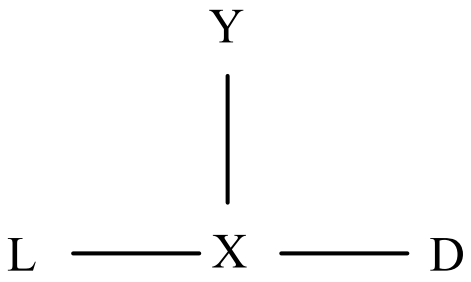
WHAT IS CLAIMED IS:

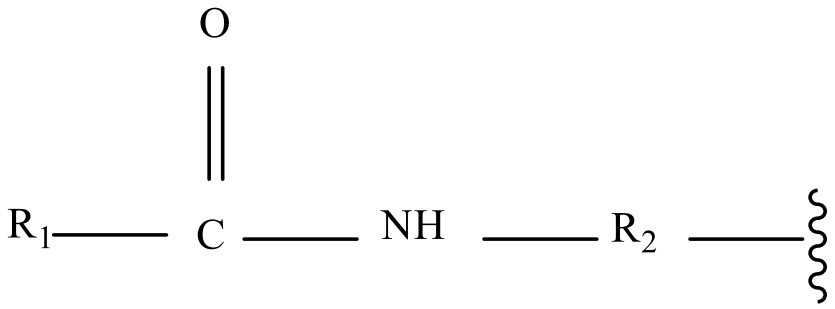
1. A conjugate of Formula I,



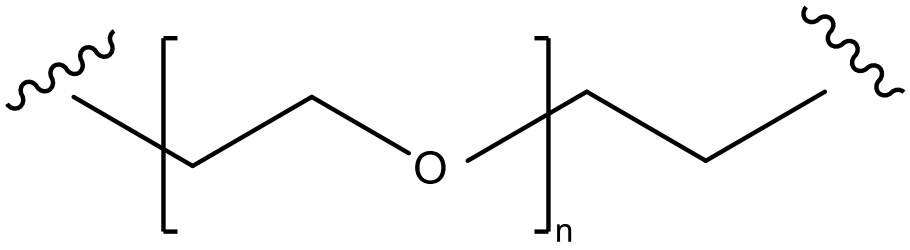
Formula I

wherein:

L is a linker represented by:

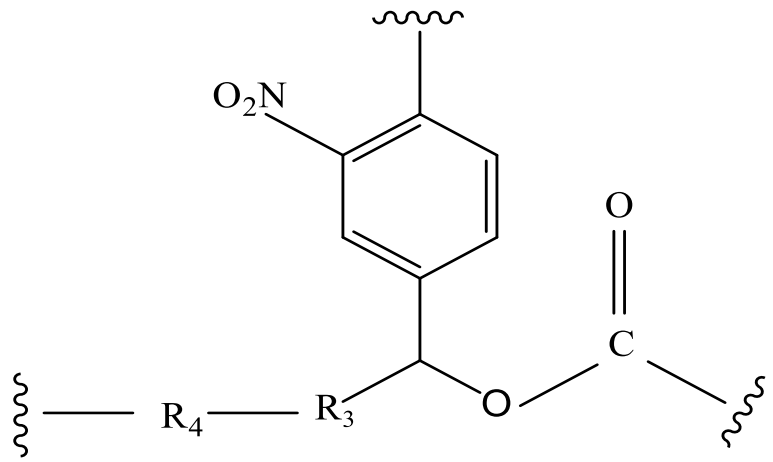


where R_1 is a nucleophilic functional group capable of coupling to an antibody, said nucleophilic functional group being selected from the group consisting of a C_1-C_{10} alkyl halide, a C_1-C_{10} alkyl alcohol, a phenol, a C_1-C_{10} alkyl thiol, and a C_1-C_{10} alkyl azide, and R_2 is a substituted or unsubstituted C_1-C_{20} alkylene, or a substituted or unsubstituted $(PEG)_n$ moiety represented by



where n is 1 to 20;

X is a substituted nitrophenyl represented by



where R_3 is a C_1-C_{20} alkylene, and R_4 is a triazole ring having a C=C double bond in the ring;

Y is a glycoside or glycoside derivative; and

D is a drug; and

Y is bonded to X at the site of a wavy line covalent attachment on the nitrophenyl ring,

D is bonded to X at the site of a wavy line covalent attachment on the carbonyl group, and

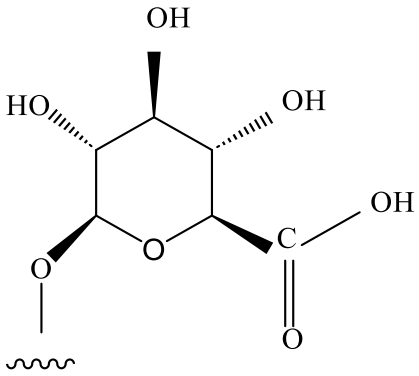
L is bonded to X via bonding of a wavy line covalent attachment between R_2 and R_4.

