any length. Unless specifically indicated

otherwise, “polypeptide,” “peptide,” and “protein” can include genetically coded and non-coded amino acids, chemically or biochemically modified or derivatized amino acids, and polypeptides having modified peptide backbones. The term includes fusion proteins, including, but not limited to, fusion proteins with a heterologous amino acid sequence, fusions with heterologous and homologous leader sequences, proteins which contain at least one N-terminal methionine residue (e.g., to facilitate production in a recombinant host cell); immunologically tagged proteins; and the like.

[00162] “Native amino acid sequence” or “parent amino acid sequence” are used interchangeably herein to refer to the amino acid sequence of a polypeptide prior to modification to include a modified amino acid residue.

[00163] The terms “amino acid analog,” “unnatural amino acid,” and the like may be used interchangeably, and include amino acid-like compounds that are similar in structure and/or overall shape to one or more amino acids commonly found in naturally occurring proteins (e.g., Ala or A, Cys or C, Asp or D, Glu or E, Phe or F, Gly or G, His or H, Ile or I, Lys or K, Leu or L, Met or M, Asn or N, Pro or P, Gln or Q, Arg or R, Ser or S, Thr or T, Val or V, Trp or W, Tyr or Y). Amino acid analogs also include natural amino acids with modified side chains or backbones. Amino acid analogs also include amino acid analogs with the same stereochemistry as in the naturally occurring D-form, as well as the L-form of amino acid analogs. In some instances, the amino acid analogs share backbone structures, and/or the side chain structures of one or more natural amino acids, with difference(s) being one or more modified groups in the molecule. Such modification may include, but is not limited to, substitution of an atom (such as N) for a related atom (such as S), addition of a group (such as methyl, or hydroxyl, etc.) or an atom (such as Cl or Br, etc.), deletion of a group, substitution of a covalent bond (single bond for double bond, etc.), or combinations thereof. For example, amino acid analogs may include α -hydroxy acids, and α -amino acids, and the like.

[00164] The terms “amino acid side chain” or “side chain of an amino acid” and the like may be used to refer to the substituent attached to the α -carbon of an amino acid residue, including natural amino acids, unnatural amino acids, and amino acid analogs. An amino acid side chain can also include an amino acid side chain as described in the context of the modified amino acids and/or conjugates described herein.

[00165] The term “carbohydrate” and the like may be used to refer to monomers units and/or polymers of monosaccharides, disaccharides, oligosaccharides, and polysaccharides. The term sugar may be used to refer to the smaller carbohydrates, such as monosaccharides, disaccharides. The term “carbohydrate derivative” includes compounds where one or more functional groups of a carbohydrate of interest are substituted (replaced by any convenient substituent), modified (converted to another group using any convenient chemistry) or absent (e.g., eliminated or replaced by H). A variety of carbohydrates and carbohydrate derivatives are available and may be adapted for use in the subject compounds and conjugates.

[00166] The term “glycoside” or “glycosyl” refers to a sugar molecule or group bound to a moiety via a glycosidic bond. For example, the moiety that the glycoside is bound to can be a cleavable linker as described herein. A glycosidic bond can link the glycoside to the other moiety through various types of bonds, such as, but not limited to, an O-glycosidic bond (an O-glycoside), an N-glycosidic bond (a glycosylamine), an S-glycosidic bond (a thioglycoside), or C-glycosidic bond (a C-glycoside or C-glycosyl). In some cases, glycosides can be cleaved from the moiety they are attached to, such as by chemically-mediated hydrolysis or enzymatically-mediated hydrolysis.

[00167] The term “antibody” is used in the broadest sense and includes monoclonal antibodies (including full length monoclonal antibodies), polyclonal antibodies, and multispecific antibodies (e.g., bispecific antibodies), humanized antibodies, single-chain antibodies, chimeric antibodies, antibody fragments (e.g., Fab fragments), and the like. An antibody is capable of binding a target antigen. (Janeway, C., Travers, P., Walport, M., Shlomchik (2001) *Immuno Biology*, 5th Ed., Garland Publishing, New York). A target antigen can have one or more binding sites, also called epitopes, recognized by complementarity determining regions (CDRs) formed by one or more variable regions of an antibody.

[00168] The term “natural antibody” refers to an antibody in which the heavy and light chains of the antibody have been made and paired by the immune system of a multi-cellular organism. Spleen, lymph nodes, bone marrow and serum are examples of tissues that produce natural antibodies. For example, the antibodies produced by the antibody producing cells isolated from a first animal immunized with an antigen are natural antibodies.

[00169] The term “humanized antibody” or “humanized immunoglobulin” refers to a non-human (e.g., mouse or rabbit) antibody containing one or more amino acids (in a framework

region, a constant region or a CDR, for example) that have been substituted with a correspondingly positioned amino acid from a human antibody. In general, humanized antibodies produce a reduced immune response in a human host, as compared to a non-humanized version of the same antibody. Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Pat. Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, *Molecular Immunology* 28(4/5):489-498 (1991); Studnicka et al., *Protein Engineering* 7(6):805-814 (1994); Roguska. et al., *PNAS* 91:969-973 (1994)), and chain shuffling (U.S. Pat. No. 5,565,332). In certain embodiments, framework substitutions are identified by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions (see, e.g., U.S. Pat. No. 5,585,089; Riechmann et al., *Nature* 332:323 (1988)). Additional methods for humanizing antibodies contemplated for use in the present invention are described in U.S. Pat. Nos. 5,750,078; 5,502,167; 5,705,154; 5,770,403; 5,698,417; 5,693,493; 5,558,864; 4,935,496; and 4,816,567, and PCT publications WO 98/45331 and WO 98/45332. In particular embodiments, a subject rabbit antibody may be humanized according to the methods set forth in US20040086979 and US20050033031. Accordingly, the antibodies described above may be humanized using methods that are well known in the art.

[00170] The term “chimeric antibodies” refer to antibodies whose light and heavy chain genes have been constructed, typically by genetic engineering, from antibody variable and constant region genes belonging to different species. For example, the variable segments of the genes from a mouse monoclonal antibody may be joined to human constant segments, such as gamma 1 and gamma 3. An example of a therapeutic chimeric antibody is a hybrid protein composed of the variable or antigen-binding domain from a mouse antibody and the constant or effector domain from a human antibody, although domains from other mammalian species may be used.

[00171] An immunoglobulin polypeptide immunoglobulin light or heavy chain variable region is composed of a framework region (FR) interrupted by three hypervariable regions, also called “complementarity determining regions” or “CDRs”. The extent of the framework region and CDRs have been defined (see, “Sequences of Proteins of Immunological Interest,” E. Kabat et al., U.S. Department of Health and Human Services, 1991). The framework region of an

antibody, that is the combined framework regions of the constituent light and heavy chains, serves to position and align the CDRs. The CDRs are primarily responsible for binding to an epitope of an antigen.

[00172] A “parent Ig polypeptide” is a polypeptide comprising an amino acid sequence which lacks an aldehyde-tagged constant region as described herein. The parent polypeptide may comprise a native sequence constant region, or may comprise a constant region with pre-existing amino acid sequence modifications (such as additions, deletions and/or substitutions).

[00173] As used herein the term “isolated” is meant to describe a compound of interest that is in an environment different from that in which the compound naturally occurs. “Isolated” is meant to include compounds that are within samples that are substantially enriched for the compound of interest and/or in which the compound of interest is partially or substantially purified.

[00174] As used herein, the term “substantially purified” refers to a compound that is removed from its natural environment and is at least 60% free, at least 75% free, at least 80% free, at least 85% free, at least 90% free, at least 95% free, at least 98% free, or more than 98% free, from other components with which it is naturally associated.

[00175] The term “physiological conditions” is meant to encompass those conditions compatible with living cells, *e.g.*, predominantly aqueous conditions of a temperature, pH, salinity, *etc.* that are compatible with living cells.

[00176] By “reactive partner” is meant a molecule or molecular moiety that specifically reacts with another reactive partner to produce a reaction product. Exemplary reactive partners include a cysteine or serine of a sulfatase motif and Formylglycine Generating Enzyme (FGE), which react to form a reaction product of a converted aldehyde tag containing a formylglycine (FGly) in lieu of cysteine or serine in the motif. Other exemplary reactive partners include an aldehyde of an fGly residue of a converted aldehyde tag (*e.g.*, a reactive aldehyde group) and an “aldehyde-reactive reactive partner”, which comprises an aldehyde-reactive group and a moiety of interest, and which reacts to form a reaction product of a modified aldehyde tagged polypeptide having the moiety of interest conjugated to the modified polypeptide through a modified fGly residue.

[00177] “N-terminus” refers to the terminal amino acid residue of a polypeptide having a free amine group, which amine group in non-N-terminus amino acid residues normally forms part of the covalent backbone of the polypeptide.

[00178] “C-terminus” refers to the terminal amino acid residue of a polypeptide having a free carboxyl group, which carboxyl group in non-C-terminus amino acid residues normally forms part of the covalent backbone of the polypeptide.

[00179] By “internal site” as used in referenced to a polypeptide or an amino acid sequence of a polypeptide means a region of the polypeptide that is not at the N-terminus or at the C-terminus.

[00180] Before the present invention is further described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[00181] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

[00182] It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. All combinations of the embodiments pertaining to the invention are specifically embraced by the present invention and are disclosed herein just as if each and every combination was individually and explicitly disclosed, to the extent that such combinations embrace subject matter that are, for example, compounds that are stable compounds (i.e.,

compounds that can be made, isolated, characterized, and tested for biological activity). In addition, all sub-combinations of the various embodiments and elements thereof (e.g., elements of the chemical groups listed in the embodiments describing such variables) are also specifically embraced by the present invention and are disclosed herein just as if each and every such sub-combination was individually and explicitly disclosed herein.

[00183] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

[00184] It must be noted that as used herein and in the appended claims, the singular forms “a,” “an,” and “the” include plural referents unless the context clearly dictates otherwise. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as “solely,” “only” and the like in connection with the recitation of claim elements, or use of a “negative” limitation.

[00185] It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination.

[00186] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

DETAILED DESCRIPTION

[00187] The present disclosure provides antibody-drug conjugate structures, that include a cleavable linker containing an ester group that links the antibody to the drug. The disclosure also encompasses methods of production of such conjugates, as well as methods of using the same.

[00188] Most payloads require cleavage from the ADC assembly in order to exhibit activity. As such, chemical linkers used in ADCs are primarily cleavable under intracellular (lysosomal) conditions. Typically, such linkers are susceptible to enzymatic cleavage by proteases and glycosidases or are acid-labile. For instance, many amine-containing drugs can be easily connected to a protease-cleavable dipeptide linker through a self-immolative spacer and carbamate group (FIG. 2, panel A). By contrast, payloads that possess only a free hydroxy group as a linkable moiety have proven difficult to use directly in ADCs. Cleavable linkers for such payloads require special design and often a laborious synthetic approach. Using a carboxylic ester group to attach a hydroxy-containing payload to an antibody appears to be the most straightforward and synthetically tractable approach for generating ADCs with this class of payloads (FIG. 2, panel B). However, esters are highly susceptible to various plasma hydrolases (esterases) and, therefore, are not considered useful linkers for ADCs, which normally require a high stability in the circulation ($t_{1/2}$ = weeks). The present disclosure provides ADCs that include the use of ester linkers to directly connect hydroxy-containing drugs to antibodies for the generation of plasma-stable conjugates.

[00189] ADCs containing carboxylic ester linkers adjacent to a monosaccharide moiety may demonstrate greater plasma stability compared to the fully exposed ester group, but may cleave efficiently upon ADC internalization and trafficking as shown in FIG. 3.

[00190] Ester cleavage by extracellular hydrolases prior to entering the targeted cell may be negligible, due to the steric hinderance provided by the neighboring glycoside, which itself is very stable in plasma. Conversely, upon ADC internalization, the monosaccharide group may be efficiently removed by lysosomal glycosidases, leaving the ester group accessible to enzymatic hydrolysis by intracellular esterases. This latter step would lead to the release of the original payload with recovery of the free hydroxy group employed for conjugation (FIG. 3). Examples of such dual-cleavage ester-glycoside linkers are shown in FIG. 4. These include a salicylic ester linker containing an *ortho*-glycoside moiety (**1**), and the structurally related derivative of α -aryl glycine (**2**). Applying the same structural design concept to serine, isoserine, and threonine

provides glycosidated ester linkers **3**, **4**, and **5**, respectively. Following the same principle, monosaccharide-containing derivatives of malic and tartaric acids (**6** and **7**, respectively) are also provided (FIG. 4).

ANTIBODY-DRUG CONJUGATES

[00191] The present disclosure provides a conjugate, e.g., an antibody-drug conjugate (ADC). By “conjugate” is meant a first moiety (e.g., an antibody) is stably associated with a second moiety (e.g., a drug or active agent). For example, an antibody-drug conjugate includes a drug or active agent stably associated with another moiety (e.g., the antibody). By “stably associated” is meant that a moiety is bound to another moiety or structure under standard conditions. In certain embodiments, the first and second moieties are bound to each other through one or more functional groups and covalent bonds. For example, the one or more functional groups and covalent bonds can include a cleavable linker as described herein.

[00192] In certain embodiments, the conjugate is a polypeptide conjugate, which includes a polypeptide (e.g., an antibody) conjugated to a second moiety. In certain embodiments, the moiety conjugated to the polypeptide can be any of a variety of moieties of interest such as, but not limited to, a drug, an active agent, a detectable label, a water-soluble polymer, or a moiety for immobilization of the polypeptide to a membrane or a surface. In certain embodiments, the conjugate is a drug conjugate, where a polypeptide is an antibody, thus providing an antibody-drug conjugate. For instance, the conjugate can be a drug conjugate, where a polypeptide is conjugated to a drug or an active agent. Various types of drugs or active agents may be used in the conjugates and are described in more detail below.

[00193] The moiety of interest (e.g., drug or active agent) can be conjugated to the polypeptide (e.g., antibody) at any desired site of the polypeptide. Thus, the present disclosure provides, for example, a modified polypeptide having a moiety conjugated at a site at or near the C-terminus of the polypeptide. Other examples include a modified polypeptide having a moiety conjugated at a position at or near the N-terminus of the polypeptide. Examples also include a modified polypeptide having a moiety conjugated at a position between the C-terminus and the N-terminus of the polypeptide (e.g., at an internal site of the polypeptide). Combinations of the above are also possible where the modified polypeptide is conjugated to two or more moieties.

[00194] In certain embodiments, a conjugate of the present disclosure includes a drug or active agent conjugated to an amino acid residue of a polypeptide at the α -carbon of an amino acid

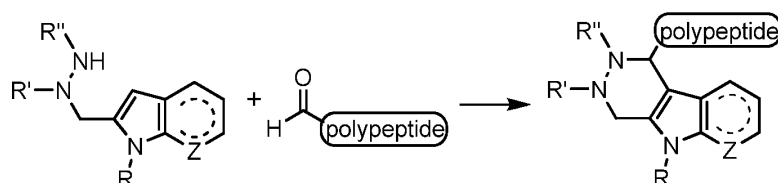
residue. Stated another way, a conjugate includes a polypeptide where the side chain of one or more amino acid residues in the polypeptide have been modified to be attached to a drug or active agent (e.g., attached to a drug or active agent through a linker as described herein). For example, a conjugate includes a polypeptide where the α -carbon of one or more amino acid residues in the polypeptide has been modified to be attached to a drug or active agent (e.g., attached to a drug or active agent through a linker as described herein).

[00195] Embodiments of the present disclosure include conjugates where a polypeptide is conjugated to one or more moieties, such as 2 moieties, 3 moieties, 4 moieties, 5 moieties, 6 moieties, 7 moieties, 8 moieties, 9 moieties, or 10 or more moieties. The moieties may be conjugated to the polypeptide at one or more sites in the polypeptide. For example, one or more moieties may be conjugated to a single amino acid residue of the polypeptide. In some cases, one moiety is conjugated to an amino acid residue of the polypeptide. In other embodiments, two moieties may be conjugated to the same amino acid residue of the polypeptide. In other embodiments, a first moiety is conjugated to a first amino acid residue of the polypeptide and a second moiety is conjugated to a second amino acid residue of the polypeptide. Combinations of the above are also possible, for example where a polypeptide is conjugated to a first moiety at a first amino acid residue and conjugated to two other moieties at a second amino acid residue. Other combinations are also possible, such as, but not limited to, a polypeptide conjugated to first and second moieties at a first amino acid residue and conjugated to third and fourth moieties at a second amino acid residue, etc.

[00196] The one or more amino acid residues of the polypeptide that are conjugated to the one or more moieties may be naturally occurring amino acids, unnatural amino acids, or combinations thereof. For instance, the conjugate may include a moiety conjugated to a naturally occurring amino acid residue of the polypeptide. In other instances, the conjugate may include a moiety conjugated to an unnatural amino acid residue of the polypeptide. One or more moieties may be conjugated to the polypeptide at a single natural or unnatural amino acid residue as described above. One or more natural or unnatural amino acid residues in the polypeptide may be conjugated to the moiety or moieties as described herein. For example, two (or more) amino acid residues (e.g., natural or unnatural amino acid residues) in the polypeptide may each be conjugated to one or two moieties, such that multiple sites in the polypeptide are modified.

[00197] As described herein, a polypeptide may be conjugated to one or more moieties. In certain embodiments, the moiety of interest is a chemical entity, such as a drug, an active agent, or a detectable label. For example, a drug (or active agent) may be conjugated to the polypeptide, or in other embodiments, a detectable label may be conjugated to the polypeptide. Thus, for instance, embodiments of the present disclosure include, but are not limited to, the following: a conjugate of a polypeptide and a drug; a conjugate of a polypeptide and an active agent; a conjugate of a polypeptide and a detectable label; a conjugate of two or more drugs and a polypeptide; a conjugate of two or more detectable labels and a polypeptide; and the like.

[00198] In certain embodiments, the polypeptide (e.g., antibody) and the moiety of interest (e.g., drug or active agent) are conjugated through a conjugation moiety. For example, the polypeptide and the moiety of interest may each be bound (e.g., covalently bonded) to the conjugation moiety, thus indirectly binding the polypeptide and the moiety of interest together through the conjugation moiety. In some cases, the conjugation moiety includes a hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl compound, or a derivative of a hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl compound. For instance, a general scheme for coupling a moiety of interest to a polypeptide through a hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl conjugation moiety is shown in the general reaction scheme below. Hydrazinyl-indolyl and hydrazinyl-pyrrolo-pyridinyl conjugation moieties are also referred to herein as a hydrazino-*iso*-Pictet-Spengler (HIPS) conjugation moiety and an aza-hydrazino-*iso*-Pictet-Spengler (azaHIPS) conjugation moiety, respectively.



[00199] In the reaction scheme above, R includes the moiety of interest (e.g., a drug or active agent) that is conjugated to the polypeptide (e.g., conjugated to the polypeptide through a cleavable linker as described herein). As shown in the reaction scheme above, a polypeptide that includes a 2-formylglycine residue (fGly) is reacted with a drug or active agent that has been modified to include a conjugation moiety (e.g., a hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl conjugation moiety) to produce a polypeptide conjugate attached to the conjugation

moiety, thus attaching the drug or active agent to the polypeptide through the conjugation moiety.

[00200] As described herein, the moiety can be any of a variety of moieties such as, but not limited to, chemical entity, such as a detectable label, or a drug or active agent. R' and R'' may each independently be any desired substituent, such as, but not limited to, hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, amino, substituted amino, carboxyl, carboxyl ester, acyl, acyloxy, acyl amino, amino acyl, alkylamide, substituted alkylamide, sulfonyl, thioalkoxy, substituted thioalkoxy, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl. Z may be CR²¹, NR²², N, O or S, where R²¹ and R²² are each independently selected from any of the substituents described for R' and R'' above.

[00201] Other hydrazinyl-indolyl or hydrazinyl-pyrrolo-pyridinyl conjugation moieties are also possible, as shown in the conjugates and compounds described herein. For example, the hydrazinyl-indolyl or hydrazinyl-pyrrolo-pyridinyl conjugation moieties may be modified to be attached (e.g., covalently attached) to a linker. As such, embodiments of the present disclosure include a hydrazinyl-indolyl or hydrazinyl-pyrrolo-pyridinyl conjugation moiety attached to a drug or active agent through a linker. Various embodiments of the linker that may couple the hydrazinyl-indolyl or hydrazinyl-pyrrolo-pyridinyl conjugation moiety to the drug or active agent are described in detail herein. For example, in some instances, the linker is a cleavable linker, such as a cleavable linker as described herein.

[00202] In certain embodiments, the polypeptide may be conjugated to a moiety of interest, where the polypeptide is modified before conjugation to the moiety of interest. Modification of the polypeptide may produce a modified polypeptide that contains one or more reactive groups suitable for conjugation to the moiety of interest. In some cases, the polypeptide may be modified at one or more amino acid residues to provide one or more reactive groups suitable for conjugation to the moiety of interest (e.g., a moiety that includes a conjugation moiety, such as a hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl conjugation moiety as described above). For example, the polypeptide may be modified to include a reactive aldehyde group (e.g., a reactive aldehyde). A reactive aldehyde may be included in an "aldehyde tag" or "ald-tag", which as used herein refers to an amino acid sequence derived from a sulfatase motif (e.g., L(C/S)TPSR) that has been converted by action of a formylglycine generating enzyme

(FGE) to contain a 2-formylglycine residue (referred to herein as “FGly”). The FGly residue generated by an FGE may also be referred to as a “formylglycine”. Stated differently, the term “aldehyde tag” is used herein to refer to an amino acid sequence that includes a “converted” sulfatase motif (i.e., a sulfatase motif in which a cysteine or serine residue has been converted to FGly by action of an FGE, e.g., L(FGly)TPSR). A converted sulfatase motif may be derived from an amino acid sequence that includes an “unconverted” sulfatase motif (i.e., a sulfatase motif in which the cysteine or serine residue has not been converted to FGly by an FGE, but is capable of being converted, e.g., an unconverted sulfatase motif with the sequence: L(C/S)TPSR). By “conversion” as used in the context of action of a formylglycine generating enzyme (FGE) on a sulfatase motif refers to biochemical modification of a cysteine or serine residue in a sulfatase motif to a formylglycine (FGly) residue (e.g., Cys to FGly, or Ser to FGly). Additional aspects of aldehyde tags and uses thereof in site-specific protein modification are described in U.S. Patent No. 7,985,783 and U.S. Patent No. 8,729,232, the disclosures of each of which are incorporated herein by reference.

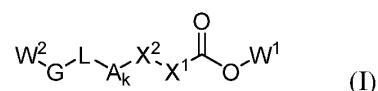
[00203] In some cases, the modified polypeptide containing the FGly residue may be conjugated to the moiety of interest by reaction of the FGly with a compound (e.g., a compound containing a hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl conjugation moiety, as described above). For example, an FGly-containing polypeptide may be contacted with a reactive partner-containing drug under conditions suitable to provide for conjugation of the drug to the polypeptide. In some instances, the reactive partner-containing drug may include a hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl conjugation moiety as described above. For example, a drug or active agent may be modified to include a hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl conjugation moiety. In some cases, the drug or active agent is attached to a hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl, such as covalently attached to a hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl through a linker, such as a cleavable linker as described in detail herein.

[00204] In certain embodiments, a conjugate of the present disclosure includes a polypeptide (e.g., an antibody) having at least one modified amino acid residue. The modified amino acid residue of the polypeptide may be coupled to a drug or active agent containing a hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl conjugation moiety as described above. In certain embodiments, the modified amino acid residue of the polypeptide (e.g., antibody) may be

derived from a cysteine or serine residue that has been converted to an FGly residue as described above. In certain embodiments, the FGly residue is conjugated to a drug or active agent containing a hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl conjugation moiety as described above to provide a conjugate of the present disclosure where the drug or active agent is conjugated to the polypeptide through the hydrazinyl-indolyl or hydrazinyl-pyrrolo-pyridinyl conjugation moiety. As used herein, the term 'FGly' refers to the modified amino acid residue of the polypeptide (e.g., antibody) that is coupled to the moiety of interest (e.g., a drug or active agent).

[00205] In certain embodiments, the conjugate includes at least one modified amino acid residue as described herein, where the modified amino acid residue is attached to a linker (cleavable linker) as described herein, which in turn is attached to a drug or active agent. For instance, the conjugate may include at least one modified amino acid residue ('FGly') as described above.

[00206] In some embodiments, the conjugate is of formula (I):



wherein:

W^1 is a drug;

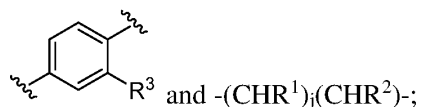
W^2 is a polypeptide;

A is an amino acid residue, where k is 0 or an integer from 1 to 5;

L is a linker;

G is a conjugation moiety;

X^1 is selected from:



R^1 is selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, substituted heterocyclyl and R^3 , wherein R^1 is optionally substituted with R^3 ;

j is 0 or an integer from 1 to 5;

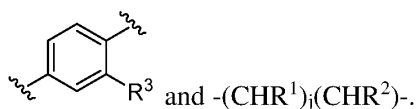
R^2 is R^3 ; or R^2 is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl, wherein R^2 is substituted with R^3 ;

X^2 is -NH- or -C(O)-;

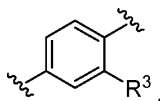
each R^3 is independently a glycoside or glycoside derivative.

[00207] The substituents related to conjugates of formula (I) are described in more detail below.

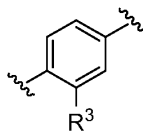
[00208] In certain embodiments, X^1 is selected from:



[00209] In some instances, X^1 is



[00210] In certain embodiments, X^1 is



[00211] In some instances, X^1 is $-(CHR^1)_j(CHR^2)-$. In certain embodiments, j is 0 or an integer from 1 to 5. In some instances, j is 0, and thus X^1 is absent. In some instances, j is 1. In some instances, j is 2. In some instances, j is 3. In some instances, j is 4. In some instances, j is 5.

[00212] In certain embodiments, R^1 is selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, substituted heterocyclyl and R^3 , wherein R^1 is optionally substituted with R^3 . In certain embodiments, R^1 is hydrogen. In certain embodiments, R^1 is alkyl or substituted alkyl, such as C_{1-6} alkyl or C_{1-6} substituted alkyl, or C_{1-4} alkyl or C_{1-4} substituted alkyl, or C_{1-3} alkyl or C_{1-3} substituted alkyl. In certain embodiments, R^1 is alkenyl or substituted alkenyl, such as C_{2-6} alkenyl or C_{2-6} substituted alkenyl, or C_{2-4} alkenyl or C_{2-4} substituted alkenyl, or C_{2-3} alkenyl or C_{2-3} substituted alkenyl. In certain embodiments, R^1 is alkynyl or substituted alkynyl, such as C_{2-6} alkynyl or C_{2-6} substituted

alkenyl, or C₂₋₄ alkenyl or C₂₋₄ substituted alkenyl, or C₂₋₃ alkenyl or C₂₋₃ substituted alkenyl. In certain embodiments, R¹ is aryl or substituted aryl, such as C₅₋₈ aryl or C₅₋₈ substituted aryl, such as a C₅ aryl or C₅ substituted aryl, or a C₆ aryl or C₆ substituted aryl. In certain embodiments, R¹ is phenyl. In certain embodiments, R¹ is heteroaryl or substituted heteroaryl, such as C₅₋₈ heteroaryl or C₅₋₈ substituted heteroaryl, such as a C₅ heteroaryl or C₅ substituted heteroaryl, or a C₆ heteroaryl or C₆ substituted heteroaryl. In certain embodiments, R¹ is cycloalkyl or substituted cycloalkyl, such as C₃₋₈ cycloalkyl or C₃₋₈ substituted cycloalkyl, such as a C₃₋₆ cycloalkyl or C₃₋₆ substituted cycloalkyl, or a C₃₋₅ cycloalkyl or C₃₋₅ substituted cycloalkyl. In certain embodiments, R¹ is heterocyclyl or substituted heterocyclyl, such as C₃₋₈ heterocyclyl or C₃₋₈ substituted heterocyclyl, such as a C₃₋₆ heterocyclyl or C₃₋₆ substituted heterocyclyl, or a C₃₋₅ heterocyclyl or C₃₋₅ substituted heterocyclyl. In certain embodiments, R¹ is R³.

[00213] In certain embodiments, R¹ is optionally substituted with R³. For example, in some instances, R¹ is not substituted with R³. In other embodiments, R¹ is substituted with R³.

