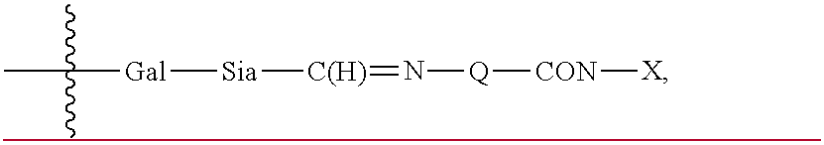
CLAIMS

~~We claim:~~

1. ~~A~~ An antibody, or antigen-binding ~~polypeptide~~fragment thereof, comprising at least one modified glycan, wherein the glycan comprises at least one moiety of Formula (IV):

~~Gal—Sia—C(H)=N—Q—CON—X~~ ~~Formula (IV),~~

Formula (IV)



wherein:

A) Q is NH or O;

B) CON is a connector moiety;

C) X is a targeting moiety~~;~~ comprising a carbohydrate or a glycopeptide that binds to a carbohydrate receptor on a cell or a glycopeptide receptor on a cell;

D) Gal is a ~~component derived from~~ galactose moiety; and

E) Sia is a ~~component derived from~~ sialic acid~~, wherein Sia is present or absent, and wherein the targeting moiety binds to a cell.~~ moiety.

2. The antibody, or antigen-binding ~~polypeptide~~fragment thereof, of claim 1, wherein ~~one or more of the following conditions are met:~~

~~a)~~ the cell is a mammalian cell~~;~~.

~~b) the~~

3. The antibody, or antigen-binding ~~polypeptide is internalized by the cell;~~

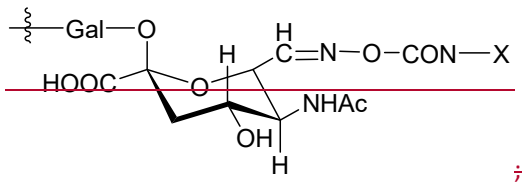
~~c) the targeting moiety binds to a mannose 6-phosphate receptor on the cell;~~

~~d) the targeting moiety binds to a sialic acid-binding immunoglobulin-type lectin (Siglec) on the cell;~~

~~e) the targeting moiety binds to a C-type lectin receptor, a galectin, or a L-type lectin receptor;~~

~~f) Q is O;~~

~~g) the glycan comprises at least one moiety of the following structural formula:~~



~~;~~

~~h) the targeting moiety is a glycopeptide capable of binding an asialoglycoprotein receptor (ASGPR) on a cell;~~

~~i) the binding protein comprises an N-glycan glycoform selected from the group consisting of: a G0 glycoform, a G1 glycoform, and a G2 glycoform;~~

~~j) the N-glycan glycoform is selected from the group consisting of: a G1S1 glycoform, a G2S1 glycoform, and a G2S2 glycoform; and~~

~~k) the N-glycan glycoform is selected from the group consisting of: a G1F glycoform, a G2F glycoform, a G1S1F glycoform, a G2S1F glycoform, and a G2S2F glycoform.~~

~~3. The binding polypeptide of~~ fragment thereof, of claim 2, wherein ~~one or more of the following conditions are met:~~

~~a)~~ the mammalian cell is selected from an immune cell, a liver cell, a tumor cell, a vascular cell, an epithelial cell, or a mesenchymal cell~~;~~.

~~b) the amount of the binding polypeptide internalized by the cell is greater than the amount of a reference binding polypeptide lacking a targeting moiety internalized by the cell;~~