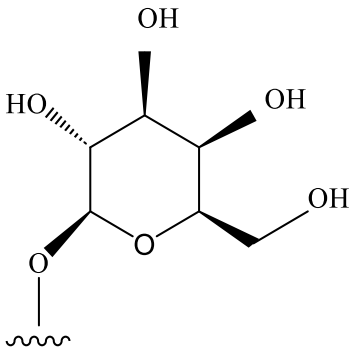
2. The conjugate of claim 1, wherein R_1 is a C_1-C_6 alkyl halide with a halide selected from the group consisting of Br, I, Cl, and F, a C_1-C_2 alkyl alcohol, a 4-phenol, or a C_1-C_2 alkyl thiol.

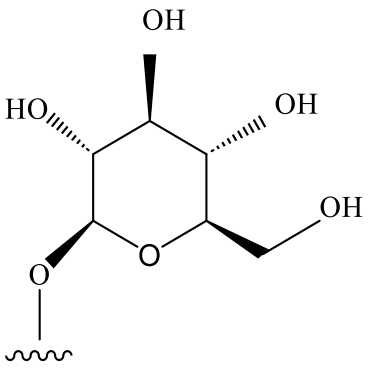
3. The conjugate of any one of claims 1 or 2, wherein R_2 is an unsubstituted C_2-C_5 alkylene, or an unsubstituted $(PEG)_n$ where n is 8, and R_1 is $-CH_2$-Br.

4. The conjugate of any one of claims 1 to 3, wherein R_3 is $-CH_2$-, and the glycoside or glycoside derivative Y is selected from the following structures:

glucuronide galactoside glucoside



,


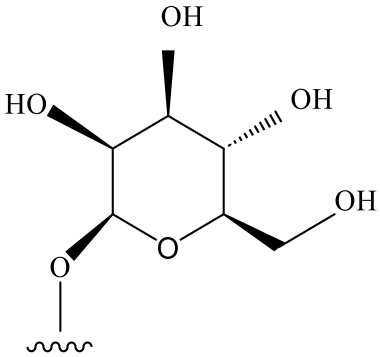
,


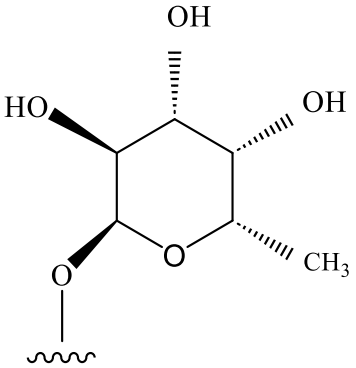
,

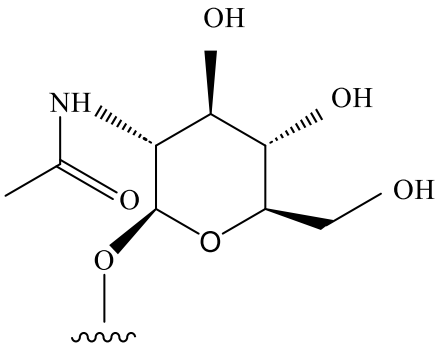
mannoside

fucoside

O-GlcNac

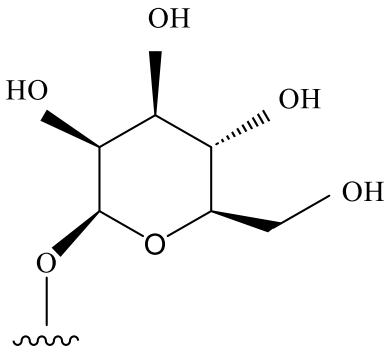


,


,


and

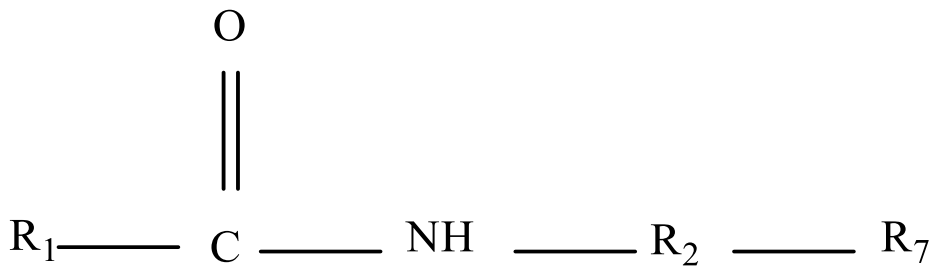
O-GalNAc



.

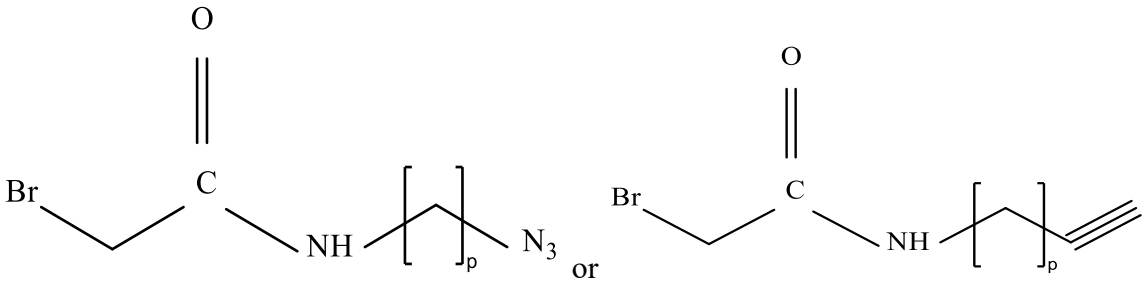
5. The conjugate of any one of claims 1 to 4, wherein the glycoside or glycoside derivative Y is a glucuronide.