[00214] In certain embodiments, R² is R³.

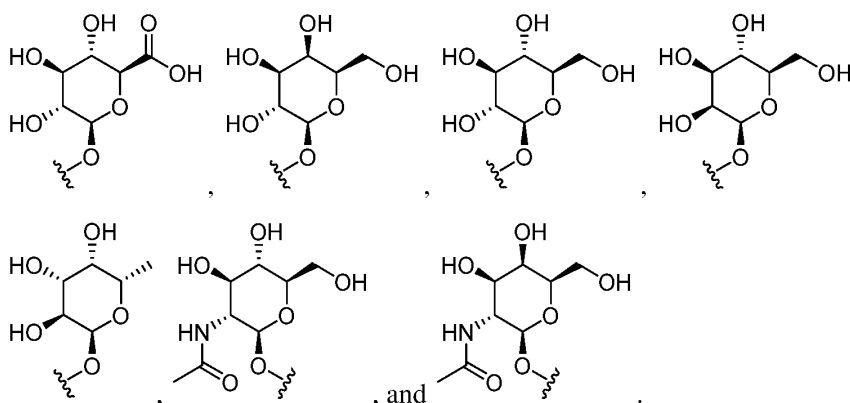
[00215] In certain embodiments, R² is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl, wherein R² is substituted with R³. In certain embodiments, R² is substituted with R³. In these instances, R² can be selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl. In certain embodiments, R² is alkyl or substituted alkyl, such as C₁₋₆ alkyl or C₁₋₆ substituted alkyl, or C₁₋₄ alkyl or C₁₋₄ substituted alkyl, or C₁₋₃ alkyl or C₁₋₃ substituted alkyl. In certain embodiments, R² is methyl. In certain embodiments, R² is ethyl. In certain embodiments, R² is alkenyl or substituted alkenyl, such as C₂₋₆ alkenyl or C₂₋₆ substituted alkenyl, or C₂₋₄ alkenyl or C₂₋₄ substituted alkenyl, or C₂₋₃ alkenyl or C₂₋₃ substituted alkenyl. In certain embodiments, R² is alkynyl or substituted alkynyl, such as C₂₋₆ alkynyl or C₂₋₆ substituted alkynyl, or C₂₋₄ alkynyl or C₂₋₄ substituted alkynyl, or C₂₋₃ alkynyl or C₂₋₃ substituted alkynyl. In certain embodiments, R² is aryl or substituted aryl, such as C₅₋₈ aryl or C₅₋₈ substituted aryl, such as a C₅ aryl or C₅ substituted aryl, or a C₆ aryl or C₆ substituted aryl. In certain embodiments, R² is phenyl. In certain embodiments, R² is heteroaryl or substituted heteroaryl, such as C₅₋₈ heteroaryl or C₅₋₈ substituted heteroaryl, such as a C₅

heteroaryl or C₅ substituted heteroaryl, or a C₆ heteroaryl or C₆ substituted heteroaryl. In certain embodiments, R² is cycloalkyl or substituted cycloalkyl, such as C₃₋₈ cycloalkyl or C₃₋₈ substituted cycloalkyl, such as a C₃₋₆ cycloalkyl or C₃₋₆ substituted cycloalkyl, or a C₃₋₅ cycloalkyl or C₃₋₅ substituted cycloalkyl. In certain embodiments, R² is heterocyclyl or substituted heterocyclyl, such as C₃₋₈ heterocyclyl or C₃₋₈ substituted heterocyclyl, such as a C₃₋₆ heterocyclyl or C₃₋₆ substituted heterocyclyl, or a C₃₋₅ heterocyclyl or C₃₋₅ substituted heterocyclyl.

[00216] In certain embodiments, X² is -NH- or -C(O)-. In some instances, X² is -NH-. In some instances, X² is -C(O)-.

[00217] In certain embodiments, each R³ is independently a glycoside or glycoside derivative. In certain embodiments, the glycoside or glycoside derivative is selected from a glucuronide, a galactoside, a glucoside, a mannoside, a fucoside, O-GlcNAc, and O-GalNAc.

[00218] For example, in some embodiments, the glycoside or glycoside derivative can be selected from the following structures:



[00219] In certain embodiments, W¹ is a drug (or an active agent). Examples of drugs and active agents that can be used in the conjugates of the present disclosure are described in more detail below.

[00220] In certain embodiments, W² is a polypeptide (e.g., an antibody). Examples of polypeptides and antibodies that can be used in the conjugates of the present disclosure are described in more detail below.

[00221] In certain embodiments, A is an amino acid residue. In certain embodiments, k is 0 or an integer from 1 to 5. In some instances, k is 0, and thus A is absent. In some instances, k

is 1. In some instances, k is 2. In some instances, k is 3. In some instances, k is 4. In some instances, k is 5.

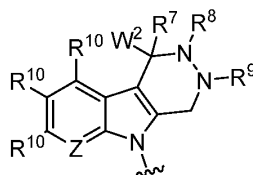
[00222] Amino acid residues include, but are not limited to amino acids found in naturally occurring proteins (e.g., Ala or A, Cys or C, Asp or D, Glu or E, Phe or F, Gly or G, His or H, Ile or I, Lys or K, Leu or L, Met or M, Asn or N, Pro or P, Gln or Q, Arg or R, Ser or S, Thr or T, Val or V, Trp or W, Tyr or Y). In some instances, the amino acid residue can be an amino acid analog or an unnatural amino acid.

[00223] The terms “amino acid analog,” “unnatural amino acid,” and the like may be used interchangeably, and include amino acid-like compounds that are similar in structure and/or overall shape to one or more amino acids commonly found in naturally occurring proteins. Amino acid analogs also include natural amino acids with modified side chains or backbones. Amino acid analogs also include amino acid analogs with the same stereochemistry as in the naturally occurring D-form, as well as the L-form of amino acid analogs. In some instances, the amino acid analogs share backbone structures, and/or the side chain structures of one or more natural amino acids, with difference(s) being one or more modified groups in the molecule. Such modification may include, but is not limited to, substitution of an atom (such as N) for a related atom (such as S), addition of a group (such as methyl, or hydroxyl, etc.) or an atom (such as Cl or Br, etc.), deletion of a group, substitution of a covalent bond (single bond for double bond, etc.), or combinations thereof. For example, amino acid analogs may include α -hydroxy acids, and α -amino acids, and the like.

[00224] In certain embodiments, L is a linker. In some instances, the linker is a cleavable linker. Examples of linkers that can be used in the conjugates of the present disclosure are described in more detail below.

[00225] In certain embodiments, G is a conjugation moiety. As described above, in the ADCs of the present disclosure, the polypeptide (e.g., antibody) and the drug or active agent can be conjugated to each other through a conjugation moiety. For example, the polypeptide (antibody) and the drug may each be bound (e.g., covalently bonded) to the conjugation moiety, thus indirectly binding the polypeptide (antibody) and the drug together through the conjugation moiety. Any convenient conjugation moiety may be used in the conjugates of the present disclosure, where the conjugation moiety is sufficient to provide stable attachment of the polypeptide (e.g., antibody) to the drug or active agent in the ADC.

[00226] In certain embodiments, the conjugation moiety includes a hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl compound, or a derivative of a hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl compound. In certain embodiments, the conjugation moiety G is:



wherein:

Z is CR¹⁰ or N,

R⁷ is selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl;

R⁸ and R⁹ are each independently selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, amino, substituted amino, carboxyl, carboxyl ester, acyl, acyloxy, acyl amino, amino acyl, alkylamide, substituted alkylamide, sulfonyl, thioalkoxy, substituted thioalkoxy, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl, or R⁸ and R⁹ are optionally cyclically linked to form a 5 or 6-membered heterocyclyl;

each R¹⁰ is independently selected from hydrogen, halogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, amino, substituted amino, carboxyl, carboxyl ester, acyl, acyloxy, acyl amino, amino acyl, alkylamide, substituted alkylamide, sulfonyl, thioalkoxy, substituted thioalkoxy, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl.

[00227] In certain embodiments, Z is CR¹⁰ or N. In certain embodiments, Z is CR¹⁰. In certain embodiments, Z is N.

[00228] In certain embodiments, R⁷ is selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl. In certain embodiments, R⁷ is hydrogen. In certain embodiments, R⁷ is alkyl or

substituted alkyl, such as C₁₋₆ alkyl or C₁₋₆ substituted alkyl, or C₁₋₄ alkyl or C₁₋₄ substituted alkyl, or C₁₋₃ alkyl or C₁₋₃ substituted alkyl. In certain embodiments, R⁷ is methyl. In certain embodiments, R⁷ is alkenyl or substituted alkenyl, such as C₂₋₆ alkenyl or C₂₋₆ substituted alkenyl, or C₂₋₄ alkenyl or C₂₋₄ substituted alkenyl, or C₂₋₃ alkenyl or C₂₋₃ substituted alkenyl. In certain embodiments, R⁷ is alkynyl or substituted alkynyl, such as C₂₋₆ alkynyl or C₂₋₆ substituted alkynyl, or C₂₋₄ alkynyl or C₂₋₄ substituted alkynyl, or C₂₋₃ alkynyl or C₂₋₃ substituted alkynyl. In certain embodiments, R⁷ is aryl or substituted aryl, such as C₅₋₈ aryl or C₅₋₈ substituted aryl, such as a C₅ aryl or C₅ substituted aryl, or a C₆ aryl or C₆ substituted aryl. In certain embodiments, R⁷ is heteroaryl or substituted heteroaryl, such as C₅₋₈ heteroaryl or C₅₋₈ substituted heteroaryl, such as a C₅ heteroaryl or C₅ substituted heteroaryl, or a C₆ heteroaryl or C₆ substituted heteroaryl. In certain embodiments, R⁷ is cycloalkyl or substituted cycloalkyl, such as C₃₋₈ cycloalkyl or C₃₋₈ substituted cycloalkyl, such as a C₃₋₆ cycloalkyl or C₃₋₆ substituted cycloalkyl, or a C₃₋₅ cycloalkyl or C₃₋₅ substituted cycloalkyl. In certain embodiments, R⁷ is heterocyclyl or substituted heterocyclyl, such as C₃₋₈ heterocyclyl or C₃₋₈ substituted heterocyclyl, such as a C₃₋₆ heterocyclyl or C₃₋₆ substituted heterocyclyl, or a C₃₋₅ heterocyclyl or C₃₋₅ substituted heterocyclyl.

[00229] In certain embodiments, R⁸ and R⁹ are each independently selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, amino, substituted amino, carboxyl, carboxyl ester, acyl, acyloxy, acyl amino, amino acyl, alkylamide, substituted alkylamide, sulfonyl, thioalkoxy, substituted thioalkoxy, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl, or R⁸ and R⁹ are optionally cyclically linked to form a 5 or 6-membered heterocyclyl.

[00230] In certain embodiments, R⁸ is selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, amino, substituted amino, carboxyl, carboxyl ester, acyl, acyloxy, acyl amino, amino acyl, alkylamide, substituted alkylamide, sulfonyl, thioalkoxy, substituted thioalkoxy, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl. In certain embodiments, R⁸ is hydrogen. In certain embodiments, R⁸ is alkyl or substituted alkyl, such as C₁₋₆ alkyl or C₁₋₆ substituted alkyl, or C₁₋₄ alkyl or C₁₋₄ substituted alkyl, or C₁₋₃ alkyl or C₁₋₃ substituted alkyl. In certain embodiments, R⁸ is methyl. In

certain embodiments, R^8 is alkenyl or substituted alkenyl, such as C_{2-6} alkenyl or C_{2-6} substituted alkenyl, or C_{2-4} alkenyl or C_{2-4} substituted alkenyl, or C_{2-3} alkenyl or C_{2-3} substituted alkenyl. In certain embodiments, R^8 is alkynyl or substituted alkynyl. In certain embodiments, R^8 is alkoxy or substituted alkoxy. In certain embodiments, R^8 is amino or substituted amino. In certain embodiments, R^8 is carboxyl or carboxyl ester. In certain embodiments, R^8 is acyl or acyloxy. In certain embodiments, R^8 is acyl amino or amino acyl. In certain embodiments, R^8 is alkylamide or substituted alkylamide. In certain embodiments, R^8 is sulfonyl. In certain embodiments, R^8 is thioalkoxy or substituted thioalkoxy. In certain embodiments, R^8 is aryl or substituted aryl, such as C_{5-8} aryl or C_{5-8} substituted aryl, such as a C_5 aryl or C_5 substituted aryl, or a C_6 aryl or C_6 substituted aryl. In certain embodiments, R^8 is heteroaryl or substituted heteroaryl, such as C_{5-8} heteroaryl or C_{5-8} substituted heteroaryl, such as a C_5 heteroaryl or C_5 substituted heteroaryl, or a C_6 heteroaryl or C_6 substituted heteroaryl. In certain embodiments, R^8 is cycloalkyl or substituted cycloalkyl, such as C_{3-8} cycloalkyl or C_{3-8} substituted cycloalkyl, such as a C_{3-6} cycloalkyl or C_{3-6} substituted cycloalkyl, or a C_{3-5} cycloalkyl or C_{3-5} substituted cycloalkyl. In certain embodiments, R^8 is heterocyclyl or substituted heterocyclyl, such as a C_{3-6} heterocyclyl or C_{3-6} substituted heterocyclyl, or a C_{3-5} heterocyclyl or C_{3-5} substituted heterocyclyl.

[00231] In certain embodiments, R^9 is selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, amino, substituted amino, carboxyl, carboxyl ester, acyl, acyloxy, acyl amino, amino acyl, alkylamide, substituted alkylamide, sulfonyl, thioalkoxy, substituted thioalkoxy, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl. In certain embodiments, R^9 is hydrogen. In certain embodiments, R^9 is alkyl or substituted alkyl, such as C_{1-6} alkyl or C_{1-6} substituted alkyl, or C_{1-4} alkyl or C_{1-4} substituted alkyl, or C_{1-3} alkyl or C_{1-3} substituted alkyl. In certain embodiments, R^9 is methyl. In certain embodiments, R^9 is alkenyl or substituted alkenyl, such as C_{2-6} alkenyl or C_{2-6} substituted alkenyl, or C_{2-4} alkenyl or C_{2-4} substituted alkenyl, or C_{2-3} alkenyl or C_{2-3} substituted alkenyl. In certain embodiments, R^9 is alkynyl or substituted alkynyl. In certain embodiments, R^9 is alkoxy or substituted alkoxy. In certain embodiments, R^9 is amino or substituted amino. In certain embodiments, R^9 is carboxyl or carboxyl ester. In certain embodiments, R^9 is acyl or acyloxy. In certain embodiments, R^9 is acyl amino or amino acyl. In certain embodiments, R^9 is

alkylamide or substituted alkylamide. In certain embodiments, R⁹ is sulfonyl. In certain embodiments, R⁹ is thioalkoxy or substituted thioalkoxy. In certain embodiments, R⁹ is aryl or substituted aryl, such as C₅₋₈ aryl or C₅₋₈ substituted aryl, such as a C₅ aryl or C₅ substituted aryl, or a C₆ aryl or C₆ substituted aryl. In certain embodiments, R⁹ is heteroaryl or substituted heteroaryl, such as C₅₋₈ heteroaryl or C₅₋₈ substituted heteroaryl, such as a C₅ heteroaryl or C₅ substituted heteroaryl, or a C₆ heteroaryl or C₆ substituted heteroaryl. In certain embodiments, R⁹ is cycloalkyl or substituted cycloalkyl, such as C₃₋₈ cycloalkyl or C₃₋₈ substituted cycloalkyl, such as a C₃₋₆ cycloalkyl or C₃₋₆ substituted cycloalkyl, or a C₃₋₅ cycloalkyl or C₃₋₅ substituted cycloalkyl. In certain embodiments, R⁹ is heterocyclyl or substituted heterocyclyl, such as C₃₋₈ heterocyclyl or C₃₋₈ substituted heterocyclyl, such as a C₃₋₆ heterocyclyl or C₃₋₆ substituted heterocyclyl, or a C₃₋₅ heterocyclyl or C₃₋₅ substituted heterocyclyl.

[00232] In certain embodiments, R⁸ and R⁹ are optionally cyclically linked to form a 5 or 6-membered heterocyclyl. In certain embodiments, R⁸ and R⁹ are cyclically linked to form a 5 or 6-membered heterocyclyl. In certain embodiments, R⁸ and R⁹ are cyclically linked to form a 5-membered heterocyclyl. In certain embodiments, R⁸ and R⁹ are cyclically linked to form a 6-membered heterocyclyl.

[00233] In certain embodiments, each R¹⁰ is independently selected from hydrogen, halogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, amino, substituted amino, carboxyl, carboxyl ester, acyl, acyloxy, acyl amino, amino acyl, alkylamide, substituted alkylamide, sulfonyl, thioalkoxy, substituted thioalkoxy, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl.

[00234] The various possibilities for each R¹⁰ are described in more detail as follows. In certain embodiments, R¹⁰ is hydrogen. In certain embodiments, each R¹⁰ is hydrogen. In certain embodiments, R¹⁰ is halogen, such as F, Cl, Br or I. In certain embodiments, R¹⁰ is F. In certain embodiments, R¹⁰ is Cl. In certain embodiments, R¹⁰ is Br. In certain embodiments, R¹⁰ is I. In certain embodiments, R¹⁰ is alkyl or substituted alkyl, such as C₁₋₆ alkyl or C₁₋₆ substituted alkyl, or C₁₋₄ alkyl or C₁₋₄ substituted alkyl, or C₁₋₃ alkyl or C₁₋₃ substituted alkyl. In certain embodiments, R¹⁰ is methyl. In certain embodiments, R¹⁰ is alkenyl or substituted alkenyl, such as C₂₋₆ alkenyl or C₂₋₆ substituted alkenyl, or C₂₋₄ alkenyl or C₂₋₄ substituted alkenyl, or C₂₋₃ alkenyl or C₂₋₃ substituted alkenyl. In certain embodiments, R¹⁰ is alkynyl or substituted alkynyl.

In certain embodiments, R¹⁰ is alkoxy or substituted alkoxy. In certain embodiments, R¹⁰ is amino or substituted amino. In certain embodiments, R¹⁰ is carboxyl or carboxyl ester. In certain embodiments, R¹⁰ is acyl or acyloxy. In certain embodiments, R¹⁰ is acyl amino or amino acyl. In certain embodiments, R¹⁰ is alkylamide or substituted alkylamide. In certain embodiments, R¹⁰ is sulfonyl. In certain embodiments, R¹⁰ is thioalkoxy or substituted thioalkoxy. In certain embodiments, R¹⁰ is aryl or substituted aryl, such as C₅₋₈ aryl or C₅₋₈ substituted aryl, such as a C₅ aryl or C₅ substituted aryl, or a C₆ aryl or C₆ substituted aryl (e.g., phenyl or substituted phenyl). In certain embodiments, R¹⁰ is heteroaryl or substituted heteroaryl, such as C₅₋₈ heteroaryl or C₅₋₈ substituted heteroaryl, such as a C₅ heteroaryl or C₅ substituted heteroaryl, or a C₆ heteroaryl or C₆ substituted heteroaryl. In certain embodiments, R¹⁰ is cycloalkyl or substituted cycloalkyl, such as C₃₋₈ cycloalkyl or C₃₋₈ substituted cycloalkyl, such as a C₃₋₆ cycloalkyl or C₃₋₆ substituted cycloalkyl, or a C₃₋₅ cycloalkyl or C₃₋₅ substituted cycloalkyl. In certain embodiments, R¹⁰ is heterocyclyl or substituted heterocyclyl, such as C₃₋₈ heterocyclyl or C₃₋₈ substituted heterocyclyl, such as a C₃₋₆ heterocyclyl or C₃₋₆ substituted heterocyclyl, or a C₃₋₅ heterocyclyl or C₃₋₅ substituted heterocyclyl.

[00235] In certain embodiments, the conjugation moiety G includes a functional group that conjugates the polypeptide (antibody) W² to the linker L. In some instances, G is selected from an acetal, a hydrazone, an oxime, a sulfide, a disulfide, a triazole, an ester, and an amide. In some instances, the conjugation moiety G is an acetal. In some instances, the conjugation moiety G is a hydrazone. In some instances, the conjugation moiety G is an oxime. In some instances, the conjugation moiety G is a sulfide. In some instances, the conjugation moiety G is a disulfide. In some instances, the conjugation moiety G is a triazole. In some instances, the conjugation moiety G is an ester. In some instances, the conjugation moiety G is an amide.

[00236] In certain embodiments, the conjugation moiety G can be described in terms of an intermediate or precursor functional group, which reacts with a corresponding reactive group on the polypeptide (antibody) to conjugate the the polypeptide (antibody) to the linker L. In these instances, the conjugation moiety G can be selected from an acetal, an NHS-ester, a PFP-ester, a TFP-ester, an isocyanate, an isothiocyanate, an acyl halide, a maleimide, an iodoacetamide, a phosphine, an alkyne, an azide, a hydrazine, an alkoxyamine, a disulfide, an ester, and an amide. In some instances, the conjugation moiety G is an acetal. In some instances, the conjugation moiety G is an NHS-ester. In some instances, the conjugation moiety G is a PFP-ester. In some

instances, the conjugation moiety G is a TFP-ester. In some instances, the conjugation moiety G is an isocyanate. In some instances, the conjugation moiety G is an isothiocyanate. In some instances, the conjugation moiety G is an acyl halide. In some instances, the conjugation moiety G is a maleimide. In some instances, the conjugation moiety G is an iodoacetamide. In some instances, the conjugation moiety G is a phosphine. In some instances, the conjugation moiety G is an alkyne. In some instances, the conjugation moiety G is an azide. In some instances, the conjugation moiety G is a hydrazine. In some instances, the conjugation moiety G is an alkoxyamine. In some instances, the conjugation moiety G is a disulfide. In some instances, the conjugation moiety G is an ester. In some instances, the conjugation moiety G is and an amide.

[00237] In certain embodiments, the conjugate of formula (I) includes a linker, L. The linker may be utilized to bind one or more moieties of interest (e.g., drug or active agent) to one or more polypeptides through a conjugation moiety. The linker may be bound (e.g., covalently bonded) to the conjugation moiety G (e.g., as described herein) at any convenient position. For example, the linker may attach a hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl conjugation moiety to a drug (e.g., a camptothecine or camptothecine derivative). The hydrazinyl-indolyl or hydrazinyl-pyrrolo-pyridinyl conjugation moiety may be used to conjugate the linker (and thus the drug) to a polypeptide, such as an antibody.

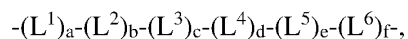
[00238] For example, as shown in formula (I) above, L is attached W^2 through a conjugation moiety G, and thus W^2 is indirectly bonded to the linker L through the conjugation moiety. As described above, W^2 is a polypeptide (e.g., an antibody), and thus L is attached through the conjugation moiety to the polypeptide (antibody), e.g., the linker L is indirectly bonded to the polypeptide (antibody) through the conjugation moiety (e.g., through a hydrazinyl-indolyl or hydrazinyl-pyrrolo-pyridinyl conjugation moiety as described herein).

[00239] Any convenient linker may be utilized for the linker L in the subject conjugates and compounds. In certain embodiments, the linker L may include a group selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, amino, substituted amino, carboxyl, carboxyl ester, acyl amino, alkylamide, substituted alkylamide, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl. In certain embodiments, the linker L may include an alkyl or substituted alkyl group. In certain embodiments, the linker L may include an alkenyl or substituted alkenyl group. In certain embodiments, the linker L may include an

alkynyl or substituted alkynyl group. In certain embodiments, the linker L may include an alkoxy or substituted alkoxy group. In certain embodiments, the linker L may include an amino or substituted amino group. In certain embodiments, the linker L may include a carboxyl or carboxyl ester group. In certain embodiments, the linker L may include an acyl amino group. In certain embodiments, the linker L may include an alkylamide or substituted alkylamide group. In certain embodiments, the linker L may include an aryl or substituted aryl group. In certain embodiments, the linker L may include a heteroaryl or substituted heteroaryl group. In certain embodiments, the linker L may include a cycloalkyl or substituted cycloalkyl group. In certain embodiments, the linker L may include a heterocyclyl or substituted heterocyclyl group.

[00240] In certain embodiments, the linker L may include a polymer. For example, the polymer may include a polyalkylene glycol and derivatives thereof, including polyethylene glycol, methoxypolyethylene glycol, polyethylene glycol homopolymers, polypropylene glycol homopolymers, copolymers of ethylene glycol with propylene glycol (e.g., where the homopolymers and copolymers are unsubstituted or substituted at one end with an alkyl group), polyvinyl alcohol, polyvinyl ethyl ethers, polyvinylpyrrolidone, combinations thereof, and the like. In certain embodiments, the polymer is a polyalkylene glycol. In certain embodiments, the polymer is a polyethylene glycol. Other linkers are also possible, as shown in the conjugates and compounds described in more detail below.

[00241] In some embodiments, L is a linker described by the formula:



wherein L^1 , L^2 , L^3 , L^4 , L^5 and L^6 are each independently a linker subunit, and a, b, c, d, e and f are each independently 0 or 1, wherein the sum of a, b, c, d, e and f is 1 to 6.

[00242] In certain embodiments, the sum of a, b, c, d, e and f is 1. In certain embodiments, the sum of a, b, c, d, e and f is 2. In certain embodiments, the sum of a, b, c, d, e and f is 3. In certain embodiments, the sum of a, b, c, d, e and f is 4. In certain embodiments, the sum of a, b, c, d, e and f is 5. In certain embodiments, the sum of a, b, c, d, e and f is 6. In certain embodiments, a, b, c, d, e and f are each 1. In certain embodiments, a, b, c, d and e are each 1 and f is 0. In certain embodiments, a, b, c and d are each 1 and e and f are each 0. In certain embodiments, a, b, and c are each 1 and d, e and f are each 0. In certain embodiments, a and b are each 1 and c, d, e and f are each 0. In certain embodiments, a is 1 and b, c, d, e and f are each 0.

[00243] In certain embodiments, the linker subunit L^1 is attached to the conjugation moiety G (e.g., as shown in formula (I) above). In certain embodiments, the linker subunit L^2 , if present, is attached to the drug or active agent W^1 . For example, L^2 can be indirectly attached to the drug or active agent W^1 through the $-A_k-X^2-X^1-C(O)O-$ portion of the structure in formula (I). In certain embodiments, the linker subunit L^3 , if present, is attached to the drug or active agent. For example, L^3 can be indirectly attached to the drug or active agent W^1 through the $-A_k-X^2-X^1-C(O)O-$ portion of the structure in formula (I). In certain embodiments, the linker subunit L^4 , if present, is attached to the drug or active agent. For example, L^4 can be indirectly attached to the drug or active agent W^1 through the $-A_k-X^2-X^1-C(O)O-$ portion of the structure in formula (I). In certain embodiments, the linker subunit L^5 , if present, is attached to the drug or active agent. For example, L^5 can be indirectly attached to the drug or active agent W^1 through the $-A_k-X^2-X^1-C(O)O-$ portion of the structure in formula (I). In certain embodiments, the linker subunit L^6 , if present, is attached to the drug or active agent. For example, L^6 can be indirectly attached to the drug or active agent W^1 through the $-A_k-X^2-X^1-C(O)O-$ portion of the structure in formula (I).

[00244] Any convenient linker subunits may be utilized in the linker L. Linker subunits of interest include, but are not limited to, units of polymers such as polyethylene glycols, polyethylenes and polyacrylates, amino acid residue(s), carbohydrate-based polymers or carbohydrate residues and derivatives thereof, polynucleotides, alkyl groups, aryl groups, heterocyclic groups, combinations thereof, and substituted versions thereof. In some embodiments, each of L^1 , L^2 , L^3 , L^4 , L^5 and L^6 (if present) comprise one or more groups independently selected from a polyethylene glycol, a modified polyethylene glycol, an amino acid residue, an alkyl group, a substituted alkyl, an aryl group, a substituted aryl group, and a diamine (e.g., a linking group that includes an alkylene diamine).

[00245] In some embodiments, L^1 (if present) comprises a polyethylene glycol, a modified polyethylene glycol, an amino acid residue, an alkyl group, a substituted alkyl, an aryl group, a substituted aryl group, or a diamine. In some embodiments, L^1 comprises a polyethylene glycol. In some embodiments, L^1 comprises a modified polyethylene glycol. In some embodiments, L^1 comprises an amino acid residue. In some embodiments, L^1 comprises an alkyl group or a substituted alkyl. In some embodiments, L^1 comprises an aryl group or a substituted aryl group.

In some embodiments, L¹ comprises a diamine (e.g., a linking group comprising an alkylene diamine).

[00246] In some embodiments, L² (if present) comprises a polyethylene glycol, a modified polyethylene glycol, an amino acid residue, an alkyl group, a substituted alkyl, an aryl group, a substituted aryl group, or a diamine. In some embodiments, L² comprises a polyethylene glycol. In some embodiments, L² comprises a modified polyethylene glycol. In some embodiments, L² comprises an amino acid residue. In some embodiments, L² comprises an alkyl group or a substituted alkyl. In some embodiments, L² comprises an aryl group or a substituted aryl group. In some embodiments, L² comprises a diamine (e.g., a linking group comprising an alkylene diamine).

[00247] In some embodiments, L³ (if present) comprises a polyethylene glycol, a modified polyethylene glycol, an amino acid residue, an alkyl group, a substituted alkyl, an aryl group, a substituted aryl group, or a diamine. In some embodiments, L³ comprises a polyethylene glycol. In some embodiments, L³ comprises a modified polyethylene glycol. In some embodiments, L³ comprises an amino acid residue. In some embodiments, L³ comprises an alkyl group or a substituted alkyl. In some embodiments, L³ comprises an aryl group or a substituted aryl group. In some embodiments, L³ comprises a diamine (e.g., a linking group comprising an alkylene diamine).

[00248] In some embodiments, L⁴ (if present) comprises a polyethylene glycol, a modified polyethylene glycol, an amino acid residue, an alkyl group, a substituted alkyl, an aryl group, a substituted aryl group, or a diamine. In some embodiments, L⁴ comprises a polyethylene glycol. In some embodiments, L⁴ comprises a modified polyethylene glycol. In some embodiments, L⁴ comprises an amino acid residue. In some embodiments, L⁴ comprises an alkyl group or a substituted alkyl. In some embodiments, L⁴ comprises an aryl group or a substituted aryl group. In some embodiments, L⁴ comprises a diamine (e.g., a linking group comprising an alkylene diamine).

[00249] In some embodiments, L⁵ (if present) comprises a polyethylene glycol, a modified polyethylene glycol, an amino acid residue, an alkyl group, a substituted alkyl, an aryl group, a substituted aryl group, or a diamine. In some embodiments, L⁵ comprises a polyethylene glycol. In some embodiments, L⁵ comprises a modified polyethylene glycol. In some embodiments, L⁵ comprises an amino acid residue. In some embodiments, L⁵ comprises an alkyl group or a

substituted alkyl. In some embodiments, L^5 comprises an aryl group or a substituted aryl group. In some embodiments, L^5 comprises a diamine (e.g., a linking group comprising an alkylene diamine).

[00250] In some embodiments, L^6 (if present) comprises a polyethylene glycol, a modified polyethylene glycol, an amino acid residue, an alkyl group, a substituted alkyl, an aryl group, a substituted aryl group, or a diamine. In some embodiments, L^6 comprises a polyethylene glycol. In some embodiments, L^6 comprises a modified polyethylene glycol. In some embodiments, L^6 comprises an amino acid residue. In some embodiments, L^6 comprises an alkyl group or a substituted alkyl. In some embodiments, L^6 comprises an aryl group or a substituted aryl group. In some embodiments, L^6 comprises a diamine (e.g., a linking group comprising an alkylene diamine).

[00251] In some embodiments, L is a linker comprising $-(L^1)_a-(L^2)_b-(L^3)_c-(L^4)_d-(L^5)_e-(L^6)_f-$, where:

$-(L^1)_a-$ is $-(T^1-V^1)_a-$;

$-(L^2)_b-$ is $-(T^2-V^2)_b-$;

$-(L^3)_c-$ is $-(T^3-V^3)_c-$;

$-(L^4)_d-$ is $-(T^4-V^4)_d-$;

$-(L^5)_e-$ is $-(T^5-V^5)_e-$; and

$-(L^6)_f-$ is $-(T^6-V^6)_f-$,

wherein T^1 , T^2 , T^3 , T^4 , T^5 and T^6 , if present, are tether groups;

V^1 , V^2 , V^3 , V^4 , V^5 and V^6 , if present, are covalent bonds or linking functional groups; and

a, b, c, d, e and f are each independently 0 or 1, wherein the sum of a, b, c, d, e and f is 1 to 6.

[00252] As described above, in certain embodiments, L^1 is attached to the conjugation moiety G (e.g., as shown in formula (I) above). As such, in certain embodiments, T^1 is attached to the conjugation moiety G (e.g., as shown in formula (I) above). In certain embodiments, V^1 is (indirectly) attached to the drug or active agent (e.g., through the $-A_k-X^2-X^1-C(O)O-$ portion of the structure in formula (I)). In certain embodiments, L^2 , if present, is attached to the drug or active agent. As such, in certain embodiments, T^2 , if present, is (indirectly) attached to the drug or active agent, or V^2 , if present, is (indirectly) attached to the drug or active agent (e.g., through the $-A_k-X^2-X^1-C(O)O-$ portion of the structure in formula (I)). In certain embodiments, L^3 , if

present, is attached to the drug or active agent. As such, in certain embodiments, T^3 , if present, is (indirectly) attached to the drug or active agent, or V^3 , if present, is (indirectly) attached to the drug or active agent (e.g., through the $-A_k-X^2-X^1-C(O)O-$ portion of the structure in formula (I)). In certain embodiments, L^4 , if present, is attached to the camptothecine or camptothecine derivative. As such, in certain embodiments, T^4 , if present, is (indirectly) attached to the drug or active agent, or V^4 , if present, is (indirectly) attached to the drug or active agent (e.g., through the $-A_k-X^2-X^1-C(O)O-$ portion of the structure in formula (I)). In certain embodiments, L^5 , if present, is attached to the camptothecine or camptothecine derivative. As such, in certain embodiments, T^5 , if present, is (indirectly) attached to the drug or active agent, or V^5 , if present, is (indirectly) attached to the drug or active agent (e.g., through the $-A_k-X^2-X^1-C(O)O-$ portion of the structure in formula (I)). In certain embodiments, L^6 , if present, is attached to the camptothecine or camptothecine derivative. As such, in certain embodiments, T^6 , if present, is (indirectly) attached to the drug or active agent, or V^6 , if present, is (indirectly) attached to the drug or active agent (e.g., through the $-A_k-X^2-X^1-C(O)O-$ portion of the structure in formula (I)).

[00253] Regarding the tether groups, T^1 , T^2 , T^3 , T^4 , T^5 and T^6 , any convenient tether groups may be utilized in the subject linkers. In some embodiments, T^1 , T^2 , T^3 , T^4 , T^5 and T^6 each comprise one or more groups independently selected from a covalent bond, a (C_1-C_{12}) alkyl, a substituted (C_1-C_{12}) alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl, $(EDA)_w$, $(PEG)_n$, $(AA)_p$, $-(CR^{13}OH)_m$, 4-amino-piperidine (4AP), meta-amino-benzyloxy (MABO), meta-amino-benzyloxycarbonyl (MABC), para-amino-benzyloxy (PABO), para-amino-benzyloxycarbonyl (PABC), para-aminobenzyl (PAB), para-amino-benzylamino (PABA), para-amino-phenyl (PAP), para-hydroxy-phenyl (PHP), an acetal group, a hydrazine, a disulfide, and an ester, where each w is an integer from 1 to 20, each n is an integer from 1 to 30, each p is an integer from 1 to 20, and each m is an integer from 1 to 12.

[00254] In certain embodiments, the tether group (e.g., T^1 , T^2 , T^3 , T^4 , T^5 and/or T^6) includes a (C_1-C_{12}) alkyl or a substituted (C_1-C_{12}) alkyl. In certain embodiments, (C_1-C_{12}) alkyl is a straight chain or branched alkyl group that includes from 1 to 12 carbon atoms, such as 1 to 10 carbon atoms, or 1 to 8 carbon atoms, or 1 to 6 carbon atoms, or 1 to 5 carbon atoms, or 1 to 4 carbon atoms, or 1 to 3 carbon atoms. In some instances, (C_1-C_{12}) alkyl may be an alkyl or substituted alkyl, such as C_1-C_{12} alkyl, or C_1-C_{10} alkyl, or C_1-C_6 alkyl, or C_1-C_3 alkyl. In some

instances, (C₁-C₁₂)alkyl is a C₂-alkyl. For example, (C₁-C₁₂)alkyl may be an alkylene or substituted alkylene, such as C₁-C₁₂ alkylene, or C₁-C₁₀ alkylene, or C₁-C₆ alkylene, or C₁-C₃ alkylene. In some instances, (C₁-C₁₂)alkyl is a C₂-alkylene (e.g., CH₂CH₂).

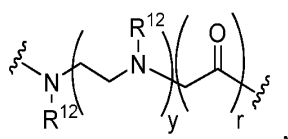
[00255] In certain embodiments, substituted (C₁-C₁₂)alkyl is a straight chain or branched substituted alkyl group that includes from 1 to 12 carbon atoms, such as 1 to 10 carbon atoms, or 1 to 8 carbon atoms, or 1 to 6 carbon atoms, or 1 to 5 carbon atoms, or 1 to 4 carbon atoms, or 1 to 3 carbon atoms. In some instances, substituted (C₁-C₁₂)alkyl may be a substituted alkyl, such as substituted C₁-C₁₂ alkyl, or substituted C₁-C₁₀ alkyl, or substituted C₁-C₆ alkyl, or substituted C₁-C₃ alkyl. In some instances, substituted (C₁-C₁₂)alkyl is a substituted C₂-alkyl. For example, substituted (C₁-C₁₂)alkyl may be a substituted alkylene, such as substituted C₁-C₁₂ alkylene, or substituted C₁-C₁₀ alkylene, or substituted C₁-C₆ alkylene, or substituted C₁-C₃ alkylene. In some instances, substituted (C₁-C₁₂)alkyl is a substituted C₂-alkylene.

[00256] In certain embodiments, the tether group (e.g., T¹, T², T³, T⁴, T⁵ and T⁶) includes an aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, or substituted heterocyclyl. In some instances, the tether group (e.g., T¹, T², T³, T⁴, T⁵ and T⁶) includes an aryl or substituted aryl. For example, the aryl can be phenyl. In some cases, the substituted aryl is a substituted phenyl. The substituted phenyl can be substituted with one or more substituents selected from (C₁-C₁₂)alkyl, a substituted (C₁-C₁₂)alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl. In some instances, the substituted aryl is a substituted phenyl, where the substituent includes a cleavable moiety as described herein (e.g., an enzymatically cleavable moiety, such as a glycoside or glycoside derivative).

[00257] In some instances, the tether group (e.g., T¹, T², T³, T⁴, T⁵ and T⁶) includes a heteroaryl or substituted heteroaryl. In some instances, the tether group (e.g., T¹, T², T³, T⁴, T⁵ and T⁶) includes a cycloalkyl or substituted cycloalkyl. In some instances, the tether group (e.g., T¹, T², T³, T⁴, T⁵ and T⁶) includes a heterocyclyl or substituted heterocyclyl. In some instances, the substituent on the substituted heteroaryl, substituted cycloalkyl or substituted heterocyclyl includes a cleavable moiety as described herein (e.g., an enzymatically cleavable moiety, such as a glycoside or glycoside derivative).

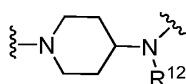
[00258] In certain embodiments, the tether group (e.g., T¹, T², T³, T⁴, T⁵ and T⁶) includes an ethylene diamine (EDA) moiety, e.g., an EDA containing tether group. In certain

embodiments, (EDA)_w includes one or more EDA moieties, such as where w is an integer from 1 to 50, such as from 1 to 40, from 1 to 30, from 1 to 20, from 1 to 12 or from 1 to 6, such as 1, 2, 3, 4, 5 or 6). The linked ethylene diamine (EDA) moieties may optionally be substituted at one or more convenient positions with any convenient substituents, e.g., with an alkyl, a substituted alkyl, an acyl, a substituted acyl, an aryl or a substituted aryl. In certain embodiments, the EDA moiety is described by the structure:



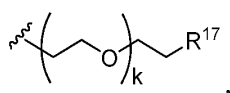
where y is an integer from 1 to 6, or is 0 or 1, and each R¹² is independently selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, amino, substituted amino, carboxyl, carboxyl ester, acyl, acyloxy, acyl amino, amino acyl, alkylamide, substituted alkylamide, sulfonyl, thioalkoxy, substituted thioalkoxy, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl. In certain embodiments, y is 1, 2, 3, 4, 5 or 6. In certain embodiments, y is 1 and r is 0. In certain embodiments, y is 1 and r is 1. In certain embodiments, y is 2 and r is 0. In certain embodiments, y is 2 and r is 1. In certain embodiments, each R¹² is independently selected from hydrogen, an alkyl, a substituted alkyl, an aryl and a substituted aryl. In certain embodiments, any two adjacent R¹² groups of the EDA may be cyclically linked, e.g., to form a piperazinyl ring. In certain embodiments, y is 1 and the two adjacent R¹² groups are an alkyl group, cyclically linked to form a piperazinyl ring. In certain embodiments, y is 1 and the adjacent R¹² groups are selected from hydrogen, an alkyl (e.g., methyl) and a substituted alkyl (e.g., lower alkyl-OH, such as ethyl-OH or propyl-OH).

[00259] In certain embodiments, the tether group (e.g., T¹, T², T³, T⁴, T⁵ and T⁶) includes a 4-amino-piperidine (4AP) moiety (also referred to herein as piperidin-4-amino, P4A). The 4AP moiety may optionally be substituted at one or more convenient positions with any convenient substituents, e.g., with an alkyl, a substituted alkyl, a polyethylene glycol moiety, an acyl, a substituted acyl, an aryl or a substituted aryl. In certain embodiments, the 4AP moiety is described by the structure:



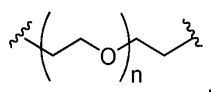
where R^{12} is selected from hydrogen, alkyl, substituted alkyl, a polyethylene glycol moiety (e.g., a polyethylene glycol or a modified polyethylene glycol), alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, amino, substituted amino, carboxyl, carboxyl ester, acyl, acyloxy, acyl amino, amino acyl, alkylamide, substituted alkylamide, sulfonyl, thioalkoxy, substituted thioalkoxy, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl. In certain embodiments, R^{12} is a polyethylene glycol moiety. In certain embodiments, R^{12} is a carboxy modified polyethylene glycol.

[00260] In certain embodiments, R^{12} includes a polyethylene glycol moiety described by the formula: $(PEG)_k$, which may be represented by the structure:



where k is an integer from 1 to 20, such as from 1 to 18, or from 1 to 16, or from 1 to 14, or from 1 to 12, or from 1 to 10, or from 1 to 8, or from 1 to 6, or from 1 to 4, or 1 or 2, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20. In some instances, k is 2. In certain embodiments, R^{17} is selected from OH, COOH, or COOR, where R is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl. In certain embodiments, R^{17} is COOH.

[00261] In certain embodiments, a tether group (e.g., T^1 , T^2 , T^3 , T^4 , T^5 and T^6) includes $(PEG)_n$, where $(PEG)_n$ is a polyethylene glycol or a modified polyethylene glycol linking unit. In certain embodiments, $(PEG)_n$ is described by the structure:



where n is an integer from 1 to 50, such as from 1 to 40, from 1 to 30, from 1 to 20, from 1 to 12 or from 1 to 6, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20. In some instances, n is 2. In some instances, n is 3. In some instances, n is 6. In some instances, n is 12.

[00262] In certain embodiments, a tether group (e.g., T^1 , T^2 , T^3 , T^4 , T^5 and T^6) includes $(AA)_p$, where AA is an amino acid residue. Any convenient amino acids may be utilized. Amino acids of interest include but are not limited to, L- and D-amino acids, naturally occurring amino acids such as any of the 20 primary alpha-amino acids and beta-alanine, non-naturally occurring

amino acids (e.g., amino acid analogs), such as a non-naturally occurring alpha-amino acid or a non-naturally occurring beta-amino acid, etc. In certain embodiments, p is an integer from 1 to 50, such as from 1 to 40, from 1 to 30, from 1 to 20, from 1 to 12 or from 1 to 6, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20. In certain embodiments, p is 1. In certain embodiments, p is 2.

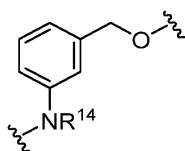
[00263] In certain embodiments, a tether group (e.g., T^1 , T^2 , T^3 , T^4 , T^5 and T^6) includes a moiety described by the formula $-(CR^{13}OH)_m-$, where m is 0 or n is an integer from 1 to 50, such as from 1 to 40, from 1 to 30, from 1 to 20, from 1 to 12 or from 1 to 6, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12. In certain embodiments, m is 1. In certain embodiments, m is 2. In certain embodiments, R^{13} is selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, amino, substituted amino, carboxyl, carboxyl ester, acyl, acyloxy, acyl amino, amino acyl, alkylamide, substituted alkylamide, sulfonyl, thioalkoxy, substituted thioalkoxy, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl. In certain embodiments, R^{13} is hydrogen. In certain embodiments, R^{13} is alkyl or substituted alkyl, such as C_{1-6} alkyl or C_{1-6} substituted alkyl, or C_{1-4} alkyl or C_{1-4} substituted alkyl, or C_{1-3} alkyl or C_{1-3} substituted alkyl. In certain embodiments, R^{13} is alkenyl or substituted alkenyl, such as C_{2-6} alkenyl or C_{2-6} substituted alkenyl, or C_{2-4} alkenyl or C_{2-4} substituted alkenyl, or C_{2-3} alkenyl or C_{2-3} substituted alkenyl. In certain embodiments, R^{13} is alkynyl or substituted alkynyl. In certain embodiments, R^{13} is alkoxy or substituted alkoxy. In certain embodiments, R^{13} is amino or substituted amino. In certain embodiments, R^{13} is carboxyl or carboxyl ester. In certain embodiments, R^{13} is acyl or acyloxy. In certain embodiments, R^{13} is acyl amino or amino acyl. In certain embodiments, R^{13} is alkylamide or substituted alkylamide. In certain embodiments, R^{13} is sulfonyl. In certain embodiments, R^{13} is thioalkoxy or substituted thioalkoxy. In certain embodiments, R^{13} is aryl or substituted aryl, such as C_{5-8} aryl or C_{5-8} substituted aryl, such as a C_5 aryl or C_5 substituted aryl, or a C_6 aryl or C_6 substituted aryl. In certain embodiments, R^{13} is heteroaryl or substituted heteroaryl, such as C_{5-8} heteroaryl or C_{5-8} substituted heteroaryl, such as a C_5 heteroaryl or C_5 substituted heteroaryl, or a C_6 heteroaryl or C_6 substituted heteroaryl. In certain embodiments, R^{13} is cycloalkyl or substituted cycloalkyl, such as C_{3-8} cycloalkyl or C_{3-8} substituted cycloalkyl, such as a C_{3-6} cycloalkyl or C_{3-6} substituted cycloalkyl, or a C_{3-5} cycloalkyl or C_{3-5} substituted cycloalkyl. In certain embodiments, R^{13} is

heterocyclyl or substituted heterocyclyl, such as C₃₋₈ heterocyclyl or C₃₋₈ substituted heterocyclyl, such as a C₃₋₆ heterocyclyl or C₃₋₆ substituted heterocyclyl, or a C₃₋₅ heterocyclyl or C₃₋₅ substituted heterocyclyl.

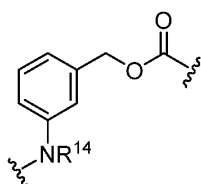
[00264] In certain embodiments, R¹³ is selected from hydrogen, alkyl, substituted alkyl, aryl, and substituted aryl. In these embodiments, alkyl, substituted alkyl, aryl, and substituted aryl are as described above for R¹³.

[00265] In certain embodiments, a tether group (e.g., T¹, T², T³, T⁴, T⁵ and T⁶) includes a meta-amino-benzyloxy (MABO), meta-amino-benzyloxycarbonyl (MABC), para-amino-benzyloxy (PABO), para-amino-benzyloxycarbonyl (PABC), para-aminobenzyl (PAB), para-amino-benzylamino (PABA), para-amino-phenyl (PAP), or para-hydroxy-phenyl (PHP).

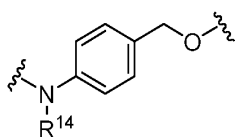
[00266] In some embodiments, a tether includes a MABO group described by the following structure:



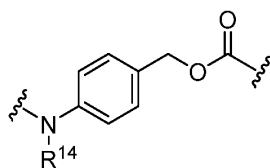
[00267] In some embodiments, a tether includes a MABC group described by the following structure:



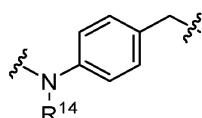
[00268] In some embodiments, a tether includes a PABO group described by the following structure:



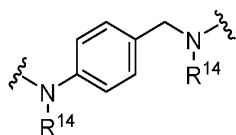
[00269] In some embodiments, a tether includes a PABC group described by the following structure:



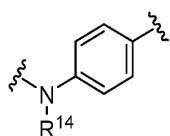
[00270] In some embodiments, a tether includes a PAB group described by the following structure:



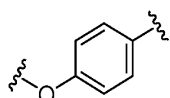
[00271] In some embodiments, a tether includes a PABA group described by the following structure:



[00272] In some embodiments, a tether includes a PAP group described by the following structure:



[00273] In some embodiments, a tether includes a PHP group described by the following structure:



[00274] In certain embodiments, each R^{14} is independently selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, amino, substituted amino, carboxyl, carboxyl ester, acyl, acyloxy, acyl amino, amino acyl, alkylamide, substituted alkylamide, sulfonyl, thioalkoxy, substituted thioalkoxy, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl.

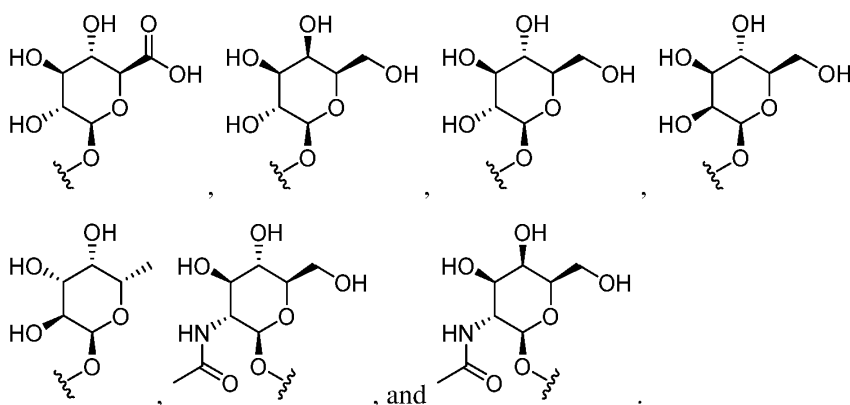
[00275] In certain embodiments, R¹⁴ is hydrogen. In certain embodiments, each R¹⁴ is hydrogen. In certain embodiments, R¹⁴ is alkyl or substituted alkyl, such as C₁₋₆ alkyl or C₁₋₆ substituted alkyl, or C₁₋₄ alkyl or C₁₋₄ substituted alkyl, or C₁₋₃ alkyl or C₁₋₃ substituted alkyl. In certain embodiments, R¹⁴ is alkenyl or substituted alkenyl, such as C₂₋₆ alkenyl or C₂₋₆ substituted alkenyl, or C₂₋₄ alkenyl or C₂₋₄ substituted alkenyl, or C₂₋₃ alkenyl or C₂₋₃ substituted alkenyl. In certain embodiments, R¹⁴ is alkynyl or substituted alkynyl. In certain embodiments, R¹⁴ is alkoxy or substituted alkoxy. In certain embodiments, R¹⁴ is amino or substituted amino. In certain embodiments, R¹⁴ is carboxyl or carboxyl ester. In certain embodiments, R¹⁴ is acyl or acyloxy. In certain embodiments, R¹⁴ is acyl amino or amino acyl. In certain embodiments, R¹⁴ is alkylamide or substituted alkylamide. In certain embodiments, R¹⁴ is sulfonyl. In certain embodiments, R¹⁴ is thioalkoxy or substituted thioalkoxy. In certain embodiments, R¹⁴ is aryl or substituted aryl, such as C₅₋₈ aryl or C₅₋₈ substituted aryl, such as a C₅ aryl or C₅ substituted aryl, or a C₆ aryl or C₆ substituted aryl. In certain embodiments, R¹⁴ is heteroaryl or substituted heteroaryl, such as C₅₋₈ heteroaryl or C₅₋₈ substituted heteroaryl, such as a C₅ heteroaryl or C₅ substituted heteroaryl, or a C₆ heteroaryl or C₆ substituted heteroaryl. In certain embodiments, R¹⁴ is cycloalkyl or substituted cycloalkyl, such as C₃₋₈ cycloalkyl or C₃₋₈ substituted cycloalkyl, such as a C₃₋₆ cycloalkyl or C₃₋₆ substituted cycloalkyl, or a C₃₋₅ cycloalkyl or C₃₋₅ substituted cycloalkyl. In certain embodiments, R¹⁴ is heterocyclyl or substituted heterocyclyl, such as C₃₋₈ heterocyclyl or C₃₋₈ substituted heterocyclyl, such as a C₃₋₆ heterocyclyl or C₃₋₆ substituted heterocyclyl, or a C₃₋₅ heterocyclyl or C₃₋₅ substituted heterocyclyl.

[00276] In some embodiments of the MABO, MABC, PABO, PABC, PAB, PABA, PAP, and PHP tether structures shown above, the phenyl ring may be substituted with one or more additional groups selected from halogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, amino, substituted amino, carboxyl, carboxyl ester, acyl, acyloxy, acyl amino, amino acyl, alkylamide, substituted alkylamide, sulfonyl, thioalkoxy, substituted thioalkoxy, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl.

[00277] In certain embodiments of the linker L, one or more of the tether groups T¹, T², T³, T⁴, T⁵ or T⁶ is each optionally substituted with a glycoside or glycoside derivative. In certain embodiments, the glycoside or glycoside derivative is selected from a glucuronide, a galactoside, a glucoside, a mannoside, a fucoside, O-GlcNAc, and O-GalNAc.

[00278] In certain embodiments, the MABO, MABC, PABO, PABC, PAB, PABA, PAP, and PHP tether structures shown above may be substituted with an one or more additional groups selected from a glycoside and a glycoside derivative. For example, in some embodiments of the MABO, MABC, PABO, PABC, PAB, PABA, PAP, and PHP tether structures shown above, the phenyl ring may be substituted with one or more additional groups selected from a glycoside and a glycoside derivative. In certain embodiments, the glycoside or glycoside derivative is selected from a glucuronide, a galactoside, a glucoside, a mannoside, a fucoside, O-GlcNAc, and O-GalNAc.

[00279] For example, in some embodiments, the glycoside or glycoside derivative can be selected from the following structures:



[00280] Regarding the linking functional groups, V^1 , V^2 , V^3 , V^4 , V^5 and V^6 , any convenient linking functional groups may be utilized in the linker L. Linking functional groups of interest include, but are not limited to, amino, carbonyl, amido, oxycarbonyl, carboxy, sulfonyl, sulfoxide, sulfonylamino, aminosulfonyl, thio, oxy, phospho, phosphoramidate, thiophosphoraidate, and the like. In some embodiments, V^1 , V^2 , V^3 , V^4 , V^5 and V^6 are each independently selected from a covalent bond, $-\text{CO}-$, $-\text{NR}^{15}-$, $-\text{NR}^{15}(\text{CH}_2)_q-$, $-\text{NR}^{15}(\text{C}_6\text{H}_4)-$, $-\text{CONR}^{15}-$, $-\text{NR}^{15}\text{CO}-$, $-\text{C}(\text{O})\text{O}-$, $-\text{OC}(\text{O})-$, $-\text{O}-$, $-\text{S}-$, $-\text{S}(\text{O})-$, $-\text{SO}_2-$, $-\text{SO}_2\text{NR}^{15}-$, $-\text{NR}^{15}\text{SO}_2-$ and $-\text{P}(\text{O})\text{OH}-$, where q is an integer from 1 to 6. In certain embodiments, q is an integer from 1 to 6 (e.g., 1, 2, 3, 4, 5 or 6). In certain embodiments, q is 1. In certain embodiments, q is 2. In certain embodiments, q is 3. In certain embodiments, q is 4. In certain embodiments, q is 5. In certain embodiments, q is 6.