6. The conjugate of any one of claims 1 to 5, wherein the triazole ring is formed with a linker-intermediate (L^{int}) having a formula represented by:



where R_1, R_2 and n are as defined in claim 1; and

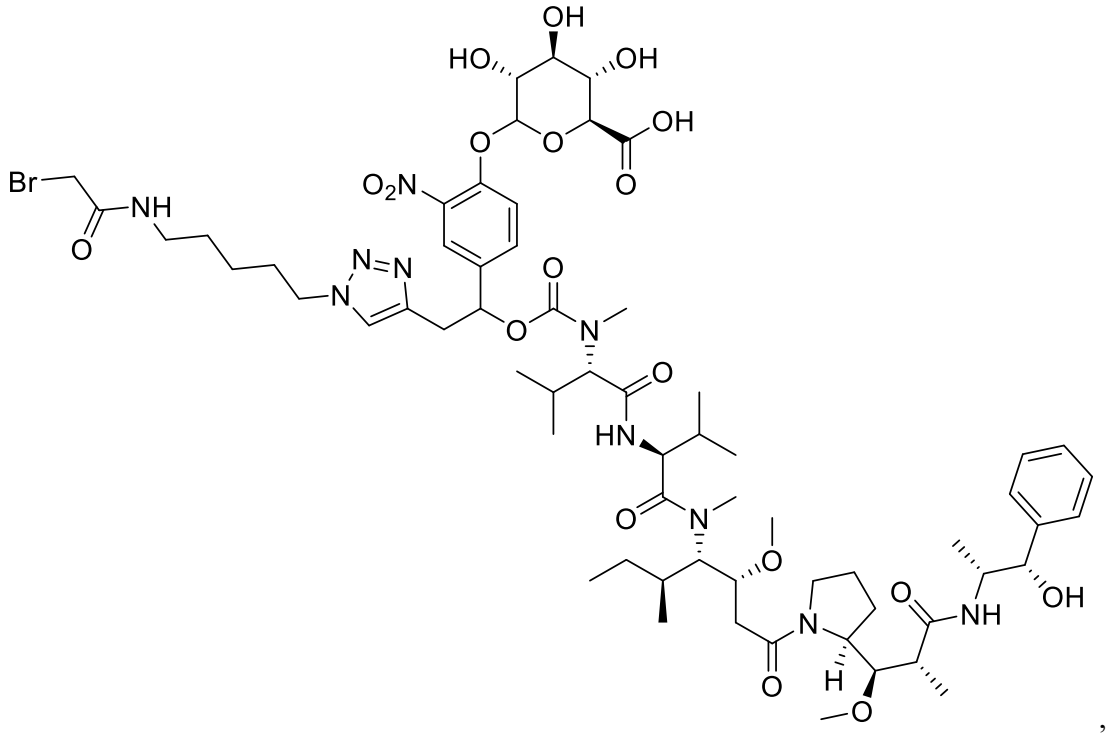
R_7 is an azide group or an acetylene group, wherein the nitrogen atoms of the azide group or the carbons of the acetylene group become ring members in the triazole ring, shown below, bonded to L:



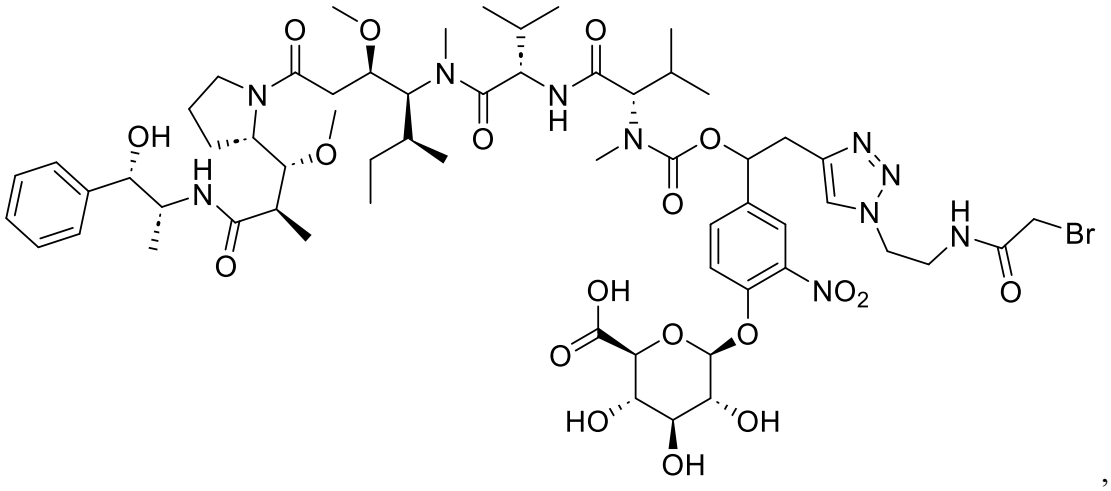
azide group acetylene group,

where p is 2 to 5.