[00281] In some embodiments, each R^{15} is independently selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted

alkoxy, amino, substituted amino, carboxyl, carboxyl ester, acyl, acyloxy, acyl amino, amino acyl, alkylamide, substituted alkylamide, sulfonyl, thioalkoxy, substituted thioalkoxy, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl.

[00282] In certain embodiments, R^{15} is hydrogen. In certain embodiments, each R^{15} is hydrogen. In certain embodiments, R^{15} is alkyl or substituted alkyl, such as C_{1-6} alkyl or C_{1-6} substituted alkyl, or C_{1-4} alkyl or C_{1-4} substituted alkyl, or C_{1-3} alkyl or C_{1-3} substituted alkyl. In certain embodiments, R^{15} is alkenyl or substituted alkenyl, such as C_{2-6} alkenyl or C_{2-6} substituted alkenyl, or C_{2-4} alkenyl or C_{2-4} substituted alkenyl, or C_{2-3} alkenyl or C_{2-3} substituted alkenyl. In certain embodiments, R^{15} is alkynyl or substituted alkynyl. In certain embodiments, R^{15} is alkoxy or substituted alkoxy. In certain embodiments, R^{15} is amino or substituted amino. In certain embodiments, R^{15} is carboxyl or carboxyl ester. In certain embodiments, R^{15} is acyl or acyloxy. In certain embodiments, R^{15} is acyl amino or amino acyl. In certain embodiments, R^{15} is alkylamide or substituted alkylamide. In certain embodiments, R^{15} is sulfonyl. In certain embodiments, R^{15} is thioalkoxy or substituted thioalkoxy. In certain embodiments, R^{15} is aryl or substituted aryl, such as C_{5-8} aryl or C_{5-8} substituted aryl, such as a C_5 aryl or C_5 substituted aryl, or a C_6 aryl or C_6 substituted aryl. In certain embodiments, R^{15} is heteroaryl or substituted heteroaryl, such as C_{5-8} heteroaryl or C_{5-8} substituted heteroaryl, such as a C_5 heteroaryl or C_5 substituted heteroaryl, or a C_6 heteroaryl or C_6 substituted heteroaryl. In certain embodiments, R^{15} is cycloalkyl or substituted cycloalkyl, such as C_{3-8} cycloalkyl or C_{3-8} substituted cycloalkyl, such as a C_{3-6} cycloalkyl or C_{3-6} substituted cycloalkyl, or a C_{3-5} cycloalkyl or C_{3-5} substituted cycloalkyl. In certain embodiments, R^{15} is heterocyclyl or substituted heterocyclyl, such as C_{3-8} heterocyclyl or C_{3-8} substituted heterocyclyl, such as a C_{3-6} heterocyclyl or C_{3-6} substituted heterocyclyl, or a C_{3-5} heterocyclyl or C_{3-5} substituted heterocyclyl.

[00283] In certain embodiments, each R^{15} is independently selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, carboxyl, carboxyl ester, acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl. In these embodiments, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, carboxyl, carboxyl ester, acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl are as described above for R^{15} .

[00284] In certain embodiments, the tether group includes an acetal group, a disulfide, a hydrazine, or an ester. In some embodiments, the tether group includes an acetal group. In some embodiments, the tether group includes a hydrazine. In some embodiments, the tether group includes a disulfide. In some embodiments, the tether group includes an ester.

[00285] As described above, in some embodiments, L is a linker comprising $-(T^1-V^1)_a-(T^2-V^2)_b-(T^3-V^3)_c-(T^4-V^4)_d-(T^5-V^5)_e-(T^6-V^6)_f-$, where a, b, c, d, e and f are each independently 0 or 1, where the sum of a, b, c, d, e and f is 1 to 6.

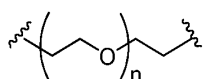
[00286] In some embodiments, in the linker L:

T^1 is selected from a (C_1-C_{12}) alkyl and a substituted (C_1-C_{12}) alkyl;

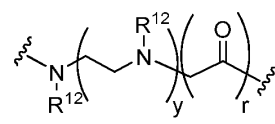
T^2 , T^3 , T^4 , T^5 and T^6 are each independently selected from (C_1-C_{12}) alkyl, substituted (C_1-C_{12}) alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl, $(EDA)_w$, $(PEG)_n$, $(AA)_p$, $-(CR^{13}OH)_m-$, 4-amino-piperidine (4AP), MABO, MABC, PABO, PABC, PAB, PABA, PAP, PHP, an acetal group, a disulfide, a hydrazine, and an ester; and

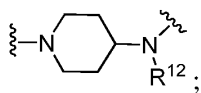
V^1 , V^2 , V^3 , V^4 , V^5 and V^6 are each independently selected from a covalent bond, $-CO-$, $-NR^{15}-$, $-NR^{15}(CH_2)_q-$, $-NR^{15}(C_6H_4)-$, $-CONR^{15}-$, $-NR^{15}CO-$, $-C(O)O-$, $-OC(O)-$, $-O-$, $-S-$, $-S(O)-$, $-SO_2-$, $-SO_2NR^{15}-$, $-NR^{15}SO_2-$ and $-P(O)OH-$, wherein q is an integer from 1 to 6;

wherein:

$(PEG)_n$ is , where n is an integer from 1 to 30;

EDA is an ethylene diamine moiety having the following structure:

, where y is an integer from 1 to 6 and r is 0 or 1;

4-amino-piperidine (4AP) is .

AA is an amino acid residue, where p is an integer from 1 to 20; and

each R^{12} is independently selected from hydrogen, an alkyl, a substituted alkyl, a polyethylene glycol moiety, an aryl and a substituted aryl, wherein any two adjacent R^{12} groups may be cyclically linked to form a piperazinyl ring;

each R^{13} is independently selected from hydrogen, alkyl, substituted alkyl, aryl, and substituted aryl; and

each R^{15} is independently selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, carboxyl, carboxyl ester, acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl.

[00287] In some embodiments, L is a linker wherein:

T^1 is (C_1-C_{12}) alkyl and V^1 is $-CO-$;

T^2 is an amino acid analog and V^2 is $-NH-$;

T^3 is $(PEG)_n$ and V^3 is $-CO-$; and

d to f are each 0; or

wherein:

T^1 is (C_1-C_{12}) alkyl and V^1 is $-CO-$;

T^2 is an amino acid analog and V^2 is $-NH-$;

T^3 is $(PEG)_n$ and V^3 is $-CONH-$;

T^4 is $(PEG)_n$ and V^4 is $-CO-$;

e and f are each 0.

[00288] In certain embodiments, the left-hand side of the above linker structure (e.g., T^1) is attached to the conjugation moiety G, and the right-hand side of the above linker structure (e.g., V^3 or V^4) is attached to the drug or active agent (e.g., through the $-A_k-X^2-X^1-C(O)O-$ portion of the structure in formula (I)).

[00289] In certain embodiments, the conjugate is an antibody-drug conjugate where the antibody and the drug are linked together by a linker as described above. In some instances, the linker is a cleavable linker. A cleavable linker is a linker that includes one or more cleavable moieties, where the cleavable moiety includes one or more bonds that can dissociate under certain conditions, thus separating the cleavable linker into two or more separable portions. For example, the cleavable moiety may include one or more covalent bonds, which under certain conditions, can dissociate or break apart to separate the cleavable linker into two or more portions. As such the linker that is included in an antibody-drug conjugate can be a cleavable

linker, such that under appropriate conditions, the cleavable linker is cleaved to separate or release the drug from the antibody at a desired target site of action for the drug.

[00290] In some instances, the cleavable linker includes two cleavable moieties, such as a first cleavable moiety and a second cleavable moiety. The cleavable moieties can be configured such that cleavage of both cleavable moieties is needed in order to separate or release the drug from the antibody at a desired target site of action for the drug. For example, cleavage of the cleavable linker can be achieved by initially cleaving one of the two cleavable moieties and then cleaving the other of the two cleavable moieties. In certain embodiments, the cleavable linker includes a first cleavable moiety and a second cleavable moiety that hinders cleavage of the first cleavable moiety. By “hinders cleavage” is meant that the presence of an uncleaved second cleavable moiety reduces the likelihood or substantially inhibits the cleavage of the first cleavable moiety, thus substantially reducing the amount or preventing the cleavage of the cleavable linker. For instance, the presence of uncleaved second cleavable moiety can hinder cleavage of the first cleavable moiety. The hinderance of cleavage of the first cleavable moiety by the presence of the second cleavable moiety, in turn, substantially reduces the amount or prevents the release of the drug from the antibody. For example, the premature release of the drug from the antibody can be substantially reduced or prevented until the antibody-drug conjugate is at or near the desired target site of action for the drug.

[00291] In some cases, since the second cleavable moiety hinders cleavage of the first cleavable moiety, cleavage of the cleavable linker can be achieved by initially cleaving the second cleavable moiety and then cleaving the first cleavable moiety. Cleavage of the second cleavable moiety can reduce or eliminate the hinderance on the cleavage of the first cleavable moiety, thus allowing the first cleavable moiety to be cleaved. Cleavage of the first cleavable moiety can result in the cleavable linker dissociating or separating into two or more portions as described above to release the drug from the antibody-drug conjugate. In some instances, cleavage of the first cleavable moiety does not substantially occur in the presence of an uncleaved second cleavable moiety. By substantially is meant that about 10% or less cleavage of the first cleavable moiety occurs in the presence of an uncleaved second cleavable moiety, such as about 9% or less, or about 8% or less, or about 7% or less, or about 6% or less, or about 5% or less, or about 4% or less, or about 3% or less, or about 2% or less, or about 1% or less, or about

0.5% or less, or about 0.1% or less cleavage of the first cleavable moiety occurs in the presence of an uncleaved second cleavable moiety.

[00292] Stated another way, the second cleavable moiety can protect the first cleavable moiety from cleavage. For instance, the presence of uncleaved second cleavable moiety can protect the first cleavable moiety from cleavage, and thus substantially reduce or prevent premature release of the drug from the antibody until the antibody-drug conjugate is at or near the desired target site of action for the drug. As such, cleavage of the second cleavable moiety exposes the first cleavable moiety (e.g., deprotects the first cleavable moiety), thus allowing the first cleavable moiety to be cleaved, which results in cleavage of the cleavable linker, which, in turn, separates or releases the drug from the antibody at a desired target site of action for the drug as described above. In certain instances, cleavage of the second cleavable moiety exposes the first cleavable moiety to subsequent cleavage, but cleavage of the second cleavable moiety does not in and of itself result in cleavage of the cleavable linker (i.e., cleavage of the first cleavable moiety is still needed in order to cleave the cleavable linker).

[00293] The cleavable moieties included in the cleavable linker may each be an enzymatically cleavable moiety. For example, the first cleavable moiety can be a first enzymatically cleavable moiety and the second cleavable moiety can be a second enzymatically cleavable moiety. An enzymatically cleavable moiety is a cleavable moiety that can be separated into two or more portions as described above through the enzymatic action of an enzyme. The enzymatically cleavable moiety can be any cleavable moiety that can be cleaved through the enzymatic action of an enzyme, such as, but not limited to, an ester, a peptide, a glycoside, and the like. In some instances, the enzyme that cleaves the enzymatically cleavable moiety is present at a desired target site of action, such as the desired target site of action of the drug that is to be released from the antibody-drug conjugate. In some cases, the enzyme that cleaves the enzymatically cleavable moiety is not present in a significant amount in other areas, such as in whole blood, plasma or serum. As such, the cleavage of an enzymatically cleavable moiety can be controlled such that substantial cleavage occurs at the desired site of action, whereas cleavage does not significantly occur in other areas or before the antibody-drug conjugate reaches the desired site of action.

[00294] For example, as described herein, antibody-drug conjugates of the present disclosure can be used for the treatment of cancer, such as for the delivery of a cancer therapeutic

drug to a desired site of action where the cancer cells are present. In some cases, enzymes, such as an esterase that cleaves ester bonds, can be a biomarker for cancer that is overexpressed in cancer cells. The overexpression, and thus localization, of certain enzymes in cancer can be used in the context of the enzymatically cleavable moieties included in the cleavable linkers of the antibody-drug conjugates of the present disclosure to specifically release the drug at the desired site of action (i.e., the site of the cancer (and overexpressed enzyme)). Thus, in some embodiments, the enzymatically cleavable moiety is a cleavable moiety (e.g., an ester) that can be cleaved by an enzyme that is overexpressed in cancer cells. For instance, the enzyme can be an esterase. As such, in some instances, the enzymatically cleavable moiety is a cleavable moiety (e.g., an ester) that can be cleaved by an esterase enzyme.

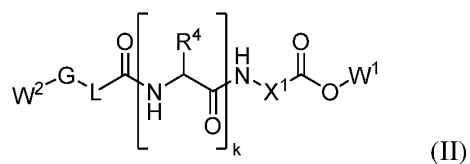
[00295] In certain embodiments, the enzymatically cleavable moiety is an ester bond. For example, the first cleavable moiety described above (i.e., the cleavable moiety protected from premature cleavage by the second cleavable moiety) can include an ester. The presence of uncleaved second cleavable moiety can protect the first cleavable moiety (ester) from cleavage by an esterase enzyme, and thus substantially reduce or prevent premature release of the drug from the antibody until the antibody-drug conjugate is at or near the desired target site of action for the drug. In some instances, a portion of the linker L adjacent to the first cleavable moiety is linked to or includes a substituent, where the substituent comprises the second cleavable moiety. In some instances, the second cleavable moiety includes a glycoside.

[00296] In some embodiments, the enzymatically cleavable moiety is sugar moiety, such as a glycoside (or glycosyl) or glycoside derivative. In some cases, the glycoside or glycoside derivative can facilitate an increase in the hydrophilicity of the cleavable linker as compared to a cleavable linker that does not include the glycoside or glycoside derivative. The glycoside or glycoside derivative can be any glycoside or glycoside derivative suitable for use in the cleavable linker and that can be cleaved through the enzymatic action of an enzyme. For example, the second cleavable moiety (i.e., the cleavable moiety that protects the first cleavable moiety from premature cleavage) can be a glycoside or glycoside derivative. For instance, in some embodiments, the first cleavable moiety includes an ester and the second cleavable moiety includes a glycoside or glycoside derivative. In certain embodiments, the second cleavable moiety is a glycoside or glycoside derivative selected from a glucuronide, a galactoside, a glucoside, a mannoside, a fucoside, O-GlcNAc, and O-GalNAc. In some instances, the second

cleavable moiety is a glucuronide. In some instances, the second cleavable moiety is a galactoside. In some instances, the second cleavable moiety is a glucoside. In some instances, the second cleavable moiety is a mannoside. In some instances, the second cleavable moiety is a fucoside. In some instances, the second cleavable moiety is O-GlcNAc. In some instances, the second cleavable moiety is O-GalNAc.

[00297] The glycoside or glycoside derivative can be attached (covalently bonded) to the cleavable linker through a glycosidic bond. The glycosidic bond can link the glycoside or glycoside derivative to the cleavable linker through various types of bonds, such as, but not limited to, an O-glycosidic bond (an O-glycoside), an N-glycosidic bond (a glycosylamine), an S-glycosidic bond (a thioglycoside), or C-glycosidic bond (a C-glycoside or C-glycosyl). In some instances, the glycosidic bond is an O-glycosidic bond (an O-glycoside). In some cases, the glycoside or glycoside derivative can be cleaved from the cleavable linker it is attached to by an enzyme (e.g., through enzymatically-mediated hydrolysis of the glycosidic bond). A glycoside or glycoside derivative can be removed or cleaved from the cleavable linker by any convenient enzyme that is able to carry out the cleavage (hydrolysis) of the glycosidic bond that attaches the glycoside or glycoside derivative to the cleavable linker. An example of an enzyme that can be used to mediate the cleavage (hydrolysis) of the glycosidic bond that attaches the glycoside or glycoside derivative to the cleavable linker is a glycosidase, such as a glucuronidase, a galactosidase, a glucosidase, a mannosidase, a fucosidase, and the like. Other suitable enzymes may also be used to mediate the cleavage (hydrolysis) of the glycosidic bond that attaches the glycoside or glycoside derivative to the cleavable linker. In some cases, the enzyme used to mediate the cleavage (hydrolysis) of the glycosidic bond that attaches the glycoside or glycoside derivative to the cleavable linker is found at or near the desired site of action for the drug of the antibody-drug conjugate. For instance, the enzyme can be a lysosomal enzyme, such as a lysosomal glycosidase, found in cells at or near the desired site of action for the drug of the antibody-drug conjugate. In some cases, the enzyme is an enzyme found at or near the target site where the enzyme that mediates cleavage of the first cleavable moiety is found.

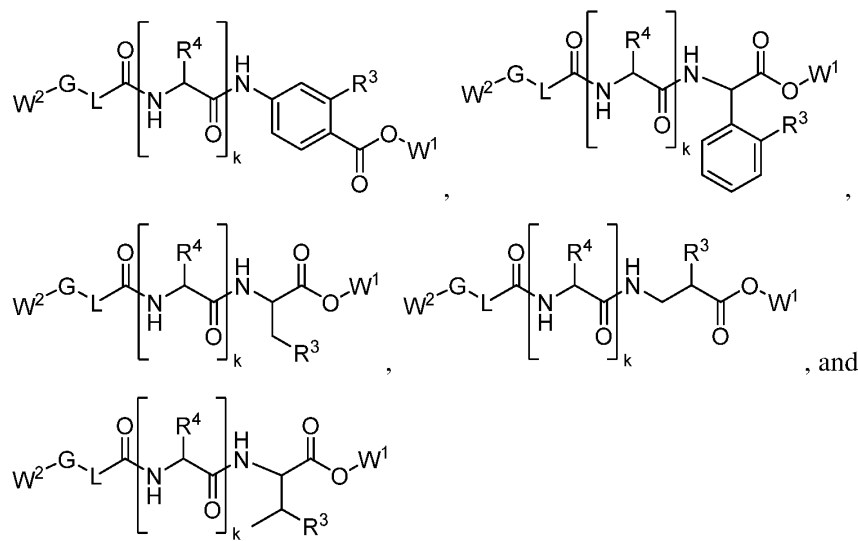
[00298] In certain embodiments, the conjugate is of formula (II):



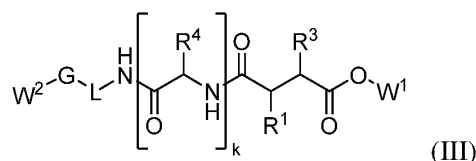
wherein R⁴ is an amino acid side chain.

[00299] In certain embodiments, R⁴ represents a side chain of an amino acid. For example, R⁴ may represent the substituent attached to the α -carbon of an amino acid residue, including natural amino acids, unnatural amino acids, and amino acid analogs. In some cases, R⁴ represents the side chain of an amino acid found in naturally occurring proteins (e.g., the side chain of Ala or A, Cys or C, Asp or D, Glu or E, Phe or F, Gly or G, His or H, Ile or I, Lys or K, Leu or L, Met or M, Asn or N, Pro or P, Gln or Q, Arg or R, Ser or S, Thr or T, Val or V, Trp or W, Tyr or Y). In certain embodiments, R⁴ represents the side chain of valine (Val); i.e., R⁴ is isopropyl. In certain embodiments, R⁴ represents the side chain of alanine (Ala); i.e., R⁴ is methyl. In certain embodiments, R⁴ represents the side chain of phenylalanine (Phe); i.e., R⁴ is benzyl. In certain embodiments, R⁴ represents the side chain of lysine (Lys); i.e., R⁴ is 4-amino-butyl.

[00300] In certain embodiments of formula (II), the conjugate is selected from:

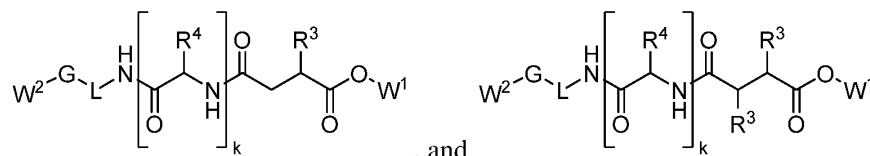


[00301] In certain embodiments, the conjugate is of formula (III):



wherein R⁴ is an amino acid side chain.

[00302] In certain embodiments of formula (III), the conjugate is selected from:



[00303] In formula (II) and formula (III), the substituents W¹, W², k, L, G, and R³ are as described above in relation to formula (I).

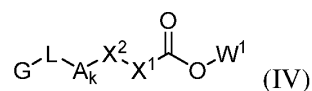
[00304] Any of the chemical entities, linkers and conjugation moieties set forth in the structures above may be adapted for use in the subject compounds and conjugates.

[00305] Additional disclosure related to hydrazinyl-indolyl and hydrazinyl-pyrrolo-pyridinyl compounds and methods for producing a conjugate is found in U.S. Patent No. 9,310,374 and U.S. Patent No. 9,493,413, the disclosures of each of which are incorporated herein by reference.

COMPOUNDS USEFUL FOR PRODUCING CONJUGATES

[00306] The present disclosure provides compounds useful for producing the conjugates described herein. In certain embodiments, the compound can be attached to a drug or active agent and may also include a conjugation moiety useful for conjugation of the drug or active agent to a polypeptide (e.g., an antibody). For example, the conjugation moiety in the compound may be conjugated to a polypeptide (e.g., antibody), thus indirectly binding the drug or active agent and the polypeptide (antibody) together.

[00307] In certain embodiments, the compound is a compound of formula (IV):



wherein:

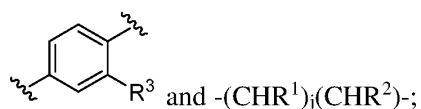
W¹ is a drug;

A is an amino acid residue, where k is 0 or an integer from 1 to 5;

L is a linker;

G is a conjugation moiety;

X¹ is selected from:



R¹ is selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, substituted heterocyclyl and R³, wherein R¹ is optionally substituted with R³;

j is 0 or an integer from 1 to 5;

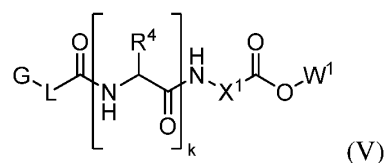
R² is R³; or R² is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl, wherein R² is substituted with R³;

X² is -NH- or -C(O)-;

each R³ is independently a glycoside or glycoside derivative.

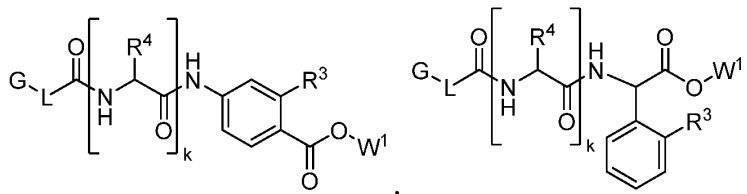
[00308] Regarding compound of formula (IV), the substituents W¹, A, k, L, G, X¹, X², R¹, j, R², and R³ are as described above in relation to the conjugates of formulae (I), (II) and (III).

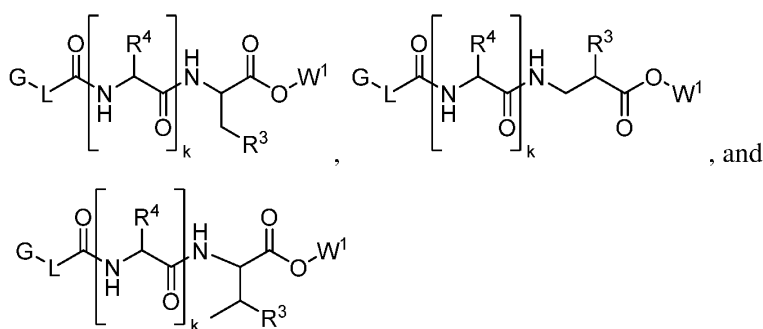
[00309] In certain embodiments, the compound is of formula (V):



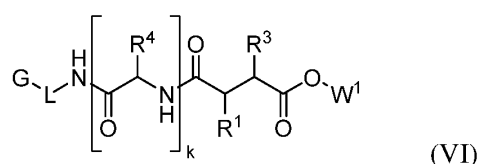
wherein R⁴ is an amino acid side chain.

[00310] In certain embodiments of formula (V), the compound is selected from:



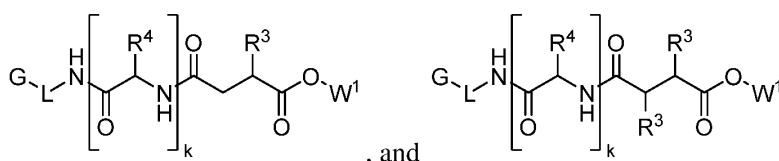


[00311] In certain embodiments, the compound is of formula (VI):



wherein R⁴ is an amino acid side chain.

[00312] In certain embodiments of formula (VI), the compound is selected from:



[00313] In formula (V) and formula (VI), the substituents W¹, k, L, G, R³, and R⁴ are as described above in relation to formulae (I), (II) and (III).

POLYPEPTIDES AND ANTIBODIES

[00314] As noted above, a subject conjugate can comprise as substituent W² a polypeptide (e.g., an antibody). The polypeptide (antibody) can be modified to include a 2-formylglycine (FGly) residue. As used herein, amino acids may be referred to by their standard name, their standard three letter abbreviation and/or their standard one letter abbreviation, such as: Alanine or Ala or A; Cysteine or Cys or C; Aspartic acid or Asp or D; Glutamic acid or Glu or E; Phenylalanine or Phe or F; Glycine or Gly or G; Histidine or His or H; Isoleucine or Ile or I; Lysine or Lys or K; Leucine or Leu or L; Methionine or Met or M; Asparagine or Asn or N; Proline or Pro or P; Glutamine or Gln or Q; Arginine or Arg or R; Serine or Ser or S; Threonine or Thr or T; Valine or Val or V; Tryptophan or Trp or W; and Tyrosine or Tyr or Y.

[00315] In certain embodiments, the amino acid sequence of a polypeptide or an antibody is modified to include a sulfatase motif that contains a serine or cysteine residue that is capable of being converted (oxidized) to a 2-formylglycine (FGly) residue by action of a formylglycine generating enzyme (FGE) either *in vivo* (e.g., at the time of translation of an aldehyde tag-containing protein in a cell) or *in vitro* (e.g., by contacting an aldehyde tag-containing protein with an FGE in a cell-free system). Such sulfatase motifs may also be referred to herein as an FGE-modification site.

Sulfatase motifs

[00316] A minimal sulfatase motif of an aldehyde tag is usually 5 or 6 amino acid residues in length, usually no more than 6 amino acid residues in length. Sulfatase motifs provided in an Ig polypeptide are at least 5 or 6 amino acid residues, and can be, for example, from 5 to 16, 6-16, 5-15, 6-15, 5-14, 6-14, 5-13, 6-13, 5-12, 6-12, 5-11, 6-11, 5-10, 6-10, 5-9, 6-9, 5-8, or 6-8 amino acid residues in length, so as to define a sulfatase motif of less than 16, 15, 14, 13, 12, 11, 10, 9, 8, 7 or 6 amino acid residues in length.

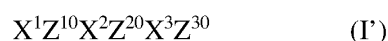
[00317] In certain embodiments, polypeptides of interest include those where one or more amino acid residues, such as 2 or more, or 3 or more, or 4 or more, or 5 or more, or 6 or more, or 7 or more, or 8 or more, or 9 or more, or 10 or more, or 11 or more, or 12 or more, or 13 or more, or 14 or more, or 15 or more, or 16 or more, or 17 or more, or 18 or more, or 19 or more, or 20 or more amino acid residues have been inserted, deleted, substituted (replaced) relative to the native amino acid sequence to provide for a sequence of a sulfatase motif in the polypeptide. In certain embodiments, the polypeptide includes a modification (insertion, addition, deletion, and/or substitution/replacement) of less than 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3 or 2 amino acid residues of the amino acid sequence relative to the native amino acid sequence of the polypeptide. Where an amino acid sequence native to the polypeptide (e.g., antibody) contains one or more residues of the desired sulfatase motif, the total number of modifications of residues can be reduced, e.g., by site-specification modification (insertion, addition, deletion, substitution/replacement) of amino acid residues flanking the native amino acid residues to provide a sequence of the desired sulfatase motif. In certain embodiments, the extent of modification of the native amino acid sequence of the target antibody is minimized, so as to minimize the number of amino acid residues that are inserted, deleted, substituted (replaced), or added (e.g., to the N- or C-terminus). Minimizing the extent of amino acid sequence

modification of the target antibody may minimize the impact such modifications may have upon antibody function and/or structure.