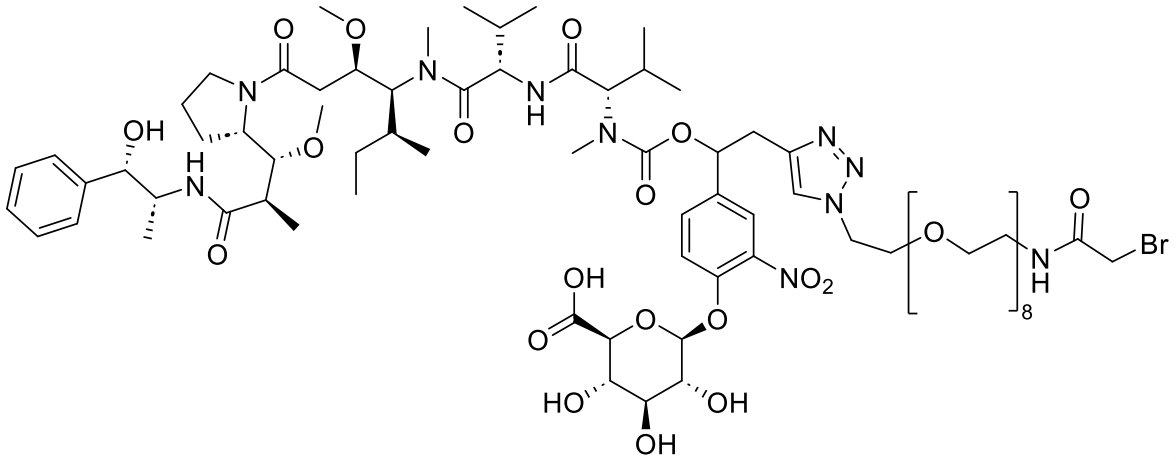
7. The conjugate of any one of claims 1 to 6, wherein the conjugate has a structure represented by SPC07:



or a structure represented by SPC19

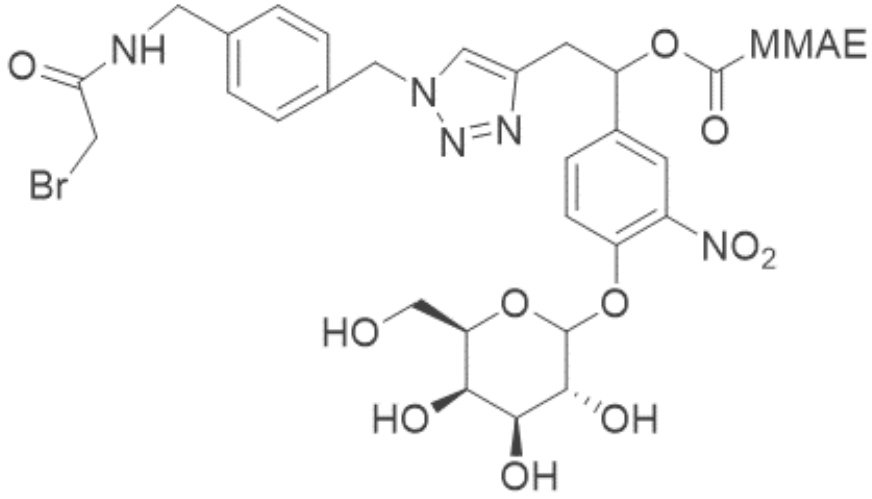


or a structure represented by SPC17



,

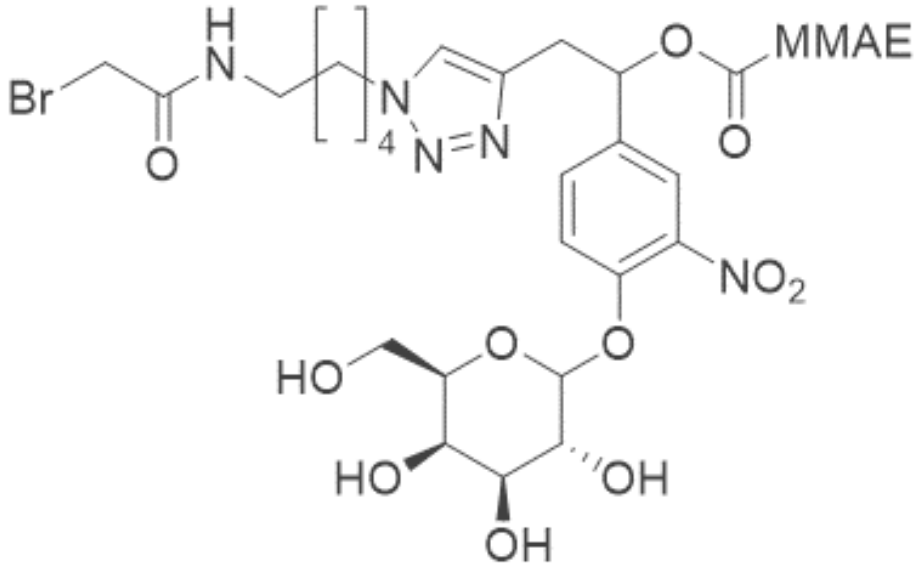
or a structure represented by SPC02



,

wherein MMAE is monomethyl auristatin E,

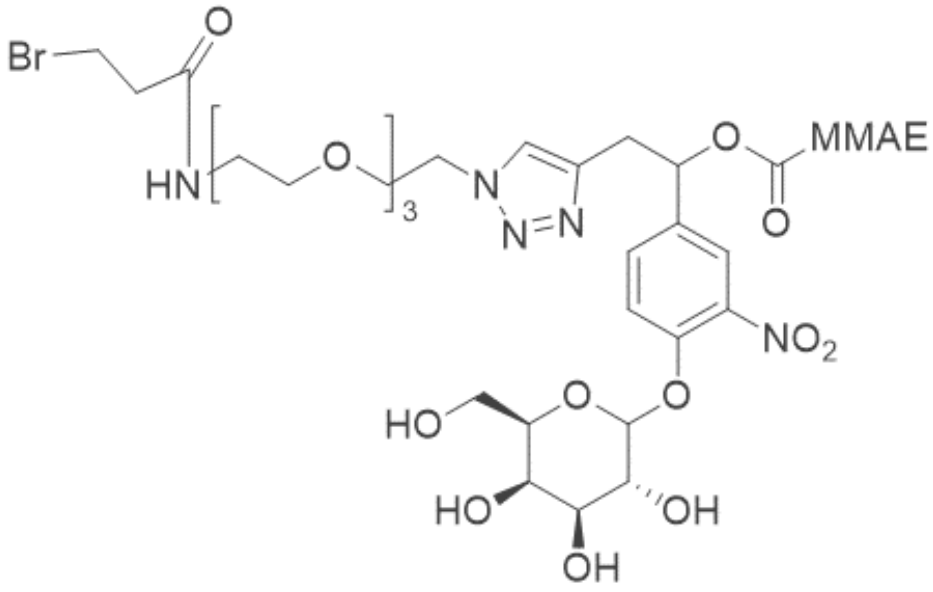
or a structure represented by SPC04



,

wherein MMAE is monomethyl auristatin E,

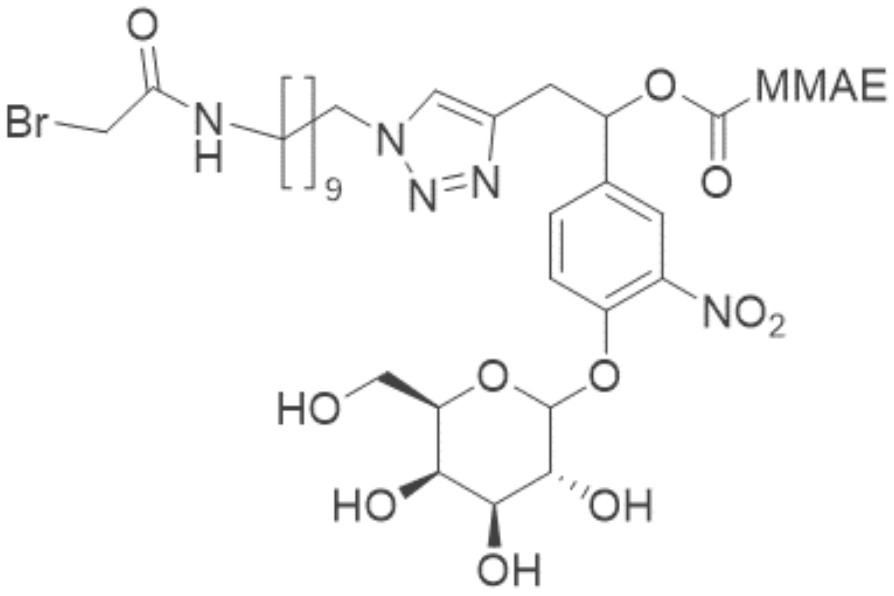
or a structure represented by SPC05



,

wherein MMAE is monomethyl auristatin E,

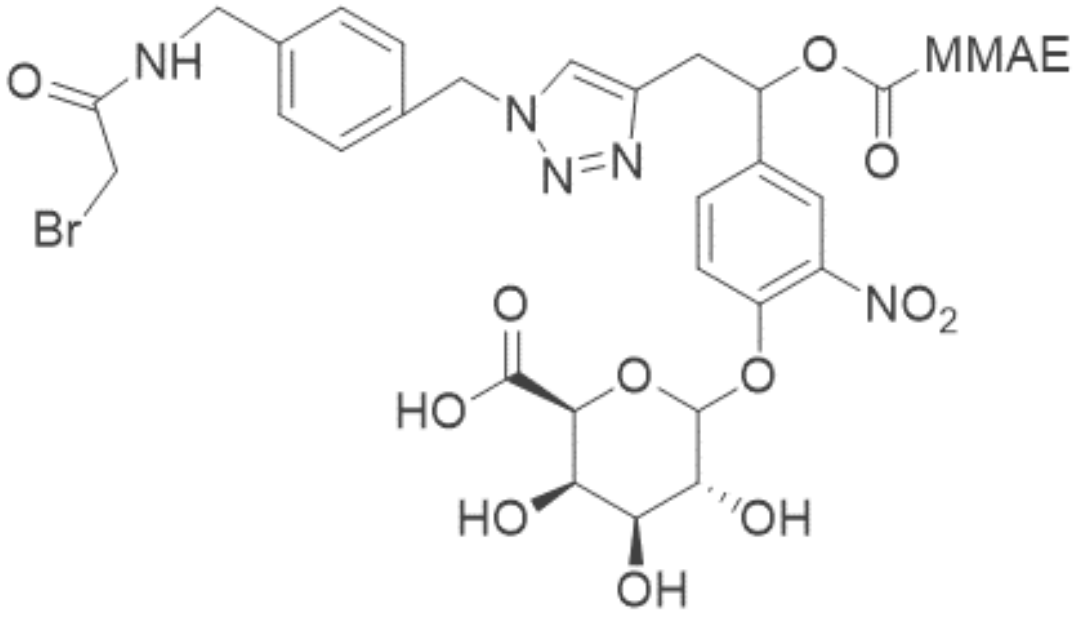
or a structure represented by SPC06



,

wherein MMAE is monomethyl auristatin E,

or a structure represented by SPC08



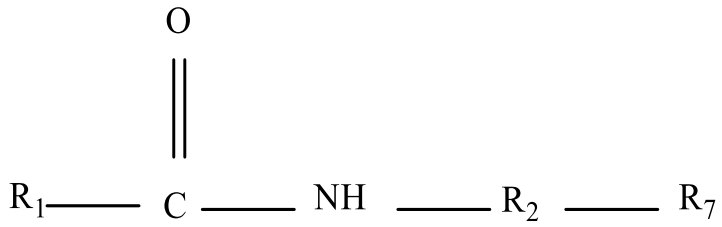
,

wherein MMAE is monomethyl auristatin E.

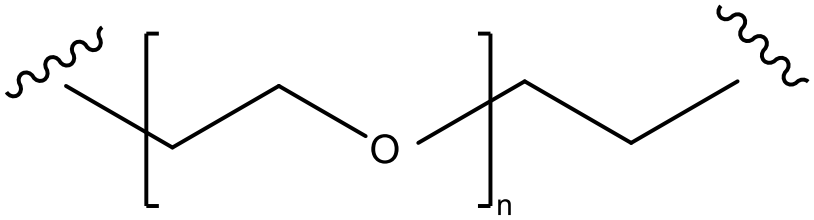
8. The conjugate of any one of claims 1 to 7, wherein Y is not a galactoside when R_2 is a substituted or unsubstituted $(PEG)_n$.