[00318] It should be noted that while aldehyde tags of particular interest are those comprising at least a minimal sulfatase motif (also referred to a “consensus sulfatase motif”), it will be readily appreciated that longer aldehyde tags are both contemplated and encompassed by the present disclosure and can find use in the compositions and methods of the present disclosure. Aldehyde tags can thus comprise a minimal sulfatase motif of 5 or 6 residues, or can be longer and comprise a minimal sulfatase motif which can be flanked at the N- and/or C-terminal sides of the motif by additional amino acid residues. Aldehyde tags of, for example, 5 or 6 amino acid residues are contemplated, as well as longer amino acid sequences of more than 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more amino acid residues.

[00319] An aldehyde tag can be present at or near the C-terminus of an Ig heavy chain; e.g., an aldehyde tag can be present within 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids of the C-terminus of a native, wild-type Ig heavy chain. An aldehyde tag can be present within a CH1 domain of an Ig heavy chain. An aldehyde tag can be present within a CH2 domain of an Ig heavy chain. An aldehyde tag can be present within a CH3 domain of an Ig heavy chain. An aldehyde tag can be present in an Ig light chain constant region, e.g., in a kappa light chain constant region or a lambda light chain constant region.

[00320] In certain embodiments, the sulfatase motif used may be described by the formula:



where

Z^{10} is cysteine or serine (which can also be represented by (C/S));

Z^{20} is either a proline or alanine residue (which can also be represented by (P/A));

Z^{30} is a basic amino acid (e.g., arginine (R), and may be lysine (K) or histidine (H), e.g., lysine), or an aliphatic amino acid (alanine (A), glycine (G), leucine (L), valine (V), isoleucine (I), or proline (P), e.g., A, G, L, V, or I;

X^1 is present or absent and, when present, can be any amino acid, e.g., an aliphatic amino acid, a sulfur-containing amino acid, or a polar, uncharged amino acid, (i.e., other than an aromatic amino acid or a charged amino acid), e.g., L, M, V, S or T, e.g., L, M, S or V, with the

proviso that when the sulfatase motif is at the N-terminus of the target polypeptide, X^1 is present; and

X^2 and X^3 independently can be any amino acid, though usually an aliphatic amino acid, a polar, uncharged amino acid, or a sulfur containing amino acid (i.e., other than an aromatic amino acid or a charged amino acid), e.g., S, T, A, V, G or C, e.g., S, T, A, V or G.

[00321] The amino acid sequence of an antibody heavy and/or light chain can be modified to provide a sequence of at least 5 amino acids of the formula $X^1Z^{10}X^2Z^{20}X^3Z^{30}$, where

Z^{10} is cysteine or serine;

Z^{20} is a proline or alanine residue;

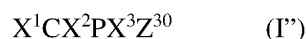
Z^{30} is an aliphatic amino acid or a basic amino acid;

X^1 is present or absent and, when present, is any amino acid, with the proviso that when the heterologous sulfatase motif is at an N-terminus of the polypeptide, X^1 is present;

X^2 and X^3 are each independently any amino acid.

[00322] The sulfatase motif is generally selected so as to be capable of conversion by a selected FGE, e.g., an FGE present in a host cell in which the aldehyde tagged polypeptide is expressed or an FGE which is to be contacted with the aldehyde tagged polypeptide in a cell-free *in vitro* method.

[00323] For example, where the FGE is a eukaryotic FGE (e.g., a mammalian FGE, including a human FGE), the sulfatase motif can be of the formula:



where

X^1 may be present or absent and, when present, can be any amino acid, e.g., an aliphatic amino acid, a sulfur-containing amino acid, or a polar, uncharged amino acid, (i.e., other than an aromatic amino acid or a charged amino acid), e.g., L, M, S or V, with the proviso that when the sulfatase motif is at the N-terminus of the target polypeptide, X^1 is present;

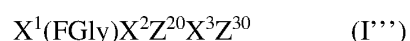
X^2 and X^3 independently can be any amino acid, e.g., an aliphatic amino acid, a sulfur-containing amino acid, or a polar, uncharged amino acid, (i.e., other than an aromatic amino acid or a charged amino acid), e.g., S, T, A, V, G, or C, e.g., S, T, A, V or G; and

Z^{30} is a basic amino acid (e.g., arginine (R), and may be lysine (K) or histidine (H), e.g., lysine), or an aliphatic amino acid (alanine (A), glycine (G), leucine (L), valine (V), isoleucine (I), or proline (P), e.g., A, G, L, V, or I.

[00324] Specific examples of sulfatase motifs include LCTPSR (SEQ ID NO://), MCTPSR (SEQ ID NO://), VCTPSR (SEQ ID NO://), LCSPSR (SEQ ID NO://), LCAPSR (SEQ ID NO://), LCVPSR (SEQ ID NO://), LCGPSR (SEQ ID NO://), ICTPAR (SEQ ID NO://), LCTPSK (SEQ ID NO://), MCTPSK (SEQ ID NO://), VCTPSK (SEQ ID NO://), LCSPSK (SEQ ID NO://), LCAPSK (SEQ ID NO://), LCVPSK (SEQ ID NO://), LCGPSK (SEQ ID NO://), LCTPSA (SEQ ID NO://), ICTPAA (SEQ ID NO://), MCTPSA (SEQ ID NO://), VCTPSA (SEQ ID NO://), LCSPSA (SEQ ID NO://), LCAPSA (SEQ ID NO://), LCVPSA (SEQ ID NO://), and LCGPSA (SEQ ID NO://).

FGly-containing sequences

[00325] Upon action of FGE on the modified antibody heavy and/or light chain, the serine or the cysteine in the sulfatase motif is modified to FGly. Thus, the FGly-containing sulfatase motif can be of the formula:



where

FGly is the formylglycine residue;

Z^{20} is either a proline or alanine residue (which can also be represented by (P/A));

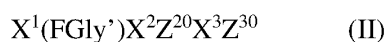
Z^{30} is a basic amino acid (e.g., arginine (R), and may be lysine (K) or histidine (H), usually lysine), or an aliphatic amino acid (alanine (A), glycine (G), leucine (L), valine (V), isoleucine (I), or proline (P), e.g., A, G, L, V, or I;

X^1 may be present or absent and, when present, can be any amino acid, e.g., an aliphatic amino acid, a sulfur-containing amino acid, or a polar, uncharged amino acid, (i.e., other than an aromatic amino acid or a charged amino acid), e.g., L, M, V, S or T, e.g., L, M or V, with the proviso that when the sulfatase motif is at the N-terminus of the target polypeptide, X^1 is present; and

X^2 and X^3 independently can be any amino acid, e.g., an aliphatic amino acid, a sulfur-containing amino acid, or a polar, uncharged amino acid, (i.e., other than an aromatic amino acid or a charged amino acid), e.g., S, T, A, V, G or C, e.g., S, T, A, V or G.

[00326] As described above, the modified polypeptide containing the FGly residue may be conjugated to a drug (e.g., a maytansinoid) by reaction of the FGly with the drug (e.g., a drug containing a hydrazinyl-indolyl or a hydrazinyl-pyrrolo-pyridinyl conjugation moiety, as described above) to produce an FGly'-containing sulfatase motif. As used herein, the term FGly'

refers to the modified amino acid residue of the sulfatase motif that is coupled to the drug, such as a maytansine or an auristatin. Thus, the FGly'-containing sulfatase motif can be of the formula:



where

FGly' is the modified amino acid residue of formula (I);

Z²⁰ is either a proline or alanine residue (which can also be represented by (P/A));

Z³⁰ is a basic amino acid (e.g., arginine (R), and may be lysine (K) or histidine (H), usually lysine), or an aliphatic amino acid (alanine (A), glycine (G), leucine (L), valine (V), isoleucine (I), or proline (P), e.g., A, G, L, V, or I;

X¹ may be present or absent and, when present, can be any amino acid, e.g., an aliphatic amino acid, a sulfur-containing amino acid, or a polar, uncharged amino acid, (i.e., other than an aromatic amino acid or a charged amino acid), e.g., L, M, V, S or T, e.g., L, M or V, with the proviso that when the sulfatase motif is at the N-terminus of the target polypeptide, X¹ is present; and

X² and X³ independently can be any amino acid, e.g., an aliphatic amino acid, a sulfur-containing amino acid, or a polar, uncharged amino acid, (i.e., other than an aromatic amino acid or a charged amino acid), e.g., S, T, A, V, G or C, e.g., S, T, A, V or G.

Site of modification

[00327] As noted above, the amino acid sequence of an antibody is modified to include a sulfatase motif that contains a serine or cysteine residue that is capable of being converted (oxidized) to an FGly residue by action of an FGE either *in vivo* (e.g., at the time of translation of an aldehyde tag-containing protein in a cell) or *in vitro* (e.g., by contacting an aldehyde tag-containing protein with an FGE in a cell-free system). The antibody used to generate a conjugate of the present disclosure include at least an Ig constant region, e.g., an Ig heavy chain constant region (e.g., at least a CH1 domain; at least a CH1 and a CH2 domain; a CH1, a CH2, and a CH3 domain; or a CH1, a CH2, a CH3, and a CH4 domain), or an Ig light chain constant region. Such Ig polypeptides are referred to herein as "target Ig polypeptides" or "target antibodies".

[00328] The site in an antibody into which a sulfatase motif is introduced can be any convenient site. As noted above, in some instances, the extent of modification of the native amino acid sequence of the target polypeptide is minimized, so as to minimize the number of

amino acid residues that are inserted, deleted, substituted (replaced), and/or added (e.g., to the N- or C-terminus). Minimizing the extent of amino acid sequence modification of the target antibody may minimize the impact such modifications may have upon antibody function and/or structure.

[00329] An antibody heavy chain constant region can include Ig constant regions of any heavy chain isotype, non-naturally occurring Ig heavy chain constant regions (including consensus Ig heavy chain constant regions). An Ig constant region can be modified to include an aldehyde tag, where the aldehyde tag is present in or adjacent a solvent-accessible loop region of the Ig constant region. An Ig constant region can be modified by insertion and/or substitution of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, or 16 amino acids, or more than 16 amino acids, to provide an amino acid sequence of a sulfatase motif as described above.

[00330] In some cases, an aldehyde-tagged antibody comprises an aldehyde-tagged Ig heavy chain constant region (e.g., at least a CH1 domain; at least a CH1 and a CH2 domain; a CH1, a CH2, and a CH3 domain; or a CH1, a CH2, a CH3, and a CH4 domain). The aldehyde-tagged Ig heavy chain constant region can include heavy chain constant region sequences of an IgA, IgM, IgD, IgE, IgG1, IgG2, IgG3, or IgG4 isotype heavy chain or any allotypic variant of same, e.g., human heavy chain constant region sequences or mouse heavy chain constant region sequences, a hybrid heavy chain constant region, a synthetic heavy chain constant region, or a consensus heavy chain constant region sequence, etc., modified to include at least one sulfatase motif that can be modified by an FGE to generate an FGly-modified Ig polypeptide. Allotypic variants of Ig heavy chains are known in the art. See, e.g., Jefferis and Lefranc (2009) *MAbs* 1:4.

[00331] In some cases, an aldehyde-tagged antibody comprises an aldehyde-tagged Ig light chain constant region. The aldehyde-tagged Ig light chain constant region can include constant region sequences of a kappa light chain, a lambda light chain, e.g., human kappa or lambda light chain constant regions, a hybrid light chain constant region, a synthetic light chain constant region, or a consensus light chain constant region sequence, etc., modified to include at least one sulfatase motif that can be modified by an FGE to generate an FGly-modified antibody. Exemplary constant regions include human gamma 1 and gamma 3 regions. With the exception of the sulfatase motif, a modified constant region may have a wild-type amino acid sequence, or it may have an amino acid sequence that is at least 70% identical (e.g., at least 80%, at least 90% or at least 95% identical) to a wild type amino acid sequence.

[00332] In some embodiments the sulfatase motif is at a position other than, or in addition to, the C-terminus of the Ig polypeptide heavy chain. As noted above, an isolated aldehyde-tagged antibody can comprise a heavy chain constant region modified to include a sulfatase motif as described above, where the sulfatase motif is in or adjacent a surface-accessible loop region of the antibody heavy chain constant region.

[00333] A sulfatase motif can be provided within or adjacent one or more of these amino acid sequences of such modification sites of an Ig heavy chain. For example, an Ig heavy chain polypeptide can be modified (e.g., where the modification includes one or more amino acid residue insertions, deletions, and/or substitutions) at one or more of these amino acid sequences to provide a sulfatase motif adjacent and N-terminal and/or adjacent and C-terminal to these modification sites. Alternatively or in addition, an Ig heavy chain polypeptide can be modified (e.g., where the modification includes one or more amino acid residue insertions, deletions, and/or substitutions) at one or more of these amino acid sequences to provide a sulfatase motif between any two residues of the Ig heavy chain modifications sites. In some embodiments, an Ig heavy chain polypeptide may be modified to include two motifs, which may be adjacent to one another, or which may be separated by one, two, three, four or more (e.g., from about 1 to about 25, from about 25 to about 50, or from about 50 to about 100, or more, amino acids. Alternatively or in addition, where a native amino acid sequence provides for one or more amino acid residues of a sulfatase motif sequence, selected amino acid residues of the modification sites of an Ig heavy chain polypeptide amino acid sequence can be modified (e.g., where the modification includes one or more amino acid residue insertions, deletions, and/or substitutions) so as to provide a sulfatase motif at the modification site.

[00334] An antibody used in an antibody-drug conjugate of the present disclosure can have any of a variety of antigen-binding specificities, including but not limited to, e.g., an antigen present on a cancer cell; an antigen present on an autoimmune cell; an antigen present on a pathogenic microorganism; an antigen present on a virus-infected cell (e.g., a human immunodeficiency virus-infected cell); an antigen present on a diseased cell; and the like. For example, an antibody conjugate can bind an antigen, where the antigen is present on the surface of the cell. An antibody conjugate of the present disclosure can bind antigen with a suitable binding affinity, e.g., from 5×10^{-6} M to 10^{-7} M, from 10^{-7} M to 5×10^{-7} M, from 5×10^{-7} M to

10^{-8} M, from 10^{-8} M to 5×10^{-8} M, from 5×10^{-8} M to 10^{-9} M, or a binding affinity greater than 10^{-9} M.

[00335] As non-limiting examples, a subject antibody conjugate can bind an antigen present on a cancer cell (e.g., a tumor-specific antigen; an antigen that is over-expressed on a cancer cell; etc.), and the conjugated moiety can be a drug, such as a cytotoxic compound (e.g., a cytotoxic small molecule, a cytotoxic synthetic peptide, etc.). For example, a subject antibody conjugate can be specific for an antigen on a cancer cell, where the conjugated moiety is a drug, such as a cytotoxic compound (e.g., a cytotoxic small molecule, a cytotoxic synthetic peptide, etc.).

[00336] As further non-limiting examples, a subject antibody conjugate can bind an antigen present on a cell infected with a virus (e.g., where the antigen is encoded by the virus; where the antigen is expressed on a cell type that is infected by a virus; etc.), and the conjugated moiety can be a drug, such as a viral fusion inhibitor. For example, a subject antibody conjugate can bind an antigen present on a cell infected with a virus, and the conjugated moiety can be a drug, such as a viral fusion inhibitor.

DRUGS FOR CONJUGATION TO A POLYPEPTIDE

[00337] As noted above, a conjugate or a compound of the present disclosure can include as substituent W¹ a drug or active agent. Any of a number of drugs are suitable for use, or can be modified to be rendered suitable for use, as a reactive partner to conjugate to an antibody. Examples of drugs include small molecule drugs and peptide drugs.

[00338] “Small molecule drug” as used herein refers to a compound, e.g., an organic compound, which exhibits a pharmaceutical activity of interest and which is generally of a molecular weight of 800 Da or less, or 2000 Da or less, but can encompass molecules of up to 5kDa and can be as large as 10 kDa. A small inorganic molecule refers to a molecule containing no carbon atoms, while a small organic molecule refers to a compound containing at least one carbon atom.

[00339] For example, the drug or active agent can be a camptothecine, or an analog or derivative thereof, or a pharmaceutically active camptothecine moiety and/or a portion thereof. A camptothecine conjugated to the polypeptide can be any of a variety of camptothecine moieties such as, but not limited to, a camptothecine and analogs and derivatives thereof as described herein. Examples of drugs that find use in the conjugates and compounds described

herein include, but are not limited to a camptothecine or a camptothecine derivative, such as SN-38, Belotecan, Exatecan, 9-aminocamptothecin (9-AC), derivatives thereof, and the like.

[00340] In other embodiments, the drug or active agent can be a maytansine. “Maytansine”, “maytansine moiety”, “maytansine active agent moiety” and “maytansinoid” refer to a maytansine and analogs and derivatives thereof, and pharmaceutically active maytansine moieties and/or portions thereof. A maytansine conjugated to the polypeptide can be any of a variety of maytansinoid moieties such as, but not limited to, maytansine and analogs and derivatives thereof as described herein (e.g., deacylmaytansine).

[00341] In other instances, the drug or active agent can be an auristatin, or an analog or derivative thereof, or a pharmaceutically active auristatin moiety and/or a portion thereof. An auristatin conjugated to the polypeptide can be any of a variety of auristatin moieties such as, but not limited to, an auristatin and analogs and derivatives thereof as described herein. Examples of drugs that find use in the conjugates and compounds described herein include, but are not limited to an auristatin or an auristatin derivative, such as monomethyl auristatin D (MMAD), monomethyl auristatin E (MMAE), monomethyl auristatin F (MMAF), derivatives thereof, and the like.

[00342] In other cases, the drug or active agent can be a duocarmycin, or an analog or derivative thereof, or a pharmaceutically active duocarmycin moiety and/or a portion thereof. A duocarmycin conjugated to the polypeptide can be any of a variety of duocarmycin moieties such as, but not limited to, a duocarmycin and analogs and derivatives thereof as described herein. Examples of drugs that find use in the conjugates and compounds described herein include, but are not limited to a duocarmycin or a duocarmycin derivative, such as duocarmycin A, duocarmycin B1, duocarmycin B2, duocarmycin C1, duocarmycin C2, duocarmycin D, duocarmycin SA, and CC-1065, derivatives thereof, and the like. In some embodiments, the duocarmycin is a duocarmycin analog, such as, but not limited to, adozelesin, bizelesin, or carzelesin.

[00343] In certain embodiments, the drug is selected from a cytotoxin, a kinase inhibitor, an immunostimulatory agent, a toll-like receptor (TLR) agonist, an oligonucleotide, an aptamer, a cytokine, a steroid, and a peptide.

[00344] For example, a cytotoxin can include any compound that leads to cell death (e.g., necrosis or apoptosis) or a decrease in cell viability.

[00345] Kinase inhibitors can include, but are not limited to, Adavosertib, Afatinib, Axitinib, Bosutinib, Cetuximab, Cobimetinib, Crizotinib, Cabozantinib, Dacomitinib, Dasatinib, Entrectinib, Erdafitinib, Erlotinib, Fostamatinib, Gefitinib, Ibrutinib, Imatinib, Lapatinib, Lenvatinib, Mubritinib, Nilotinib, Pazopanib, Pegaptanib, Ruxolitinib, Sorafenib, Sunitinib, Tucatinib, Vandetanib, Vemurafenib, and the like.

[00346] Immunostimulatory agents can include, but are not limited to, vaccines (e.g., bacterial or viral vaccines), colony stimulating factors, interferons, interleukins, and the like. TLR agonists include, but are not limited to, imiquimod, resiquimod, and the like.

[00347] Oligonucleotide drugs include, but are not limited to, fomivirsen, pegaptanib, mipomersen, eteplirsen, defibrotide, nusinersen, golodirsen, viltolarsen, volanesorsen, inotersen, tofersen, tominersen, and the like.

[00348] Aptamer drugs include, but are not limited to, pegaptanib, AS1411, REG1, ARC1779, NU172, ARC1905, E10030, NOX-A12, NOX-E36, and the like.

[00349] Cytokines include, but are not limited to, Albinterferon Alfa-2B, Aldesleukin, ALT-801, Anakinra, Ancestim, Avotermin, Balugrastim, Bempegaldesleukin, Binetrakin, Cintredekin Besudotox, CTCE-0214, Darbepoetin alfa, Denileukin diftitox, Dulanermin, Edodekin alfa, Emfilermin, Epoetin delta, Erythropoietin, Human interleukin-2, Interferon alfa, Interferon alfa-2c, Interferon alfa-n1, Interferon alfa-n3, Interferon alfacon-1, Interferon beta-1a, Interferon beta-1b, Interferon gamma-1b, Interferon Kappa, Interleukin-1 alpha, Interleukin-10, Interleukin-7, Lenograstim, Leridistim, Lipegfilgrastim, Lorukafusp alfa, Maxy-G34, Methoxy polyethylene glycol-epoetin beta, Molgramostim, Muplestim, Nagrestipen, Oprelvekin, Pegfilgrastim, Pegilodecakin, Peginterferon alfa-2a, Peginterferon alfa-2b, Peginterferon beta-1a, Peginterferon lambda-1a, Recombinant CD40-ligand, Regramostim, Romiplostim, Sargramostim, Thrombopoietin, Tucotuzumab celmoleukin, Viral Macrophage-Inflammatory Protein, and the like.

[00350] Steroid drugs include, but are not limited to, prednisolone, betamethasone, dexamethasone, hydrocortisone, methylprednisolone, deflazacort, and the like.

[00351] “Peptide drug” as used herein refers to amino-acid containing polymeric compounds, and is meant to encompass naturally-occurring and non-naturally-occurring peptides, oligopeptides, cyclic peptides, polypeptides, and proteins, as well as peptide mimetics. The peptide drugs may be obtained by chemical synthesis or be produced from a genetically

encoded source (e.g., recombinant source). Peptide drugs can range in molecular weight, and can be from 200 Da to 10 kDa or greater in molecular weight. Suitable peptides include, but are not limited to, cytotoxic peptides; angiogenic peptides; anti-angiogenic peptides; peptides that activate B cells; peptides that activate T cells; anti-viral peptides; peptides that inhibit viral fusion; peptides that increase production of one or more lymphocyte populations; anti-microbial peptides; growth factors; growth hormone-releasing factors; vasoactive peptides; anti-inflammatory peptides; peptides that regulate glucose metabolism; an anti-thrombotic peptide; an anti-nociceptive peptide; a vasodilator peptide; a platelet aggregation inhibitor; an analgesic; and the like.

[00352] Additional examples of drugs that find use in the conjugates and compounds described herein include, but are not limited to Tubulysin M, Calicheamicin, a STAT3 inhibitor, alpha-Amanitin, an aurora kinase inhibitor, belotecan, and an anthracycline.

[00353] Other examples of drugs include small molecule drugs, such as a cancer chemotherapeutic agent. For example, where the polypeptide is an antibody (or fragment thereof) that has specificity for a tumor cell, the antibody can be modified as described herein to include a modified amino acid, which can be subsequently conjugated to a cancer chemotherapeutic agent. Cancer chemotherapeutic agents include non-peptidic (i.e., non-proteinaceous) compounds that reduce proliferation of cancer cells, and encompass cytotoxic agents and cytostatic agents. Non-limiting examples of chemotherapeutic agents include alkylating agents, nitrosoureas, antimetabolites, antitumor antibiotics, plant (vinca) alkaloids, and steroid hormones. Peptidic compounds can also be used.

[00354] Suitable cancer chemotherapeutic agents include dolastatin and active analogs and derivatives thereof; and auristatin and active analogs and derivatives thereof (e.g., Monomethyl auristatin D (MMAD), monomethyl auristatin E (MMAE), monomethyl auristatin F (MMAF), and the like). See, e.g., WO 96/33212, WO 96/14856, and U.S. 6,323,315. For example, dolastatin 10 or auristatin PE can be included in an antibody-drug conjugate of the present disclosure. Suitable cancer chemotherapeutic agents also include maytansinoids and active analogs and derivatives thereof (see, e.g., EP 1391213; and Liu et al (1996) *Proc. Natl. Acad. Sci. USA* 93:8618-8623); duocarmycins and active analogs and derivatives thereof (e.g., including the synthetic analogues, KW-2189 and CB 1-TM1); and benzodiazepines and active analogs and derivatives thereof (e.g., pyrrolobenzodiazepine (PBD)).

[00355] Agents that act to reduce cellular proliferation are known in the art and widely used. Such agents include alkylating agents, such as nitrogen mustards, nitrosoureas, ethylenimine derivatives, alkyl sulfonates, and triazenes, including, but not limited to, mechlorethamine, cyclophosphamide (CytosanTM), melphalan (L-sarcolysin), carmustine (BCNU), lomustine (CCNU), semustine (methyl-CCNU), streptozocin, chlorozotocin, uracil mustard, chlormethine, ifosfamide, chlorambucil, pipobroman, triethylenemelamine, triethylenethiophosphoramine, busulfan, dacarbazine, and temozolomide.

[00356] Antimetabolite agents include folic acid analogs, pyrimidine analogs, purine analogs, and adenosine deaminase inhibitors, including, but not limited to, cytarabine (CYTOSAR-U), cytosine arabinoside, fluorouracil (5-FU), floxuridine (FudR), 6-thioguanine, 6-mercaptopurine (6-MP), pentostatin, 5-fluorouracil (5-FU), methotrexate, 10-propargyl-5,8-dideazafolate (PDDF, CB3717), 5,8-dideazatetrahydrofolic acid (DDATHF), leucovorin, fludarabine phosphate, pentostatin, and gemcitabine.

[00357] Suitable natural products and their derivatives, (e.g., vinca alkaloids, antitumor antibiotics, enzymes, lymphokines, and epipodophyllotoxins), include, but are not limited to, Ara-C, paclitaxel (Taxol[®]), docetaxel (Taxotere[®]), deoxycoformycin, mitomycin-C, L-asparaginase, azathioprine; brequinar; alkaloids, e.g. vincristine, vinblastine, vinorelbine, vindesine, etc.; podophyllotoxins, e.g. etoposide, teniposide, etc.; antibiotics, e.g. anthracycline, daunorubicin hydrochloride (daunomycin, rubidomycin, cerubidine), idarubicin, doxorubicin, epirubicin and morpholino derivatives, etc.; phenoxizone biscyclopeptides, e.g. dactinomycin; basic glycopeptides, e.g. bleomycin; anthraquinone glycosides, e.g. plicamycin (mithramycin); anthracenediones, e.g. mitoxantrone; azirinopyrrolo indoleiones, e.g. mitomycin; macrocyclic immunosuppressants, e.g. cyclosporine, FK-506 (tacrolimus, prograf), rapamycin, etc.; and the like.

[00358] Other anti-proliferative cytotoxic agents are navelbene, CPT-11, anastrozole, letrozole, capecitabine, reloxafine, cyclophosphamide, ifosfamide, and droloxafine.

[00359] Microtubule affecting agents that have antiproliferative activity are also suitable for use and include, but are not limited to, allocolchicine (NSC 406042), Halichondrin B (NSC 609395), colchicine (NSC 757), colchicine derivatives (e.g., NSC 33410), dolstatin 10 (NSC 376128), maytansine (NSC 153858), rhizoxin (NSC 332598), paclitaxel (Taxol[®]), Taxol[®] derivatives, docetaxel (Taxotere[®]), thiocolchicine (NSC 361792), trityl cysterin, vinblastine

sulfate, vincristine sulfate, natural and synthetic epothilones including but not limited to, eopthilone A, eopthilone B, discodermolide; estramustine, nocodazole, and the like.

[00360] Hormone modulators and steroids (including synthetic analogs) that are suitable for use include, but are not limited to, adrenocorticosteroids, e.g. prednisone, dexamethasone, etc.; estrogens and progestins, e.g. hydroxyprogesterone caproate, medroxyprogesterone acetate, megestrol acetate, estradiol, clomiphene, tamoxifen; etc.; and adrenocortical suppressants, e.g. aminoglutethimide; 17 α -ethinylestradiol; diethylstilbestrol, testosterone, fluoxymesterone, dromostanolone propionate, testolactone, methylprednisolone, methyl-testosterone, prednisolone, triamcinolone, chlorotrianisene, hydroxyprogesterone, aminoglutethimide, estramustine, medroxyprogesterone acetate, leuprolide, Flutamide (Drogenil), Toremifene (Fareston), and Zoladex®. Estrogens stimulate proliferation and differentiation; therefore compounds that bind to the estrogen receptor are used to block this activity. Corticosteroids may inhibit T cell proliferation.

[00361] Other suitable chemotherapeutic agents include metal complexes, e.g. cisplatin (cis-DDP), carboplatin, etc.; ureas, e.g. hydroxyurea; and hydrazines, e.g. N-methylhydrazine; epidophyllotoxin; a topoisomerase inhibitor; procarbazine; mitoxantrone; leucovorin; tegafur; etc. Other anti-proliferative agents of interest include immunosuppressants, e.g. mycophenolic acid, thalidomide, desoxyspergualin, azasporine, leflunomide, mizoribine, azaspirane (SKF 105685); Iressa® (ZD 1839, 4-(3-chloro-4-fluorophenylamino)-7-methoxy-6-(3-(4-morpholinyl)propoxy)quinazoline); etc.

[00362] Taxanes are suitable for use. "Taxanes" include paclitaxel, as well as any active taxane derivative or pro-drug. "Paclitaxel" (which should be understood herein to include analogues, formulations, and derivatives such as, for example, docetaxel, TAXOL™, TAXOTERE™ (a formulation of docetaxel), 10-desacetyl analogs of paclitaxel and 3'-N-desbenzoyl-3'-N-t-butoxycarbonyl analogs of paclitaxel) may be readily prepared utilizing techniques known to those skilled in the art (see also WO 94/07882, WO 94/07881, WO 94/07880, WO 94/07876, WO 93/23555, WO 93/10076; U.S. Pat. Nos. 5,294,637; 5,283,253; 5,279,949; 5,274,137; 5,202,448; 5,200,534; 5,229,529; and EP 590,267), or obtained from a variety of commercial sources, including for example, Sigma Chemical Co., St. Louis, Mo. (T7402 from *Taxus brevifolia*; or T-1912 from *Taxus yunnanensis*).

[00363] Paclitaxel should be understood to refer to not only the common chemically available form of paclitaxel, but analogs and derivatives (e.g., Taxotere™ docetaxel, as noted above) and paclitaxel conjugates (e.g., paclitaxel-PEG, paclitaxel-dextran, or paclitaxel-xylose).

[00364] Also included within the term “taxane” are a variety of known derivatives, including both hydrophilic derivatives, and hydrophobic derivatives. Taxane derivatives include, but not limited to, galactose and mannose derivatives described in International Patent Application No. WO 99/18113; piperazino and other derivatives described in WO 99/14209; taxane derivatives described in WO 99/09021, WO 98/22451, and U.S. Patent No. 5,869,680; 6-thio derivatives described in WO 98/28288; sulfenamide derivatives described in U.S. Patent No. 5,821,263; and taxol derivative described in U.S. Patent No. 5,415,869. It further includes prodrugs of paclitaxel including, but not limited to, those described in WO 98/58927; WO 98/13059; and U.S. Patent No. 5,824,701.

[00365] Biological response modifiers suitable for use include, but are not limited to, (1) inhibitors of tyrosine kinase (RTK) activity; (2) inhibitors of serine/threonine kinase activity; (3) tumor-associated antigen antagonists, such as antibodies that bind specifically to a tumor antigen; (4) apoptosis receptor agonists; (5) interleukin-2; (6) IFN- α ; (7) IFN- γ ; (8) colony-stimulating factors; and (9) inhibitors of angiogenesis.

[00366] Examples of drugs include small molecule drugs, such as a cancer chemotherapeutic agent. For example, where the polypeptide is an antibody (or fragment thereof) that has specificity for a tumor cell, the antibody can be modified as described herein to include a modified amino acid, which can be subsequently conjugated to a cancer chemotherapeutic agent, such as a microtubule affecting agent. In certain embodiments, the drug is a microtubule affecting agent that has antiproliferative activity, such as a maytansinoid.

[00367] Embodiments of the present disclosure include conjugates where an antibody is conjugated to one or more drug moieties, such as 2 drug moieties, 3 drug moieties, 4 drug moieties, 5 drug moieties, 6 drug moieties, 7 drug moieties, 8 drug moieties, 9 drug moieties, or 10 or more drug moieties. The drug moieties may be conjugated to the antibody at one or more sites in the antibody, as described herein. In certain embodiments, the conjugates have an average drug-to-antibody ratio (DAR) (molar ratio) in the range of from 0.1 to 10, or from 0.5 to 10, or from 1 to 10, such as from 1 to 9, or from 1 to 8, or from 1 to 7, or from 1 to 6, or from 1 to 5, or from 1 to 4, or from 1 to 3, or from 1 to 2. In certain embodiments, the conjugates have

an average DAR from 1 to 2, such as 1, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9 or 2. In certain embodiments, the conjugates have an average DAR of 1 to 5. In certain embodiments, the conjugates have an average DAR of 1 to 4. In certain embodiments, the conjugates have an average DAR of 1 to 3. In certain embodiments, the conjugates have an average DAR of 1 to 2. By average is meant the arithmetic mean.

[00368] Drugs to be conjugated to a polypeptide may be modified to incorporate a reactive partner for reaction with the polypeptide. Where the drug is a peptide drug, the reactive moiety (e.g., aminooxy or hydrazide) can be positioned at an N-terminal region, the N-terminus, a C-terminal region, the C-terminus, or at a position internal to the peptide. For example, an example of a method involves synthesizing a peptide drug having an aminooxy group. In this example, the peptide is synthesized from a Boc-protected precursor. An amino group of a peptide can react with a compound comprising a carboxylic acid group and oxy-N-Boc group. As an example, the amino group of the peptide reacts with 3-(2,5-dioxopyrrolidin-1-yloxy)propanoic acid. Other variations on the compound comprising a carboxylic acid group and oxy-N-protecting group can include different number of carbons in the alkylene linker and substituents on the alkylene linker. The reaction between the amino group of the peptide and the compound comprising a carboxylic acid group and oxy-N-protecting group occurs through standard peptide coupling chemistry. Examples of peptide coupling reagents that can be used include, but not limited to, DCC (dicyclohexylcarbodiimide), DIC (diisopropylcarbodiimide), di-p-toluoylcarbodiimide, BDP (1-benzotriazole diethylphosphate-1-cyclohexyl-3-(2-morpholinylethyl)carbodiimide), EDC (1-(3-dimethylaminopropyl-3-ethyl-carbodiimide hydrochloride), cyanuric fluoride, cyanuric chloride, TFFH (tetramethyl fluoroformamidinium hexafluorophosphate), DPPA (diphenylphosphorazidate), BOP (benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate), HBTU (O-benzotriazol-1-yl-N,N,N',N'-tetramethyluronium hexafluorophosphate), TBTU (O-benzotriazol-1-yl-N,N,N',N'-tetramethyluronium tetrafluoroborate), TSTU (O-(N-succinimidyl)-N,N,N',N'-tetramethyluronium tetrafluoroborate), HATU (N-[(dimethylamino)-1-H-1,2,3-triazolo[4,5,6]-pyridin-1-ylmethylene]-N-methylmethanaminium hexafluorophosphate N-oxide), BOP-Cl (bis(2-oxo-3-oxazolidinyl)phosphinic chloride), PyBOP ((1-H-1,2,3-benzotriazol-1-yloxy)-tris(pyrrolidino)phosphonium tetrafluorophosphate), BrOP (bromotris(dimethylamino)phosphonium hexafluorophosphate), DEPBT (3-

(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3H)-one) PyBrOP (bromotris(pyrrolidino)phosphonium hexafluorophosphate). As a non-limiting example, HOBt and DIC can be used as peptide coupling reagents.

[00369] Deprotection to expose the amino-oxy functionality is performed on the peptide comprising an N-protecting group. Deprotection of the N-oxysuccinimide group, for example, occurs according to standard deprotection conditions for a cyclic amide group. Deprotecting conditions can be found in Greene and Wuts, *Protective Groups in Organic Chemistry*, 3rd Ed., 1999, John Wiley & Sons, NY and Harrison et al. Certain deprotection conditions include a hydrazine reagent, amino reagent, or sodium borohydride. Deprotection of a Boc protecting group can occur with TFA. Other reagents for deprotection include, but are not limited to, hydrazine, methylhydrazine, phenylhydrazine, sodium borohydride, and methylamine. The product and intermediates can be purified by conventional means, such as HPLC purification.

[00370] The ordinarily skilled artisan will appreciate that factors such as pH and steric hindrance (i.e., the accessibility of the amino acid residue to reaction with a reactive partner of interest) are of importance. Modifying reaction conditions to provide for optimal conjugation conditions is well within the skill of the ordinary artisan, and is routine in the art. Where conjugation is conducted with a polypeptide present in or on a living cell, the conditions are selected so as to be physiologically compatible. For example, the pH can be dropped temporarily for a time sufficient to allow for the reaction to occur but within a period tolerated by the cell (e.g., from about 30 min to 1 hour). Physiological conditions for conducting modification of polypeptides on a cell surface can be similar to those used in a ketone-azide reaction in modification of cells bearing cell-surface azides (see, e.g., U.S. 6,570,040).

[00371] Small molecule compounds containing, or modified to contain, an α -nucleophilic group that serves as a reactive partner with a compound or conjugate disclosed herein are also contemplated for use as drugs in the polypeptide-drug conjugates of the present disclosure. General methods are known in the art for chemical synthetic schemes and conditions useful for synthesizing a compound of interest (see, e.g., Smith and March, *March's Advanced Organic Chemistry: Reactions, Mechanisms, and Structure*, Fifth Edition, Wiley-Interscience, 2001; or Vogel, *A Textbook of Practical Organic Chemistry, Including Qualitative Organic Analysis*, Fourth Edition, New York: Longman, 1978).

FORMULATIONS

[00372] The conjugates of the present disclosure can be formulated in a variety of different ways. In general, where the conjugate is an antibody-drug conjugate, the conjugate is formulated in a manner compatible with the drug, the antibody, the condition to be treated, and the route of administration to be used.

[00373] In some embodiments, provided is a pharmaceutical composition that includes any of the conjugates of the present disclosure and a pharmaceutically-acceptable excipient.

[00374] The conjugate (e.g., antibody-drug conjugate) can be provided in any suitable form, e.g., in the form of a pharmaceutically acceptable salt, and can be formulated for any suitable route of administration, e.g., oral, topical or parenteral administration. Where the conjugate is provided as a liquid injectable (such as in those embodiments where they are administered intravenously or directly into a tissue), the conjugate can be provided as a ready-to-use dosage form, or as a reconstitutable storage-stable powder or liquid composed of pharmaceutically acceptable carriers and excipients.

[00375] Methods for formulating conjugates can be adapted from those readily available. For example, conjugates can be provided in a pharmaceutical composition comprising a therapeutically effective amount of a conjugate and a pharmaceutically acceptable carrier (e.g., saline). The pharmaceutical composition may optionally include other additives (e.g., buffers, stabilizers, preservatives, and the like). In some embodiments, the formulations are suitable for administration to a mammal, such as those that are suitable for administration to a human.

METHODS OF TREATMENT

[00376] The antibody-drug conjugates of the present disclosure find use in treatment of a condition or disease in a subject that is amenable to treatment by administration of the parent drug (i.e., the drug prior to conjugation to the antibody).

[00377] In some embodiments, provided are methods that include administering to a subject an effective amount (e.g., a therapeutically effective amount) of any of the conjugates of the present disclosure.

[00378] In certain aspects, provided are methods of delivering a drug to a target site in a subject, the method including administering to the subject a pharmaceutical composition including any of the conjugates of the present disclosure, where the administering is effective to

release a therapeutically effective amount of the drug from the conjugate at the target site in the subject. For example, as described herein, antibody-drug conjugates of the present disclosure can include a cleavable linker, such as an enzymatically cleavable linker that includes a first enzymatically cleavable moiety and a second enzymatically cleavable moiety. In some instances, the cleavable linker can be cleaved under appropriate conditions to separate or release the drug from the antibody at a desired target site of action for the drug. For example, the second cleavable linker, which protects the first cleavable linker from cleavage, may be cleaved in order to allow the first cleavable moiety to be cleaved, which results in cleavage of the cleavable linker into two or more portions, thus releasing the drug from the antibody-drug conjugate at a desired site of action.

[00379] In certain embodiments, the first cleavable moiety can be an enzymatically cleavable moiety. In some instances, the enzyme that facilitates cleavage of the first cleavable moiety is an enzyme that is administered to the subject to be treated (i.e., exogenous to the subject to be treated). For example, a first enzyme can be administered before, concurrently with, or after administration of an antibody-drug conjugate described herein.

[00380] In certain embodiments, the second cleavable moiety can be an enzymatically cleavable moiety. In some instances, the enzyme that facilitates cleavage of the second cleavable moiety is an enzyme that is administered to the subject to be treated (i.e., exogenous to the subject to be treated). For example, a second enzyme can be administered before, concurrently with, or after administration of an antibody-drug conjugate described herein. In certain embodiments, the first enzyme and the second enzyme are different enzymes.

[00381] In other instances, the first enzyme that facilitates cleavage of the first cleavable moiety is an enzyme that is present in the subject to be treated (i.e., endogenous to the subject to be treated). For instance, the first enzyme may be present at the desired site of action for the drug of the antibody-drug conjugate. The antibody of the antibody-drug conjugate may be specifically targeted to a desired site of action (e.g., may specifically bind to an antigen present at a desired site of action), where the desired site of action also includes the presence of the first enzyme. In some instances, the first enzyme is present in an overabundance at the desired site of action as compared to other areas in the body of the subject to be treated. For example, the first enzyme may be overexpressed at the desired site of action as compared to other areas in the body of the subject to be treated. In some instances, the first enzyme is present in an overabundance at

the desired site of action due to localization of the first enzyme at a particular area or location. For instance, the first enzyme may be associated with a certain structure within the desired site of action, such as lysosomes. In some cases, the first enzyme is present in an overabundance in lysosomes as compared to other areas in the body of the subject. In some embodiments, the lysosomes that include the first enzyme, are found at a desired site of action for the drug of the antibody-drug conjugate, such as the site of a cancer or tumor that is to be treated with the drug. In certain embodiments, the first enzyme is an esterase.

[00382] In certain embodiments, the second enzyme that facilitates cleavage of the second cleavable moiety is an enzyme that is present in the subject to be treated (i.e., endogenous to the subject to be treated). For instance, the second enzyme may be present at the desired site of action for the drug of the antibody-drug conjugate. The antibody of the antibody-drug conjugate may be specifically targeted to a desired site of action (e.g., may specifically bind to an antigen present at a desired site of action), where the desired site of action also includes the presence of the second enzyme. In some instances, the second enzyme is present in an overabundance at the desired site of action as compared to other areas in the body of the subject to be treated. For example, the second enzyme may be overexpressed at the desired site of action as compared to other areas in the body of the subject to be treated. In some instances, the second enzyme is present in an overabundance at the desired site of action due to localization of the second enzyme at a particular area or location. For instance, the second enzyme may be associated with a certain structure within the desired site of action, such as lysosomes. In some cases, the second enzyme is present in an overabundance in lysosomes as compared to other areas in the body of the subject. In some embodiments, the lysosomes that include the second enzyme, are found at a desired site of action for the drug of the antibody-drug conjugate, such as the site of a cancer or tumor that is to be treated with the drug. In certain embodiments, the second enzyme is a glucuronidase, a galactosidase, a glucosidase, a mannosidase, a fucosidase, and the like.

[00383] Any suitable enzymes can be used for cleavage of the first cleavable moiety and the second cleavable moiety of the antibody-drug conjugates described herein. Other enzymes may also be suitable for use in cleavage of the first cleavable moiety and the second cleavable moiety of the antibody-drug conjugates described herein, such as but not limited to, enzymes from other vertebrates (e.g., primates, mice, rats, cats, pigs, quails, goats, dogs, etc.).

[00384] In certain embodiments, the antibody-drug conjugate is substantially stable under standard conditions. By substantially stable is meant that the cleavable linker of the antibody-drug conjugate does not undergo a significant amount of cleavage in the absence of a first enzyme and a second enzyme as described above. For example, as described above, the second cleavable moiety can protect the first cleavable moiety from being cleaved, and as such the cleavable linker of the antibody-drug conjugate does not undergo a significant amount of cleavage in the absence of a second enzyme as described above. For instance, the cleavable linker of the antibody-drug conjugate may be substantially stable such that 25% or less of the antibody-drug conjugate is cleaved in the absence of the first enzyme and/or second enzyme, such as 20% or less, or 15% or less, or 10% or less, or 5% or less, or 4% or less, or 3% or less, or 2% or less, or 1% or less. In some cases, the antibody-drug conjugate is substantially stable such that the cleavable linker of the antibody-drug conjugate does not undergo a significant amount of cleavage in the absence of the first enzyme and/or second enzyme, but can be cleaved when in the presence of the first enzyme and the second enzyme. For example, the antibody-drug conjugate can be substantially stable after administration to a subject. In some cases, the antibody-drug conjugate is substantially stable after administration to a subject, and then, when the antibody-drug conjugate is in the presence of the second enzyme at a desired site of action, the second cleavable moiety can be cleaved from the cleavable linker, thus exposing the first cleavable moiety to subsequent cleavage by the first enzyme, which in turn releases the drug at the desired site of action. In certain embodiments, after administration to a subject the antibody-drug conjugate is stable for an extended period of time in the absence of the first enzyme and/or second enzyme, such as 1 hr or more, or 2 hrs or more, or 3 hrs or more, or 4 hrs or more, or 5 hrs or more, or 6 hrs or more, or 7 hrs or more, or 8 hrs or more, or 9 hrs or more, or 10 hrs or more, or 15 hrs or more, or 20 hrs or more, or 24 hrs (1 day) or more, or 2 days or more, or 3 days or more, or 4 days or more, or 5 days or more, or 6 days or more, or 7 days (1 week) or more. In certain embodiments, the antibody-drug conjugate is stable at a range pH values for an extended period of time in the absence of the first enzyme and/or second enzyme, such as at a pH ranging from 2 to 10, or from 3 to 9, or from 4 to 8, or from 5 to 8, or from 6 to 8, or from 7 to 8.

[00385] As described above, the antibody-drug conjugates of the present disclosure find use in treatment of a condition or disease in a subject that is amenable to treatment by administration of the parent drug. By “treatment” is meant that at least an amelioration of the

symptoms associated with the condition afflicting the host is achieved, where amelioration is used in a broad sense to refer to at least a reduction in the magnitude of a parameter, e.g. symptom, associated with the condition being treated. As such, treatment also includes situations where the pathological condition, or at least symptoms associated therewith, are completely inhibited, e.g., prevented from happening, or stopped, e.g. terminated, such that the host no longer suffers from the condition, or at least the symptoms that characterize the condition. Thus treatment includes: (i) prevention, that is, reducing the risk of development of clinical symptoms, including causing the clinical symptoms not to develop, e.g., preventing disease progression to a harmful state; (ii) inhibition, that is, arresting the development or further development of clinical symptoms, e.g., mitigating or completely inhibiting an active disease; and/or (iii) relief, that is, causing the regression of clinical symptoms.

[00386] The subject to be treated can be one that is in need of therapy, where the subject to be treated is one amenable to treatment using the parent drug. Accordingly, a variety of subjects may be amenable to treatment using the antibody-drug conjugates disclosed herein. Generally, such subjects are “mammals”, with humans being of interest. Other subjects can include domestic pets (e.g., dogs and cats), livestock (e.g., cows, pigs, goats, horses, and the like), rodents (e.g., mice, guinea pigs, and rats, e.g., as in animal models of disease), as well as non-human primates (e.g., chimpanzees and monkeys).

[00387] The amount of antibody-drug conjugate administered can be initially determined based on guidance of a dose and/or dosage regimen of the parent drug. In general, the antibody-drug conjugates can provide for targeted delivery and/or enhanced serum half-life of the bound drug, thus providing for at least one of reduced dose or reduced administrations in a dosage regimen. Thus, the antibody-drug conjugates can provide for reduced dose and/or reduced administration in a dosage regimen relative to the parent drug prior to being conjugated in an antibody-drug conjugate of the present disclosure.

[00388] Furthermore, as noted above, because the antibody-drug conjugates can provide for controlled stoichiometry of drug delivery, dosages of antibody-drug conjugates can be calculated based on the number of drug molecules provided on a per antibody-drug conjugate basis.

[00389] In some embodiments, multiple doses of an antibody-drug conjugate are administered. The frequency of administration of an antibody-drug conjugate can vary depending

on any of a variety of factors, e.g., severity of the symptoms, condition of the subject, etc. For example, in some embodiments, an antibody-drug conjugate is administered once per month, twice per month, three times per month, every other week, once per week (qwk), twice per week, three times per week, four times per week, five times per week, six times per week, every other day, daily (qd/od), twice a day (bds/bid), or three times a day (tds/tid), etc.

EXAMPLES

[00390] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Celsius, and pressure is at or near atmospheric. By “average” is meant the arithmetic mean. Standard abbreviations may be used, e.g., bp, base pair(s); kb, kilobase(s); pl, picoliter(s); s or sec, second(s); min, minute(s); h or hr, hour(s); aa, amino acid(s); kb, kilobase(s); bp, base pair(s); nt, nucleotide(s); i.m., intramuscular(ly); i.p., intraperitoneal(ly); s.c., subcutaneous(ly); and the like.

General Synthetic Procedures

[00391] Many general references providing commonly known chemical synthetic schemes and conditions useful for synthesizing the disclosed compounds are available (see, e.g., Smith and March, *March's Advanced Organic Chemistry: Reactions, Mechanisms, and Structure*, Fifth Edition, Wiley-Interscience, 2001; or Vogel, *A Textbook of Practical Organic Chemistry*, Including Qualitative Organic Analysis, Fourth Edition, New York: Longman, 1978).

[00392] Compounds as described herein can be purified by any purification protocol known in the art, including chromatography, such as HPLC, preparative thin layer chromatography, flash column chromatography and ion exchange chromatography. Any suitable stationary phase can be used, including normal and reversed phases as well as ionic resins. In certain embodiments, the disclosed compounds are purified via silica gel and/or alumina chromatography. See, e.g.,

Introduction to Modern Liquid Chromatography, 2nd Edition, ed. L. R. Snyder and J. J. Kirkland, John Wiley and Sons, 1979; and Thin Layer Chromatography, ed E. Stahl, Springer-Verlag, New York, 1969.

[00393] During any of the processes for preparation of the subject compounds, it may be necessary and/or desirable to protect sensitive or reactive groups on any of the molecules concerned. This may be achieved by means of conventional protecting groups as described in standard works, such as J. F. W. McOmie, "Protective Groups in Organic Chemistry", Plenum Press, London and New York 1973, in T. W. Greene and P. G. M. Wuts, "Protective Groups in Organic Synthesis", Third edition, Wiley, New York 1999, in "The Peptides"; Volume 3 (editors: E. Gross and J. Meienhofer), Academic Press, London and New York 1981, in "Methoden der organischen Chemie", Houben-Weyl, 4th edition, Vol. 15/I, Georg Thieme Verlag, Stuttgart 1974, in H.-D. Jakubke and H. Jescheit, "Aminosäuren, Peptide, Proteine", Verlag Chemie, Weinheim, Deerfield Beach, and Basel 1982, and/or in Jochen Lehmann, "Chemie der Kohlenhydrate: Monosaccharide and Derivate", Georg Thieme Verlag, Stuttgart 1974. The protecting groups may be removed at a convenient subsequent stage using methods known from the art.

[00394] The subject compounds can be synthesized via a variety of different synthetic routes using commercially available starting materials and/or starting materials prepared by conventional synthetic methods. A variety of examples of synthetic routes that can be used to synthesize the compounds disclosed herein are described in the schemes below.

EXAMPLE 1

Synthesis of dual-cleavage ester linkers

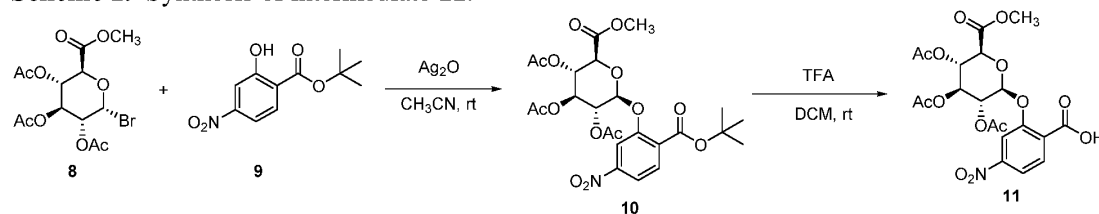
Materials and Methods

General Information

[00395] Synthetic reagents were purchased from Sigma-Aldrich, Acros, AK Scientific, or other commercial sources and were used without purification. Anhydrous solvents were obtained from commercial sources in sealed bottles. Cytotoxin SN-38 (**12**) was purchased from MedChemExpress and used as received. Cytotoxins **28** and **41** were purchased from commercial sources and used as received. Compound **9** was synthesized using previously reported procedure. Synthetic intermediates **16**, **19**, **20**, **22**, **25** and **48** were obtained commercially from

Shanghai Medicilon and used without purification. All other starting materials were purchased from commercial sources or synthesized using previously reported procedures. In all cases, solvent was removed under reduced pressure with a Buchi Rotovapor R-114 equipped with a Buchi V-700 vacuum pump. Column chromatography was performed with a Biotage chromatography purification system. Preparative HPLC purifications were performed using Waters preparative HPLC unit equipped with Phenomenex Kinetex 5 μ m EVO C18 150 x 21.2 mm column. HPLC analyses were conducted on an Agilent 1100 Series Analytical HPLC equipped with a Model G1322A Degasser, Model G1311A Quaternary Pump, Model G1329A Autosampler, Model G1314 Variable Wavelength Detector, Agilent Poroshell 120 SB C18, 4.6 mm x 50 mm column at 30 °C using a 10-100% gradient of water and acetonitrile containing 0.05% trifluoroacetic acid. HPLCs were monitored at 254 or 205 nm. Low-resolution mass spectra (LRMS) were acquired on Agilent Technology 6120 Quadrupole LC/MS, equipped with Agilent 1260 Infinity HPLC system, G1314 variable wavelength detector, and Agilent Poroshell 120 SB C18, 4.6 mm x 50 mm column at 30 °C using 10-100% gradient of water and acetonitrile containing 0.1% formic acid.

Scheme 1. Synthesis of intermediate **11**.



*Preparation of (2S,3R,4S,5S,6S)-2-(2-(tert-butoxycarbonyl)-5-nitrophenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (**10**)*

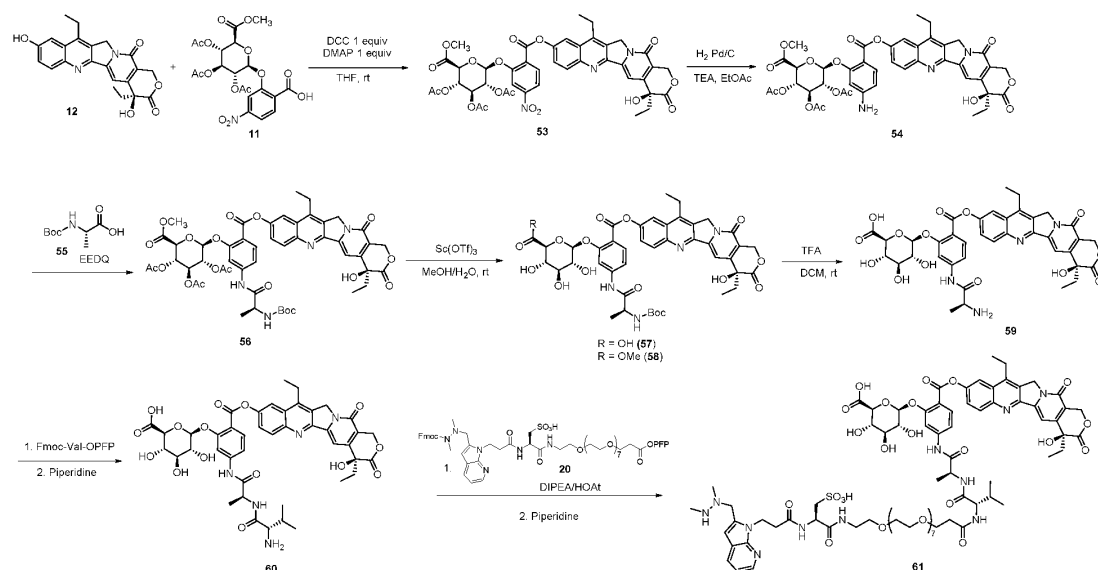
[00396] To a mixture of *tert*-butyl 2-hydroxy-4-nitrobenzoate (**9**, 160 mg, 0.67 mmol) acetobromo-D-glucuronic acid methyl ester (**8**, 797 mg, 2.0 mmol) in 20 mL of anhydrous acetonitrile were added silver(I) oxide (463 mg, 2.0 mmol). The resulting mixture was vigorously stirred in the dark for 24 hours. Reaction mixture was filtered through a pad of silica gel, eluted with ethyl acetate. Combined filtrates were concentrated to dryness, and the residue was purified on silica gel (ethyl acetate-hexane, 0-30% v/v gradient) to obtain 210 mg of product

10 (0.38 mmol, 56% yield) as a white solid. LRMS (ESI) m/z 578.1 $[M+Na]^+$, Calcd for $C_{24}H_{29}NO_{14}$ 578.2.