9. The conjugate of any one of claims 1 to 8, wherein the drug D is selected from the group consisting of monomethyl auristatin E (MMAE) and monomethyl auristatin F (MMAF), a dolastatin, a maytansinoid, a calicheamicin, a pyrrolobenzodiazepine, an anthracycline, a ribonuclease, and a DNA endonuclease.

10. A linker–intermediate (L^{int}) having a formula represented by



where R_1 is a nucleophilic functional group capable of coupling to an antibody selected from the group consisting of a C_1-C_{10} alkyl halide, a C_1-C_{10} alkyl alcohol, a phenol, a C_1-C_{10} alkyl thiol, and a C_1-C_{10} alkyl azide,
R_2 is a substituted or unsubstituted C_1-C_{20} alkylene, or a substituted or unsubstituted $(PEG)_n$ moiety represented by:

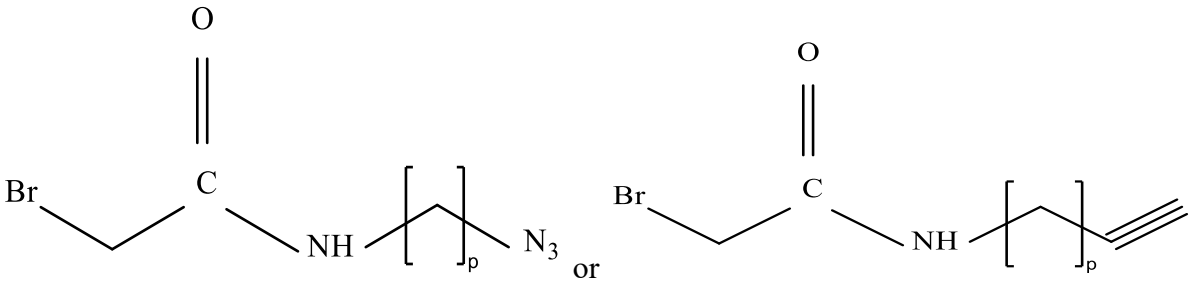


where n is 1 to 20, and
R_7 is an azide group.

11. The linker–intermediate (L^{int}) of claim 10, wherein R_2 is an unsubstituted C_2-C_5 alkylene.

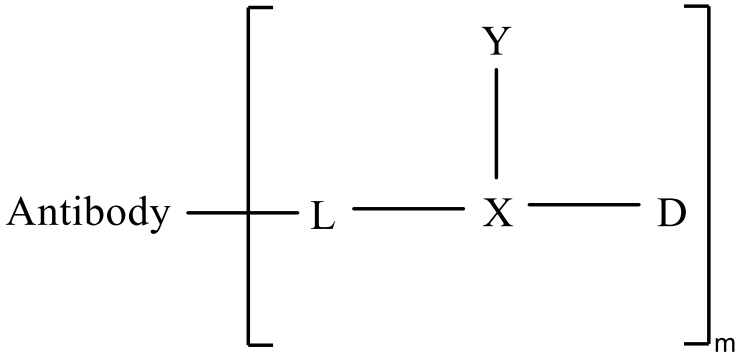
12. The linker–intermediate (L^{int}) of any one of claims 10 or 11, wherein R_2 is an unsubstituted $(PEG)_n$ where n is 8.