Preparation of 4-nitro-2-(((2S,3R,4S,5S,6S)-3,4,5-triacetoxy-6-(methoxycarbonyl)tetrahydro-2H-pyran-2-yl)oxy)benzoic acid (11)

[00397] *tert*-Butyl ester **10** (180 mg, 0.32 mmol) was dissolved in 4 mL of TFA-DCM mixture (1:1) at RT. The resulting solution was allowed to stand for 30 minutes, then solvents were removed under vacuum, and the residue was purified on silica gel (methanol-DCM, 0-5% v/v gradient) to give 155 mg of product **11** (0.31 mmol, 97% yield) as a pink foamy solid. LRMS (ESI) m/z 522.1 $[M+Na]^+$, Calcd for $C_{20}H_{21}NO_{14}$ 522.1.

Scheme 2. Synthesis of construct **61** bearing SN-38 cytotoxin and phenolic ester dual-cleavage linker



Preparation of (2S,3R,4S,5S,6S)-2-(2-(((S)-4,11-diethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl)oxy)carbonyl)-5-nitrophenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (53)

[00398] To a mixture of SN-38 (**12**, 20 mg, 51 μ mole) and carboxylic acid **11** (26 mg, 51 μ mole) in 2 mL of anhydrous THF were added 10.5 mg of DCC (51 μ mole), followed by DMAP (6 mg, 51 μ mole) at room temperature. Reaction mixture was stirred for 24 hours, then solids

were filtered off, the resulting filtrate was concentrated under vacuum and purified on silica gel (MeOH-DCM 0-10% v/v gradient) to obtain 40 mg of product **53** (46 μ mole, 90% yield) as an off-white solid. LRMS (ESI) m/z 874.2 $[M+H]^+$, Calcd for $C_{42}H_{39}N_3O_{18}$ 874.2.

Preparation of (2S,3R,4S,5S,6S)-2-(5-amino-2-(((S)-4,11-diethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl)oxy)carbonyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (54)

[00399] To a stirred solution of compound **53** (40 mg, 46 μ mole) in 5 mL of ethyl acetate were added 5 mg of 10 wt. % palladium on carbon (4.6 μ mole) and 50 μ L (0.36 mmol) of triethylamine. Reaction flask was flushed with argon, sealed with a rubber septum, and equipped with a hydrogen balloon. Reaction mixture was stirred overnight at room temperature, then filtered through a pad of celite, concentrated under vacuum, and purified on silica gel (MeOH-DCM 0-10% v/v gradient) to give 28 mg of compound **54** (34 μ mole, 73% yield) as a yellowish solid. LRMS (ESI) m/z 844.3 $[M+H]^+$, Calcd for $C_{42}H_{41}N_3O_{16}$ 844.3.

Preparation of (2S,3R,4S,5S,6S)-2-(5-((S)-2-((tert-butoxycarbonyl)amino)propanamido)-2-(((S)-4,11-diethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl)oxy)carbonyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (56)

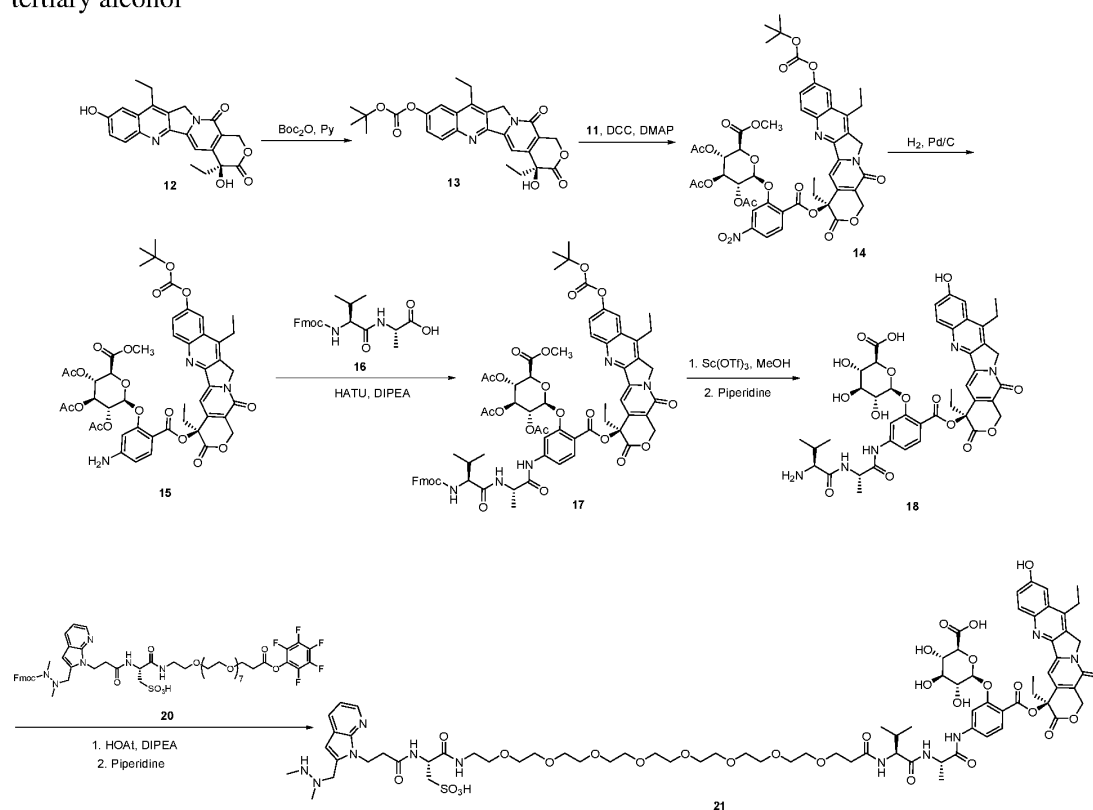
[00400] To a mixture of compound **54** (28 mg, 34 μ mole) and Boc-L-Ala-OH (7 mg, 34 μ mole) in 1 mL of DCM were added EEDQ (10 mg, 40 μ mole) at room temperature. The resulting mixture was stirred in the dark for 24 hours, then directly purified on silica gel (MeOH-DCM 0-10% v/v gradient) to give 24 mg of product **56** (24 μ mole, 70% yield) as a tan solid. LRMS (ESI) m/z 1015.4 $[M+H]^+$, Calcd for $C_{50}H_{54}N_4O_{19}$ 1015.3.

Preparation of (2S,3S,4S,5R,6S)-6-(5-((S)-2-((tert-butoxycarbonyl)amino)propanamido)-2-(((S)-4,11-diethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl)oxy)carbonyl)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (57)

[00401] To a solution of compound **55** (19 mg, 19 μ mole) in 2 mL of methanol-water mixture (4:1) were added scandium(III) triflate (19 mg, 38 μ mole) at room temperature. The resulting

mixture was stirred at 30 °C for 4 days, then solvents were removed under vacuum and the residue was purified by reversed phase HPLC (C18 column, CH₃CN-H₂O with 0.05% TFA, 0-50% v/v gradient) to give 3.5 mg of an approx. 1:1 mixture of the desired product **57** and the corresponding partially hydrolyzed methyl ester **58** (21% yield of the mixture). The mixture was taken to the next step without additional purification. LRMS (ESI) m/z 875.4 [M+H]⁺, Calcd for C₄₃H₄₆N₄O₁₆ (**57**) 875.3; LRMS (ESI) m/z 889.4 [M+H]⁺, Calcd for C₄₄H₄₈N₄O₁₆ (**58**) 889.3.

Scheme 3. Synthesis of construct **21** bearing SN-38 cytotoxin and ester dual-cleavage linker at tertiary alcohol



*Preparation of (S)-tert-butyl (4,11-diethyl-4-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-9-yl) carbonate (**13**)*

[00402] To a mixture of SN-38 **12** (158 mg, 0.40 mmol) and Boc_2O (114 mg, 1.3 mmol) in dichloromethane (16 mL) was added pyridine (0.98 mL, 12 mmol) at 0 °C. After one hour the solution was allowed to warm up to room temperature and stirred for two hours. Reaction

mixture was then concentrated under vacuum, and the residue was purified by silica gel chromatography (hexane-EtOAc, 100:0 to 0:100 v/v) to yield **13** (160 mg, 0.33 mmol, 83%) as an off-white solid. LRMS (ESI): m/z 493.2 $[M+H]^+$, Calcd for $C_{27}H_{29}N_2O_7$ m/z 493.2.

*Preparation of (2S,3R,4S,5S,6S)-2-(2-(((S)-9-((tert-butoxycarbonyl)oxy)-4,11-diethyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl)oxy)carbonyl)-5-nitrophenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (**14**)*

[00403] To a solution of carboxylic acid **11** (18 mg, 36 μ mol) in dichloromethane (1 mL) and DMF (0.5 mL) were added Boc-protected SN-38 **13** (14 mg, 28 μ mol), followed by DCC (6 mg, 29 μ mol) and DMAP (3 mg, 25 μ mol) at 0 °C. After 1 h, reaction mixture was allowed to warm to room temperature, and stirring continued for 2 h. Reaction mixture was purified by reversed-phase chromatography using C18 column (H_2O/CH_3CN with 0.05% TFA, 100:0 to 0:100 v/v) to yield compound **14** (25 mg, 26 μ mol, 93% yield) as a yellow solid. LRMS (ESI): m/z 974.3 $[M+H]^+$, Calcd for $C_{47}H_{47}N_3O_{20}$ m/z 974.3.

*Preparation of (2S,3R,4S,5S,6S)-2-(5-amino-2-(((S)-9-((tert-butoxycarbonyl)oxy)-4,11-diethyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl)oxy)carbonyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (**15**)*

[00404] To a solution of compound **14** (35 mg, 36 μ mol) in EtOAc (0.5 mL) was added Pd/C (10 wt.%, 2 mg) and triethylamine (2 μ L, 22 μ mol). The flask was then evacuated and filled with hydrogen gas from a balloon, in three repeating cycles. Reaction mixture was vigorously stirred for 48 h at room temperature with H_2 balloon attached. Solids were removed by filtration through a celite pad, the filtrate was concentrated and dried under vacuum to give 35 mg of crude compound **15**, which was used in the next step without purification. LRMS (ESI): m/z 944.3 $[M+H]^+$, Calcd for $C_{47}H_{49}N_3O_{18}$ m/z 944.3.

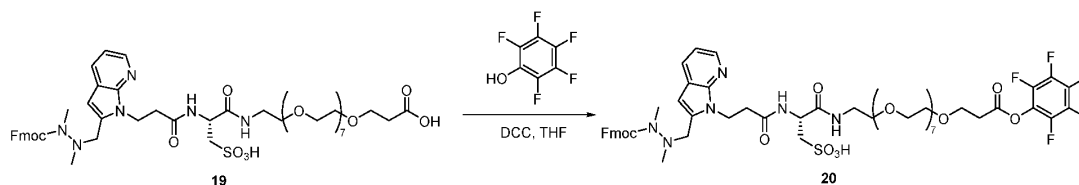
*Preparation of (2S,3R,4S,5S,6S)-2-(5-((S)-2-((S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)propanamido)-2-(((S)-9-((tert-butoxycarbonyl)oxy)-4,11-diethyl-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl)oxy)carbonyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (**17**)*

[00405] To a mixture of crude amine **15** (35 mg) and Fmoc-Val-Ala-OH **16** (60 mg, 0.15 mmol) in DMF (0.5 mL) were added HATU (56 mg, 0.15 mmol) and DIPEA (51 μ L, 0.30 mmol) at room temperature. Reaction mixture was stirred overnight and purified by reversed-phase chromatography on C18 column (H₂O/CH₃CN with 0.05% TFA, 100:0 to 0:100 v/v) to yield compound **17** (46 mg, 34 μ mol, 94% yield over two steps) as a yellow solid. LRMS (ESI): m/z 1336.5 [M+H]⁺, Calcd for C₇₀H₇₃N₅O₂₂ m/z 1336.5.

*Preparation of (2S,3S,4S,5R,6S)-6-(5-((S)-2-((S)-2-amino-3-methylbutanamido)propanamido)-2-(((S)-4,11-diethyl-9-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl)oxy)carbonyl)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (**18**)*

[00406] To a solution of compound **17** (20 mg, 15 μ mol) in a MeOH-H₂O mixture (4:1 v/v, 1 mL) were added Sc(OTf)₃ (180 mg, 0.36 mmol) at room temperature. The resulting mixture was stirred for 2 days and concentrated under vacuum. The residue was reconstituted in DMF-piperidine mixture (10:1 v/v, 1.1 mL) and stirred for 1 hour at room temperature. Reaction mixture was purified by reversed-phase chromatography on C18 column (H₂O/CH₃CN with 0.05% TFA, 90:10 to 35:65 v/v) to yield compound **18** (8 mg, 9 μ mol, 60% yield). LRMS (ESI): m/z 874.3 [M+H]⁺, Calcd for C₄₃H₄₇N₅O₁₅ m/z 874.3.

*Preparation of (R)-2-(3-(2-((2-(((9H-fluoren-9-yl)methoxy)carbonyl)-1,2-dimethylhydrazinyl)methyl)-1H-pyrrolo[2,3-b]pyridin-1-yl)propanamido)-3,31-dioxo-31-(perfluorophenoxy)-7,10,13,16,19,22,25,28-octa-4-azahentriacontane-1-sulfonic acid (**20**)*

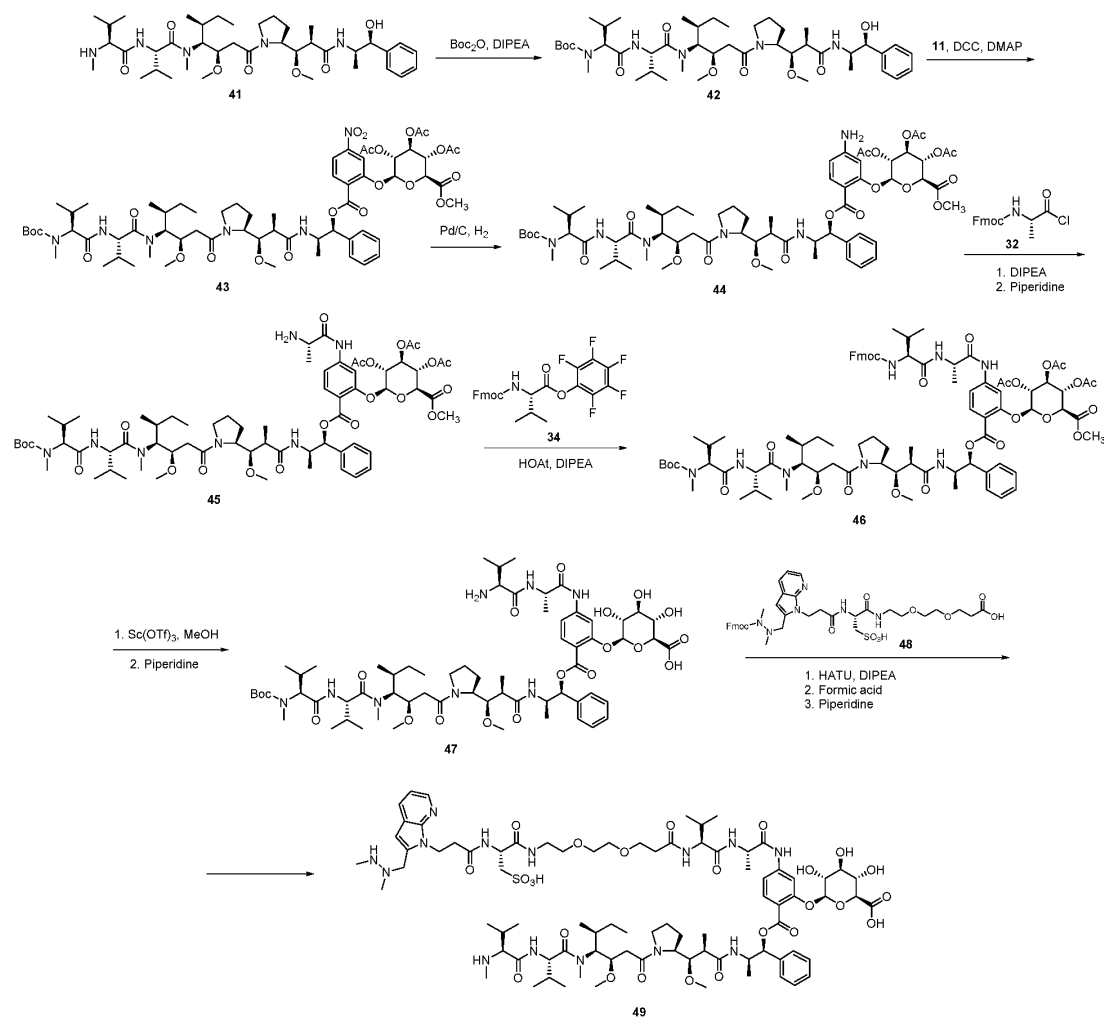


[00407] To a mixture of carboxylic acid **19** (180 mg, 0.17 mmol) and pentafluorophenol (125 mg, 0.68 mmol) in 4 mL of anhydrous THF were added DCC (68 mg, 0.33 mmol) at room temperature. The resulting mixture was stirred overnight, filtered through a pad of celite, concentrated under vacuum, and purified by reversed phase chromatography (C18, 0-80%

acetonitrile-water/0.05% TFA) to give 100 mg of PFP ester **20** as a colorless oil (0.08 mmol, 47% yield). LRMS (ESI): m/z 1225.4 $[M+H]^+$, Calcd for $C_{56}H_{69}F_5N_6O_{17}S$ m/z 1225.4.

*Preparation of (2S,3S,4S,5R,6S)-6-(2-((((S)-4,11-diethyl-9-hydroxy-3,14-dioxo-3,4,12,14-tetrahydro-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinolin-4-yl)oxy)carbonyl)-5-((2S,5S,36R)-40-(2-((1,2-dimethylhydrazinyl)methyl)-1H-pyrrolo[2,3-b]pyridin-1-yl)-5-isopropyl-2-methyl-4,7,35,38-tetraoxo-36-(sulfomethyl)-10,13,16,19,22,25,28,31-octaoxa-3,6,34,37-tetraazatetracontanamido)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (**21**)*

[00408] To a mixture of amine **18** (8 mg, 9 μ mol) and PFP ester **20** (12 mg, 10 μ mol) in DMF (0.5 mL) were added HOAt (1.2 mg, 9 μ mol) and DIPEA (5 μ L, 27 μ mol) at ambient temperature. Reaction mixture was stirred for one hour, then DMF (0.5 mL) was added to the mixture, followed by piperidine (50 μ L). After stirring for 15 minutes at room temperature, reaction mixture was directly purified by reversed phase HPLC using C18 column (H_2O/CH_3CN with 0.05% TFA, 90:10 to 45:55 v/v). Fractions containing the desired compound were pooled lyophilized to yield compound **21** (3.5 mg, 2 μ mol, 22% yield) as a yellow solid. LRMS (ESI): m/z 1692.7 $[M+H]^+$, Calcd for $C_{78}H_{105}N_{11}O_{29}S$ m/z 1692.7.

Scheme 4. Synthesis of MMAE construct **49** bearing ester linker at secondary alcohol

*Preparation of tert-butyl ((S)-1-(((S)-1-(((3R,4S,5S)-1-((S)-2-((1R,2R)-3-(((1S,2R)-1-hydroxy-1-phenylpropan-2-yl)amino)-1-methoxy-2-methyl-3-oxopropyl)pyrrolidin-1-yl)-3-methoxy-5-methyl-1-oxoheptan-4-yl)(methyl)amino)-3-methyl-1-oxobutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)(methyl)carbamate (**42**)*

[00409] To a mixture of MMAE **41** (100 mg, 0.14 mmol) and Boc_2O (56 mg, 0.26 mmol) in CH_3CN (2 mL) were added DIPEA (44 μL , 0.26 mmol) at room temperature. After stirring overnight, the reaction mixture was purified using silica gel column chromatography

(DCM/MeOH, 100:0 to 90:10 v/v) to yield compound **42** (100 mg, 0.12 mmol, 86%) as an off-white solid. LRMS (ESI): m/z 818.6 $[M+H]^+$, Calcd for $C_{44}H_{75}N_5O_9$ m/z 818.6.

*Preparation of (2S,3R,4S,5S,6S)-2-(5-amino-2-(((1S,2R)-2-((2R,3R)-3-((S)-1-((6S,9S,12S,13R)-12-((S)-sec-butyl)-6,9-diisopropyl-13-methoxy-2,2,5,11-tetramethyl-4,7,10-trioxo-3-oxa-5,8,11-triazapentadecan-15-yl)pyrrolidin-2-yl)-3-methoxy-2-methylpropanamido)-1-phenylpropoxy)carbonyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (**44**)*

[00410] To a solution of carboxylic acid **11** (160 mg, 320 μ mol) in dichloromethane (1 mL) and DMF (0.5 mL) were added Boc-MMAE **42** (100 mg, 120 μ mol), followed by DCC (54 mg, 210 μ mol) and DMAP (32 mg, 210 μ mol) at 0 °C. After 1 h, the reaction was allowed to warm to room temperature and stirred overnight. The mixture was purified by silica gel flash chromatography (0-6% MeOH in DCM) to give semi-pure compound **43**, which was dissolved in 2 mL of ethyl acetate. To this solution were added Pd/C (10 wt. %, 40 mg) and triethylamine (44 μ L, 440 μ mol). The flask was then evacuated and filled with hydrogen gas from a balloon, in three repeating cycles. Reaction mixture was vigorously stirred for 48 h at room temperature with H₂ balloon attached. After the catalyst was removed by filtration through a pad of celite, the filtrate was concentrated under vacuum and purified by silica gel flash chromatography (0-5% MeOH in DCM) to yield compound **44** (120 mg, 95 μ mol, 79% yield) as a yellow solid. LRMS (ESI): m/z 1269.7 $[M+H]^+$, Calcd for $C_{64}H_{96}N_6O_{20}$ m/z 1269.7.

*Preparation of 2S,3R,4S,5S,6S)-2-(5-((S)-2-((S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-methylbutanamido)propanamido)-2-(((1S,2R)-2-((2R,3R)-3-((S)-1-((6S,9S,12S,13R)-12-((S)-sec-butyl)-6,9-diisopropyl-13-methoxy-2,2,5,11-tetramethyl-4,7,10-trioxo-3-oxa-5,8,11-triazapentadecan-15-yl)pyrrolidin-2-yl)-3-methoxy-2-methylpropanamido)-1-phenylpropoxy)carbonyl)phenoxy)-6-(methoxycarbonyl)tetrahydro-2H-pyran-3,4,5-triyl triacetate (**46**)*

[00411] To a mixture of amine **44** (120 mg, 94 μ mol) and Fmoc-Ala-Cl **32** (63 mg, 180 μ mol) in anhydrous DMF (1 mL) were added DIPEA (32 μ L, 180 μ mol) at room temperature. After 30 minutes, reaction mixture was briefly purified by silica gel flash chromatography (0-5% MeOH in DCM). Next, to a solution of compound **45** in CH₃CN (5 mL) were added piperidine (80 μ L).

After 1 hour, reaction mixture was concentrated and reconstituted in CH₃CN (1 mL). To this solution were added Fmoc-Val-OPfp **34** (94 mg, 180 μ mol) and DIPEA (22 μ L, 120 μ mol). After 20 minutes, the reaction was purified by silica gel chromatography (0 to 5% v/v MeOH in DCM) to afford compound **46** (120 mg, 72 μ mol, 77% yield) as a yellow solid. LRMS (ESI): m/z 1661.9 [M+H]⁺, Calcd for C₈₇H₁₂₀N₈O₂₄ m/z 1661.8.

*Preparation of (2S,3S,4S,5R,6S)-6-(5-((S)-2-((S)-2-amino-3-methylbutanamido)propanamido)-2-(((1S,2R)-2-((2R,3R)-3-((S)-1-((6S,9S,12S,13R)-12-((S)-sec-butyl)-6,9-diisopropyl-13-methoxy-2,2,5,11-tetramethyl-4,7,10-trioxo-3-oxa-5,8,11-triazapentadecan-15-oyl)pyrrolidin-2-yl)-3-methoxy-2-methylpropanamido)-1-phenylpropoxy)carbonyl)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (**47**)*

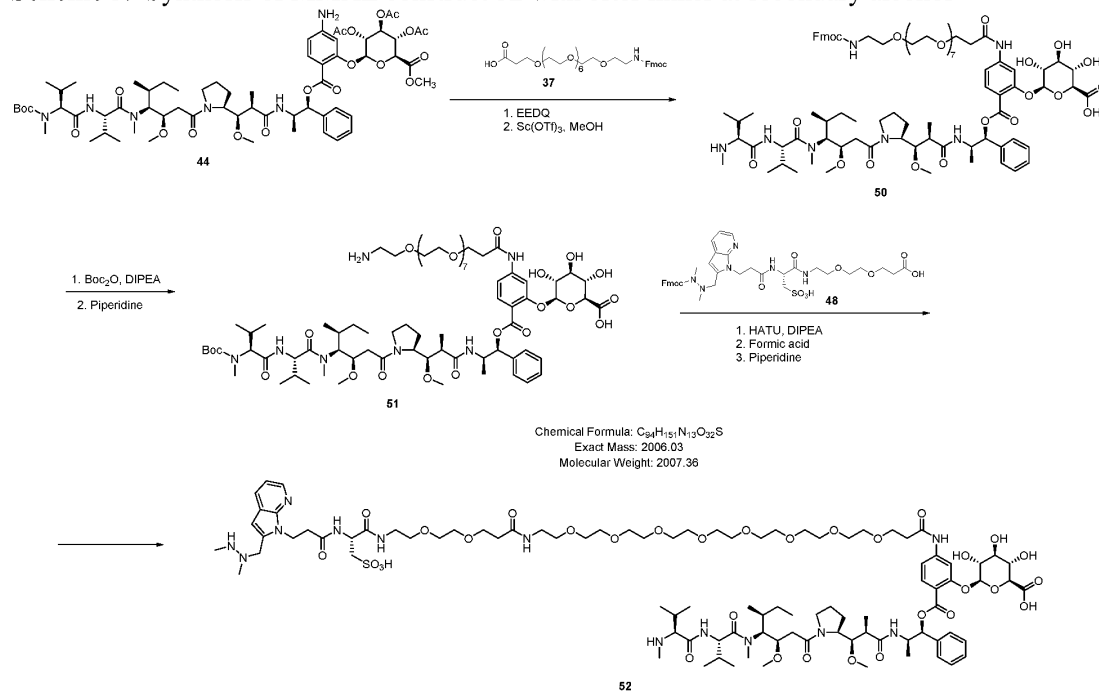
[00412] To a solution of compound **46** (15 mg, 9 μ mol) in a 4:1 (v/v) mixture of MeOH/H₂O (0.3 mL) was added ScOTf₃ (100 mg, 0.20 mmol) at room temperature while stirring. After two days, reaction mixture was concentrated under vacuum, reconstituted in DMF-piperidine mixture (10:1 v/v, 1.1 mL), and stirred for 1 hour at room temperature. The mixture was then purified by reversed-phase chromatography on C18 column (H₂O/CH₃CN with 0.05% TFA, 90:10 to 20:80 v/v). Fractions containing the desired compound were pooled and concentrated under vacuum to yield compound **47** (1.5 mg, 13% yield). LRMS (ESI): m/z 1299.7 [M+H]⁺, Calcd for C₆₅H₁₀₂N₈O₁₉ m/z 1299.7.