13. The linker intermediate (L^{int}) of claim 12, wherein the linker has a structure represented by



where p is 2 to 5.

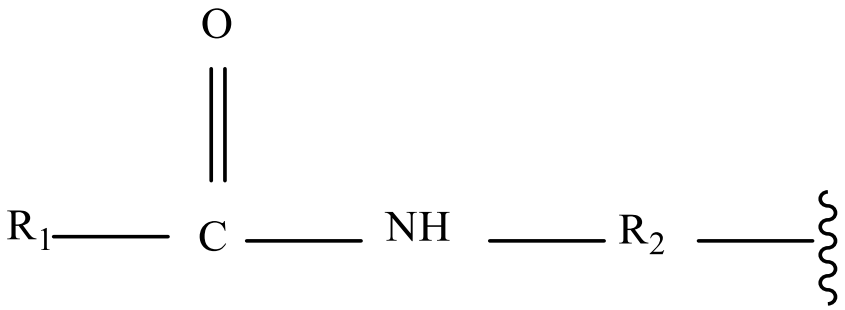
14. An immunoconjugate of Formula II,



Formula II

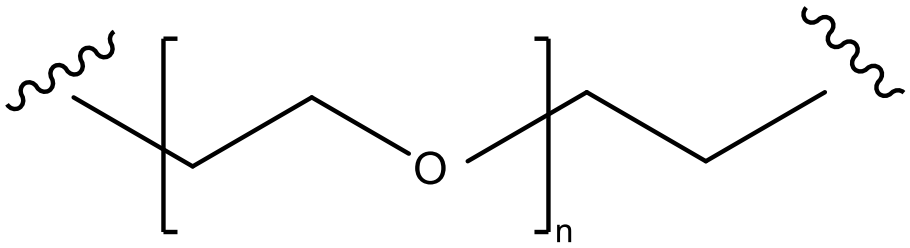
wherein:

L is represented by:



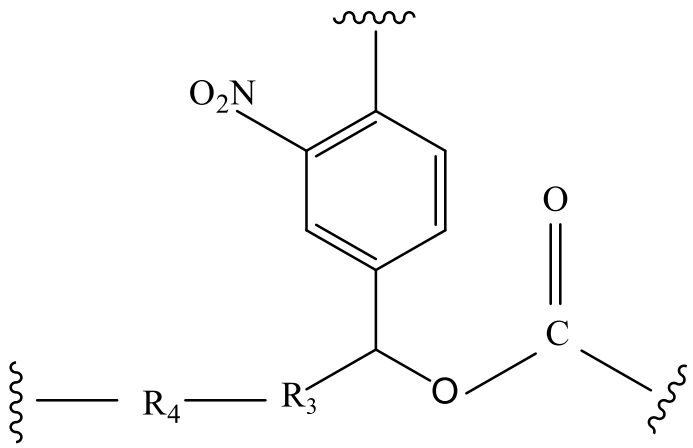
where R_1 is nucleophilic functional group capable of coupling to an antibody selected from the group consisting of a C_1-C_{10} alkyl halide, a C_1-C_{10} alkyl alcohol, a phenol, a C_1-C_{10} alkyl thiol, and a C_1-C_{10} alkyl azide,

R_2 is a substituted or unsubstituted C_1-C_{20} alkylene, or a substituted or unsubstituted $(PEG)_n$ moiety represented by



where n is 1 to 20, and

X is a substituted nitrophenyl represented by



where R_3 is a C_1-C_{20} alkylene; R_4 is a triazole ring having a C=C double bond in the ring;

Y is a glycoside or glycoside derivative;

D is a drug selected from the group consisting of an auristatin, a dolastatin, a maytansinoid, a calicheamicin, a pyrrolobenzodiazepine, an anthracycline, a ribonuclease, and a DNA endonuclease;

the wavy lines indicate a site of covalent attachment; and

m is 1 to 10,

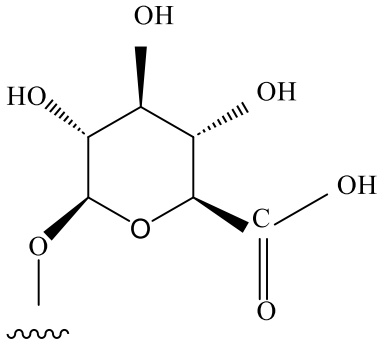
wherein the term “antibody” in Formula II represents an antibody or antibody fragment.

15. The immunoconjugate of claim 14, wherein

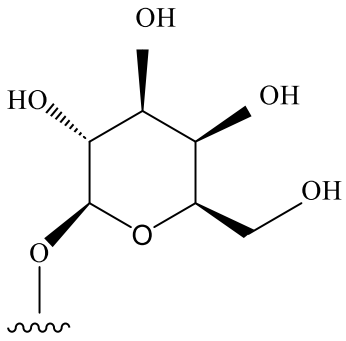
R_1 is C_1-C_6 alkyl halide with a halide selected from the group consisting of Br, I, Cl, and F, a C_1-C_2 alkyl alcohol, a 4-phenol, or a C_1-C_2 alkyl thiol,

R_2 is an unsubstituted C_2-C_5 alkylene or an unsubstituted $(PEG)_n$ where n is 8, wherein the glycoside or glycoside derivative Y is selected from the following structures:

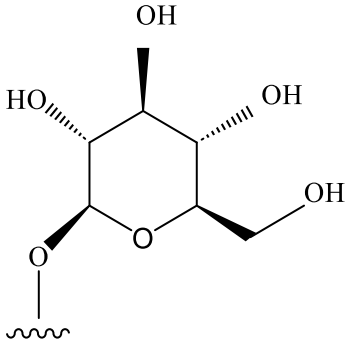
glucuronide



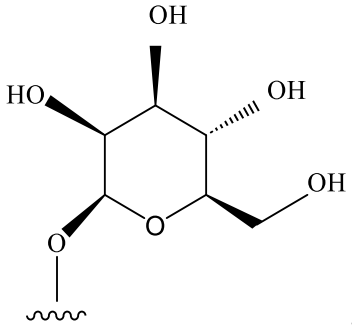
galactoside



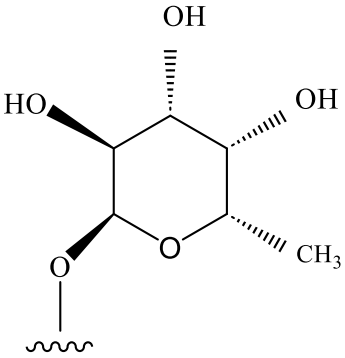
glucoside



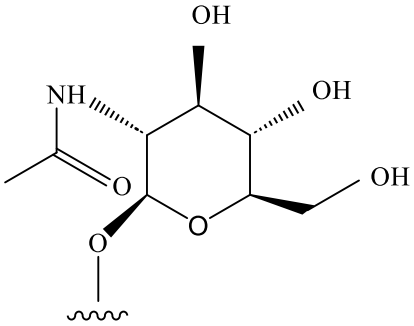
mannoside



fucoside

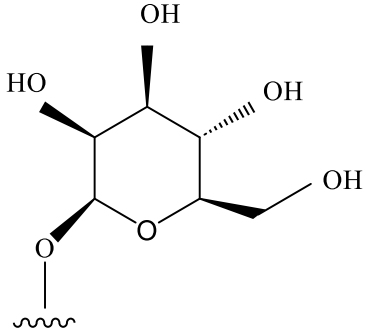


O-GlcNac



and

O-GalNAc



.

16. The immunoconjugate of claims 14 or 15, wherein the antibody is an antibody or antibody fragment that specifically binds to Nectin-4 has a higher binding affinity to Nectin-4 protein at a pH in a tumor microenvironment in comparison with a pH that occurs in a non-tumor microenvironment, and comprises a heavy chain variable region including three complementarity determining regions (CDRs) having sequences H1, H2, and H3, wherein:

the H1 sequence is GFTFSSYNX_1N (SEQ ID NO: 1);

the H2 sequence is ISSSSSTIYYADSVKG (SEQ ID NO: 2); and

the H3 sequence is AYYYGX$_2$DX$_3$ (SEQ ID NO: 3);

wherein X$_1$ is M or D; X$_2$ is M or D; X$_3$ is V or K, with the proviso that the heavy and light chain variable regions cannot be SEQ ID NOS: 18 and 31 or SEQ ID NOS: 18 and 56, in combination and a light chain variable region including three CDRs having sequences L1, L2, and L3, wherein:

the L1 sequence is X$_4$ASQGISGWX$_5$A (SEQ ID NO: 4);

the L2 sequence is AASTLQS (SEQ ID NO: 5); and

the L3 sequence is QQANSX$_6$PX$_7$T (SEQ ID NO: 6),

wherein X$_4$ is R or H; X$_5$ is L or E; X$_6$ is F or E; and X$_7$ is P or D, and with the proviso that X$_1$, X$_2$, X$_3$, X$_4$, X$_5$, X$_6$ and X$_7$ cannot simultaneously be, M, M, V, R, L, F and P, respectively.

17. The immunoconjugate of claim 16, wherein the antibody or antibody fragment further comprises six anti-CD3 complementarity determining regions L4, L5, L6, L7, L8, and L9, wherein:

the L4 sequence is GFTFNTYAMN (SEQ ID NO: 44),

the L5 sequence is RIRSKYNNYATYYADSVKD (SEQ ID NO: 45),

the L6 sequence is HX$_{11}$NFX$_{12}$NSX$_{13}$VSWFX$_{14}$Y (SEQ ID NO: 46),

the L7 sequence is RSSTGAVTTSNYX$_{15}$N (SEQ ID NO: 47),

the L8 sequence is GTNKRAP (SEQ ID NO: 48), and

the L9 sequence is ALWYSNLWV (SEQ ID NO: 49),

wherein X$_{11}$ is G or S, X$_{12}$ is G or P, X$_{13}$ is Y or K, X$_{14}$ is A or Q and X$_{15}$ is A or D.

18. A pharmaceutical composition comprising:

the immunoconjugate of any one of claims 14 to 17; and

a pharmaceutically acceptable carrier.

19. The pharmaceutical composition of claim 18, further comprising an immune checkpoint inhibitor molecule.

20. Use of the immunoconjugate of any one of claims 14 to 17, or the pharmaceutical composition of any one of claims 18 or 19, in the manufacture of a medicament for the treatment of cancer.

21. The immunoconjugate of any one of claims 14 to 17, or the pharmaceutical composition of any one of claims 18 or 19, for use in therapy.