*Preparation of (2S,3S,4S,5R,6S)-6-(2-(((1S,2R)-2-((2R,3R)-3-((S)-1-((3R,4S,5S)-4-((S)-N,3-dimethyl-2-((S)-3-methyl-2-(methylamino)butanamido)butanamido)-3-methoxy-5-methylheptanoyl)pyrrolidin-2-yl)-3-methoxy-2-methylpropanamido)-1-phenylpropoxy)carbonyl)-5-((2S,5S,18R)-22-(2-((1,2-dimethylhydrazinyl)methyl)-1H-pyrrolo[2,3-b]pyridin-1-yl)-5-isopropyl-2-methyl-4,7,17,20-tetraoxo-18-(sulfomethyl)-10,13-dioxo-3,6,16,19-tetraazadocosanamido)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (**49**)*

[00413] To a mixture of carboxylic acid **48** (1 mg, 1.3 μ mol) and HATU (0.4 mg, 1.1 μ mol) in DMF (0.5 mL) were added DIPEA (1.0 μ L, 6.1 μ mol). Reaction mixture was stirred for 30 minutes at room temperature, then amine **47** (1.5 mg, 1.2 μ mol) was added to the mixture. After 1 hour, reaction mixture was concentrated and reconstituted in formic acid (1 mL). After 30 minutes, solvents were removed in vacuum. The residue was dissolved in DMF (1 mL) and

treated with piperidine (50 μ L) at room temperature. After stirring for 15 minutes at room temperature, the reaction mixture was directly purified by reversed phase HPLC using C18 column ($\text{H}_2\text{O}/\text{CH}_3\text{CN}$ with 0.05% TFA, 90:10 to 35:65 v/v). Fractions containing the desired compound were pooled and lyophilized to yield compound **49** (0.7 mg, 35% yield). LRMS (ESI): m/z 1753.9 $[\text{M}+\text{H}]^+$, Calcd for $\text{C}_{84}\text{H}_{129}\text{N}_{13}\text{O}_{25}\text{S}$ m/z 1753.9.

Scheme 5. Synthesis of MMAE construct **52** with ester linker at secondary alcohol



Preparation of (2S,3S,4S,5R,6S)-6-(5-(1-(9H-fluoren-9-yl)-3-oxo-2,7,10,13,16,19,22,25,28-nona-4-azahentriacontan-31-amido)-2-(((1S,2R)-2-((2R,3R)-3-((S)-1-((3R,4S,5S)-4-((S)-N,3-dimethyl-2-((S)-3-methyl-2-(methylamino)butanamido)butanamido)-3-methoxy-5-methylheptanoyl)pyrrolidin-2-yl)-3-methoxy-2-methylpropanamido)-1-phenylpropoxy)carbonyl)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (50) [00414] To a mixture of compound **44** (32 mg, 25 μ mol) and carboxylic acid **37** (52 mg, 77 μ mol) in DCM (0.5 mL) and MeOH (50 μ L) were added EEDQ (25 mg, 100 μ mol) at room temperature. The resulting mixture was stirred overnight, concentrated in vacuum, and purified by silica gel chromatography (0-10% MeOH/DCM). The obtained intermediate was dissolved in 300 μ L of MeOH and 75 μ L of water. This solution was treated with scandium(III) triflate (310

mg), stirred at room temperature for 2 days, then directly purified by reversed phase chromatography (C18, 0-80% acetonitrile-water, 0.05% TFA) to afford 7 mg of compound **50** (4 μ mol, 16 % yield) as a colorless solid. LRMS (ESI): m/z 1674.9 [M+H]⁺, Calcd for C₈₆H₁₂₇N₇O₂₆ m/z 1674.9.

Preparation of (2S,3S,4S,5R,6S)-6-(5-(1-amino-3,6,9,12,15,18,21,24-octaoxaheptacosan-27-amido)-2-(((1S,2R)-2-((2R,3R)-3-((S)-1-((6S,9S,12S,13R)-12-((S)-sec-butyl)-6,9-diisopropyl-13-methoxy-2,2,5,11-tetramethyl-4,7,10-trioxo-3-oxa-5,8,11-triazapentadecan-15-oyl)pyrrolidin-2-yl)-3-methoxy-2-methylpropanamido)-1-phenylpropoxy)carbonyl)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (51)

[00415] A solution of compound **50** (7 mg, 4 μ mol) in 1.5 mL of anhydrous acetonitrile was treated with Boc₂O (10 mg, 40 μ mol) and DIPEA (7.3 μ L, 40 μ mol) at room temperature. Reaction mixture was stirred for 4 hours, then directly treated with piperidine (100 μ L). After 30 minutes, reaction mixture was purified by reversed phase chromatography (C18, 0-70% acetonitrile-water/0.05% TFA) to give 3.6 mg of compound **51** as a colorless solid (2 μ mol, 50% yield). LRMS (ESI): m/z 1553.8 [M+H]⁺, Calcd for C₇₆H₁₂₅N₇O₂₆ m/z 1553.9.

Preparation of (2S,3S,4S,5R,6S)-6-(2-(((1S,2R)-2-((2R,3R)-3-((S)-1-((3R,4S,5S)-4-((S)-N,3-dimethyl-2-((S)-3-methyl-2-(methylamino)butanamido)butanamido)-3-methoxy-5-methylheptanoyl)pyrrolidin-2-yl)-3-methoxy-2-methylpropanamido)-1-phenylpropoxy)carbonyl)-5-((R)-44-(2-((1,2-dimethylhydrazinyl)methyl)-1H-pyrrolo[2,3-b]pyridin-1-yl)-29,39,42-trioxo-40-(sulfomethyl)-4,7,10,13,16,19,22,25,32,35-decaoxa-28,38,41-triazatetratetracontanamido)phenoxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid (52)

[00416] A solution of carboxylic acid **48** (3.6 mg, 4.5 μ mol) in 0.3 mL of anhydrous DMF was treated with HATU (1.5 mg, 4 μ mol) and DIPEA (3 μ L) at room temperature. The resulting mixture was stirred for 30 minutes and combined with compound **51** (3.5 mg, 2 μ mol). After stirring for 1 h at room temperature, reaction mixture was concentrated under vacuum and reconstituted in 1 mL of formic acid. After 1 h at room temperature, formic acid was removed in vacuum, the residue was dissolved in 1 mL of DMF and treated with piperidine (100 μ L) at room temperature. After 30 minutes, reaction mixture was purified by reversed phase prep HPLC (0-

70% acetonitrile-water/0.05% TFA). Pure fractions were combined and lyophilized to afford 3.6 mg (1.8 μ mol, 90% yield) of compound **52** as a white solid. LRMS (ESI): m/z 1004.6 [M+2H]²⁺, Calcd for C₉₄H₁₅₁N₁₃O₃₂S m/z 1004.5.

EXAMPLE 2

Preparation of conjugates

[00417] Cytotoxin constructs **21**, **49**, and **52** containing dual-cleavage ester linkers were conjugated to a set of aldehyde-tagged monoclonal antibodies using HIPS ligation (FIG. 5). Analytical characterization of the resulting conjugates is shown in FIGS. 6-21.

EXAMPLE 3

Bioconjugation, Purification, and HPLC Analytics

[00418] Aldehyde-tagged antibodies (15 mg/mL) were conjugated to linker-payloads (8-28 mol. equivalents drug:antibody) for 72 h at 37 °C in a sodium citrate/sodium chloride buffer (pH 5.3-5.5) containing 0.85% DMA. In some cases, to improve linker-payload solubility, 3% sucrose, 0.05% Tween-20 and/or up to a maximum of 10% vol/vol DMA was added. After conjugation, free drug was removed by using multiple rounds of dilution into conjugation buffer and concentration using Amicon 0.5 mL 30 kD MWCO centrifugal filters (Millipore Sigma #UFC5030BK) or Zeba desalting columns (Fisher #PI87766). To determine the DAR of the final product, ADCs were examined by analytical HIC or PLRP. The HIC column (Tosoh #14947) was run with mobile phase A: 1.5 M ammonium sulfate, 25 mM sodium phosphate pH 7.0, and mobile phase B: 25% isopropanol, 18.75 mM sodium phosphate pH 7.0. The PLRP column (Agilent #PL1912-1802) was run with mobile phase A: 0.1% trifluoroacetic acid in H₂O, and mobile phase B: 0.1% trifluoroacetic acid in CH₃CN with the column heated to 80 °C. To determine aggregation, samples were analyzed using analytical size exclusion chromatography (SEC; Tosoh #08541) with a mobile phase of 300 mM NaCl, 25 mM sodium phosphate pH 6.8, 5% isopropanol.

Maleimide conjugation of untagged (wild-type) antibodies

[00419] Antibodies (5 mg/mL) were reduced using 2.5-4 mol. equivalents of TCEP for 90 min at 37° C in in PBS, pH 8.0, 1 mM DTPA. TCEP was removed and the protein was exchanged

into PBS, pH 7.4, 1 mM DTPA. Reduced antibody (3 mg/mL) was conjugated with 10 mol. equiv of CL2A-SN-38 for 60 min on ice. Free drug was removed and final ADC was exchanged into PBS, pH 7.4.

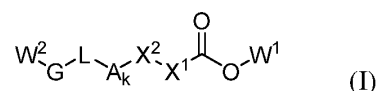
In vitro cytotoxicity assays

[00420] Cell lines were plated on Day -1 in 96-well plates (Corning #3603) at a density of 4 x 10³ cells/well in 100 μ L of growth media and cultured overnight. On Day 0, serial dilution of test samples was performed in growth media at 6x the final concentration and 20 μ L was added to the cells. After incubation at 37 °C with 5% CO₂ for 5 days, viability was measured using Promega CellTiter-Glo® according to the manufacturer's recommendations. GI50 curves were calculated in GraphPad Prism normalized to the payload concentration. Graphs of the cytotoxicity assays (% viability vs. drug concentration (nM)) are shown in FIGS. 22-31.

[00421] While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

WHAT IS CLAIMED IS:

1. A conjugate of formula (I):



wherein:

W^1 is a drug;

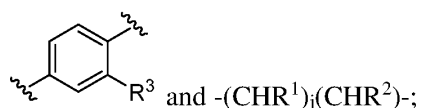
W^2 is a polypeptide;

A is an amino acid residue, where k is 0 or an integer from 1 to 5;

L is a linker;

G is a conjugation moiety;

X^1 is selected from:



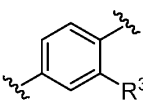
R^1 is selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, substituted heterocyclyl and R^3 , wherein R^1 optionally substituted with R^3 ;

j is 0 or an integer from 1 to 5;

R^2 is R^3 ; or R^2 is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl, wherein R^2 is substituted with R^3 ;

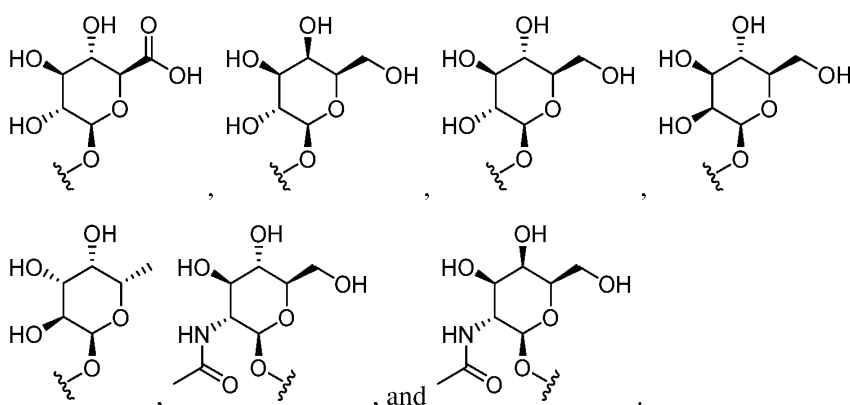
X^2 is $-NH-$ or $-C(O)-$;

each R^3 is independently a glycoside or glycoside derivative.

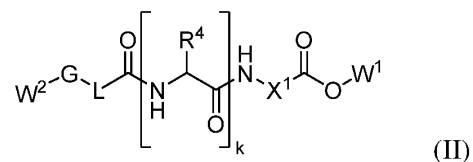
2. The conjugate of Claim 1, wherein X^1 is .

3. The conjugate of Claim 1, wherein X^1 is $-(CHR^1)_j(CHR^2)-$.

4. The conjugate of Claim 3, wherein j is 0.
5. The conjugate of Claim 4, wherein R² is alkyl.
6. The conjugate of Claim 4, wherein R² is aryl.
7. The conjugate of Claim 3, wherein j is 1.
8. The conjugate of Claim 7, wherein R¹ is hydrogen.
9. The conjugate of Claim 7, wherein R¹ is substituted with R³.
10. The conjugate of Claim 1, wherein each R³ is independently selected from:

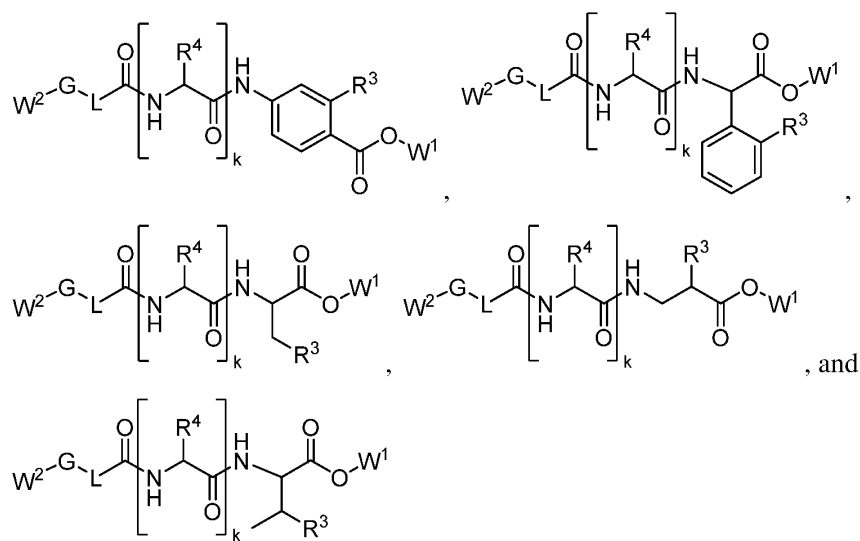


11. The conjugate of Claim 1, wherein the conjugate is of formula (II):

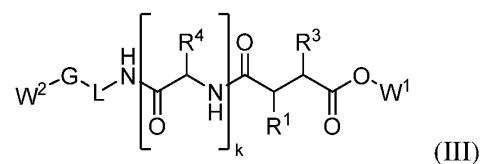


wherein R⁴ is an amino acid side chain.

12. The conjugate of Claim 11, wherein the conjugate is selected from:

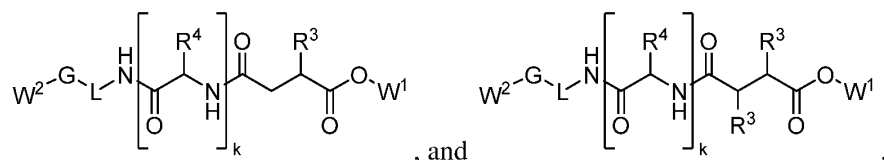


13. The conjugate of Claim 1, wherein the conjugate is of formula (III):



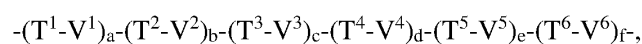
wherein R^4 is an amino acid side chain.

14. The conjugate of Claim 13, wherein the conjugate is selected from:



15. The conjugate of any of Claims 11-14, wherein k is 2.

16. The conjugate of any of Claims 1-15, wherein L comprises:



wherein

a, b, c, d, e and f are each independently 0 or 1;

T^1 , T^2 , T^3 , T^4 , T^5 and T^6 are each independently selected from a covalent bond, (C_1-C_{12}) alkyl, substituted (C_1-C_{12}) alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl, $(EDA)_w$, $(PEG)_n$, $(AA)_p$, $-(CR^{13}OH)_m-$, 4-amino-piperidine (4AP), meta-amino-benzyloxy (MABO), meta-amino-benzyloxycarbonyl (MABC), para-amino-benzyloxy (PABO), para-amino-benzyloxycarbonyl (PABC), para-aminobenzyl (PAB), para-amino-benzylamino (PABA), an acetal, a hydrazine, a disulfide, and an ester, wherein EDA is an ethylene diamine moiety, PEG is a polyethylene glycol, and AA is an amino acid residue or an amino acid analog, wherein each w is an integer from 1 to 20, each n is an integer from 1 to 30, each p is an integer from 1 to 20, and each m is an integer from 1 to 12;

V^1 , V^2 , V^3 , V^4 , V^5 and V^6 are each independently selected from the group consisting of a covalent bond, $-CO-$, $-NR^{15}-$, $-NR^{15}(CH_2)_q-$, $-NR^{15}(C_6H_4)-$, $-CONR^{15}-$, $-NR^{15}CO-$, $-C(O)O-$, $-OC(O)-$, $-O-$, $-S-$, $-S(O)-$, $-SO_2-$, $-SO_2NR^{15}-$, $-NR^{15}SO_2-$ and $-P(O)OH-$, wherein each q is an integer from 1 to 6;

each R^{13} is independently selected from hydrogen, an alkyl, a substituted alkyl, an aryl, and a substituted aryl; and

each R^{15} is independently selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, carboxyl, carboxyl ester, acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl.

17. The conjugate of Claim 16,

wherein:

T^1 is (C_1-C_{12}) alkyl and V^1 is $-CO-$;

T^2 is an amino acid analog and V^2 is $-NH-$;

T^3 is $(PEG)_n$ and V^3 is $-CO-$; and

d to f are each 0; or

wherein:

T^1 is (C_1-C_{12}) alkyl and V^1 is $-CO-$;

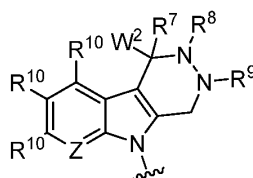
T^2 is an amino acid analog and V^2 is $-NH-$;

T^3 is $(PEG)_n$ and V^3 is $-CONH-$;

T⁴ is (PEG)_n and V⁴ is -CO-;

e and f are each 0.

18. The conjugate of any of Claims 1-17, wherein G is:



wherein:

Z is CR¹⁰ or N,

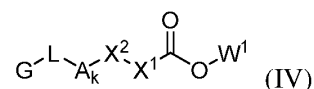
R⁷ is selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl;

R⁸ and R⁹ are each independently selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, amino, substituted amino, carboxyl, carboxyl ester, acyl, acyloxy, acyl amino, amino acyl, alkylamide, substituted alkylamide, sulfonyl, thioalkoxy, substituted thioalkoxy, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl, or R⁸ and R⁹ are optionally cyclically linked to form a 5 or 6-membered heterocyclyl;

each R¹⁰ is independently selected from hydrogen, halogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, amino, substituted amino, carboxyl, carboxyl ester, acyl, acyloxy, acyl amino, amino acyl, alkylamide, substituted alkylamide, sulfonyl, thioalkoxy, substituted thioalkoxy, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl.

19. The conjugate of Claim 1, wherein G is selected from an acetal, a hydrazone, an oxime, a sulfide, a disulfide, a triazole, an ester, and an amide.

20. A compound of formula (IV):



wherein:

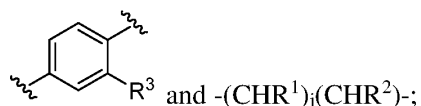
W^1 is a drug;

A is an amino acid residue, where k is 0 or an integer from 1 to 5;

L is a linker;

G is a conjugation moiety;

X^1 is selected from:



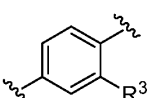
R^1 is selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, substituted heterocyclyl and R^3 , wherein R^1 is optionally substituted with R^3 ;

j is 0 or an integer from 1 to 5;

R^2 is R^3 ; or R^2 is selected from alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl, wherein R^2 is substituted with R^3 ;

X^2 is $-NH-$ or $-C(O)-$;

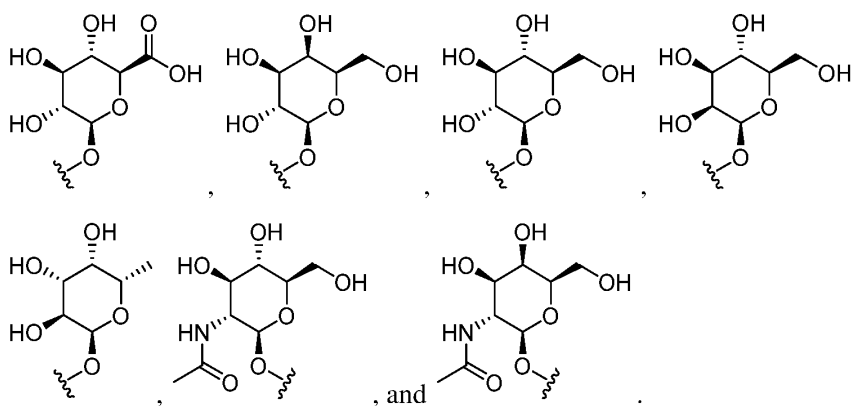
each R^3 is independently a glycoside or glycoside derivative.

21. The compound of Claim 20, wherein X^1 is .

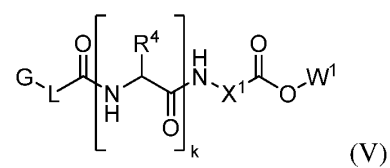
22. The compound of Claim 20, wherein X^1 is $-(CHR^1)_j(CHR^2)-$.

23. The compound of Claim 22, wherein j is 0.

24. The compound of Claim 23, wherein R^2 is alkyl.
25. The compound of Claim 23, wherein R^2 is aryl.
26. The compound of Claim 22, wherein j is 1.
27. The compound of Claim 26, wherein R^1 is hydrogen.
28. The compound of Claim 26, wherein R^1 is substituted with R^3 .
29. The compound of Claim 20, wherein each R^3 is independently selected from:

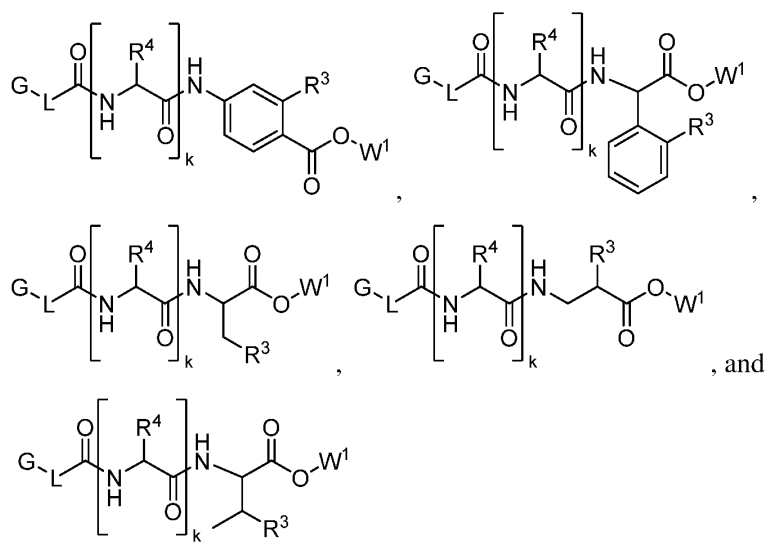


30. The compound of Claim 20, wherein the compound is of formula (V):

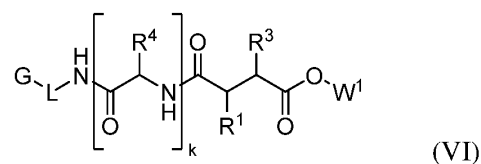


wherein R^4 is an amino acid side chain.

31. The compound of Claim 30, wherein the compound is selected from:

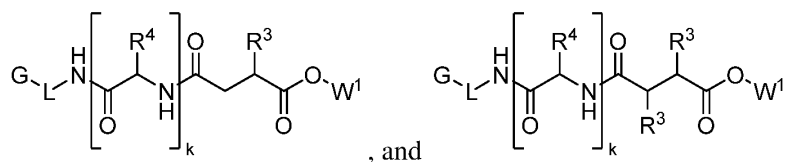


32. The compound of Claim 20, wherein the compound is of formula (VI):



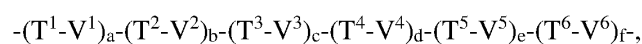
wherein R^4 is an amino acid side chain.

33. The compound of Claim 32, wherein the compound is selected from:



34. The compound of any of Claims 30-33, wherein k is 2.

35. The compound of any of Claims 20-34, wherein L comprises:



wherein

a , b , c , d , e and f are each independently 0 or 1;

T^1 , T^2 , T^3 , T^4 , T^5 and T^6 are each independently selected from a covalent bond, (C_1-C_{12}) alkyl, substituted (C_1-C_{12}) alkyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl, $(EDA)_w$, $(PEG)_n$, $(AA)_p$, $-(CR^{13}OH)_m-$, 4-amino-piperidine (4AP), meta-amino-benzyloxy (MABO), meta-amino-benzyloxycarbonyl (MABC), para-amino-benzyloxy (PABO), para-amino-benzyloxycarbonyl (PABC), para-aminobenzyl (PAB), para-amino-benzylamino (PABA), an acetal, a hydrazine, a disulfide, and an ester, wherein EDA is an ethylene diamine moiety, PEG is a polyethylene glycol, and AA is an amino acid residue or an amino acid analog, wherein each w is an integer from 1 to 20, each n is an integer from 1 to 30, each p is an integer from 1 to 20, and each m is an integer from 1 to 12;

V^1 , V^2 , V^3 , V^4 , V^5 and V^6 are each independently selected from the group consisting of a covalent bond, $-CO-$, $-NR^{15}-$, $-NR^{15}(CH_2)_q-$, $-NR^{15}(C_6H_4)-$, $-CONR^{15}-$, $-NR^{15}CO-$, $-C(O)O-$, $-OC(O)-$, $-O-$, $-S-$, $-S(O)-$, $-SO_2-$, $-SO_2NR^{15}-$, $-NR^{15}SO_2-$ and $-P(O)OH-$, wherein each q is an integer from 1 to 6;

each R^{13} is independently selected from hydrogen, an alkyl, a substituted alkyl, an aryl, and a substituted aryl; and

each R^{15} is independently selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, carboxyl, carboxyl ester, acyl, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl.

36. The compound of Claim 35,
wherein:

T^1 is (C_1-C_{12}) alkyl and V^1 is $-CO-$;

T^2 is an amino acid analog and V^2 is $-NH-$;

T^3 is $(PEG)_n$ and V^3 is $-CO-$; and

d to f are each 0; or

wherein:

T^1 is (C_1-C_{12}) alkyl and V^1 is $-CO-$;

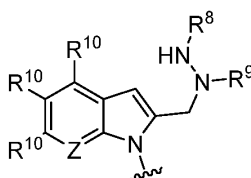
T^2 is an amino acid analog and V^2 is $-NH-$;

T^3 is $(PEG)_n$ and V^3 is $-CONH-$;

T⁴ is (PEG)_n and V⁴ is -CO-;

e and f are each 0.

37. The compound of any of Claims 20-36, wherein G is:



wherein:

Z is CR¹⁰ or N,

R⁸ and R⁹ are each independently selected from hydrogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, amino, substituted amino, carboxyl, carboxyl ester, acyl, acyloxy, acyl amino, amino acyl, alkylamide, substituted alkylamide, sulfonyl, thioalkoxy, substituted thioalkoxy, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl, or R⁸ and R⁹ are optionally cyclically linked to form a 5 or 6-membered heterocyclyl;

each R¹⁰ is independently selected from hydrogen, halogen, alkyl, substituted alkyl, alkenyl, substituted alkenyl, alkynyl, substituted alkynyl, alkoxy, substituted alkoxy, amino, substituted amino, carboxyl, carboxyl ester, acyl, acyloxy, acyl amino, amino acyl, alkylamide, substituted alkylamide, sulfonyl, thioalkoxy, substituted thioalkoxy, aryl, substituted aryl, heteroaryl, substituted heteroaryl, cycloalkyl, substituted cycloalkyl, heterocyclyl, and substituted heterocyclyl.

38. The compound of Claim 20, wherein G is selected from an acetal, an NHS-ester, a PFP-ester, a TFP-ester, an isocyanate, an isothiocyanate, an acyl halide, a maleimide, an iodoacetamide, a phosphine, an alkyne, an azide, a hydrazine, an alkoxyamine, a disulfide, an ester, and an amide.

39. A pharmaceutical composition comprising:
a conjugate of any one of Claims 1 to 19; and

a pharmaceutically-acceptable excipient.

40. A method comprising:
administering to a subject an effective amount of a conjugate of any one of Claims 1
to 19.