~~c)~~

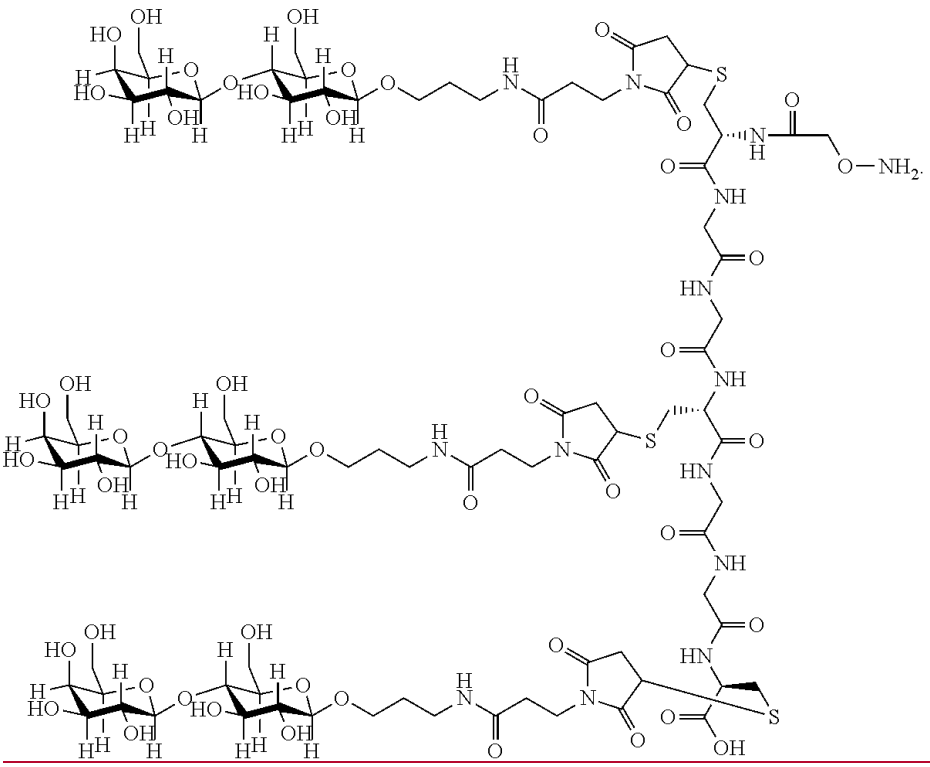
4. The antibody, or antigen-binding fragment thereof, of claim 2, wherein the mammalian cell is selected from a B cell, a T cell, a dendritic cell, a natural killer (NK) cell, a macrophage, a neutrophil, a hepatocyte, a liver sinusoidal endothelial cell, or a hepatoma cell.

5. The antibody, or antigen-binding fragment thereof, of claim 1, wherein the targeting moiety comprises an α2,3-sialyllactose moiety or an α2,6-sialyllactose moiety.

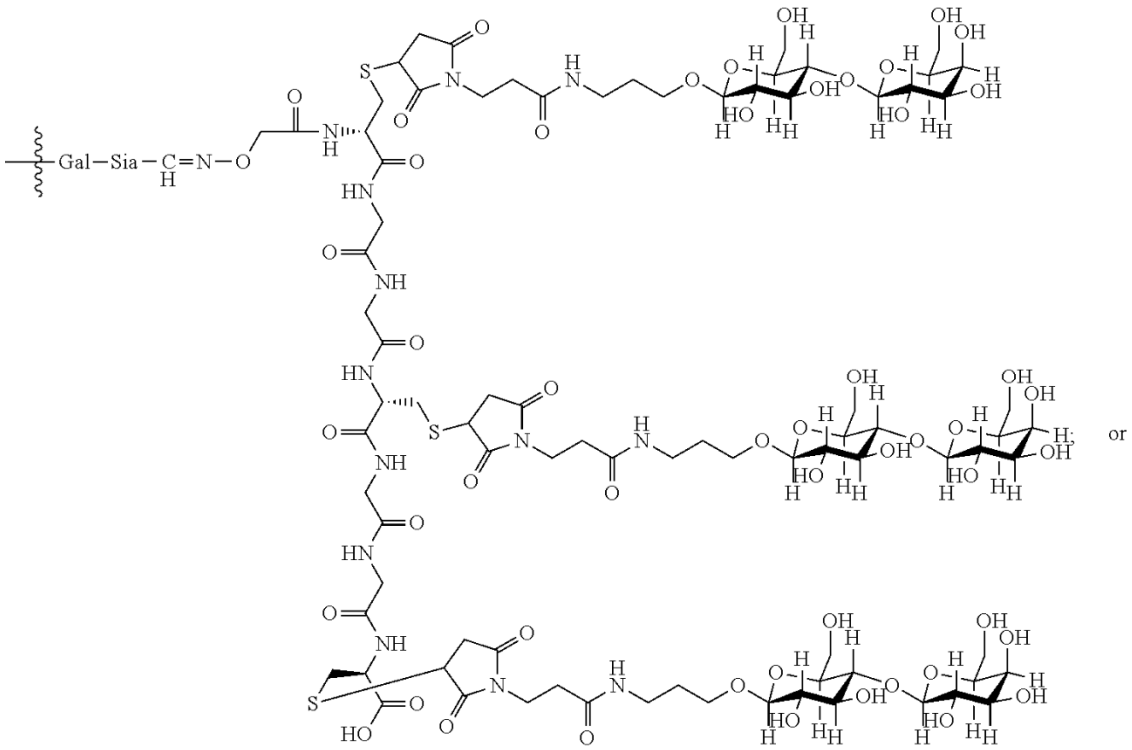
6. The antibody, or antigen-binding fragment thereof, of claim 3, wherein the targeting moiety is a tri-galactosylated glycopeptide or lactose$_3$-Cys$_3$Gly$_4$.

7. The antibody, or antigen-binding fragment thereof, of claim 6, wherein the lactose$_3$-Cys$_3$Gly$_4$ moiety is represented by Formula V:

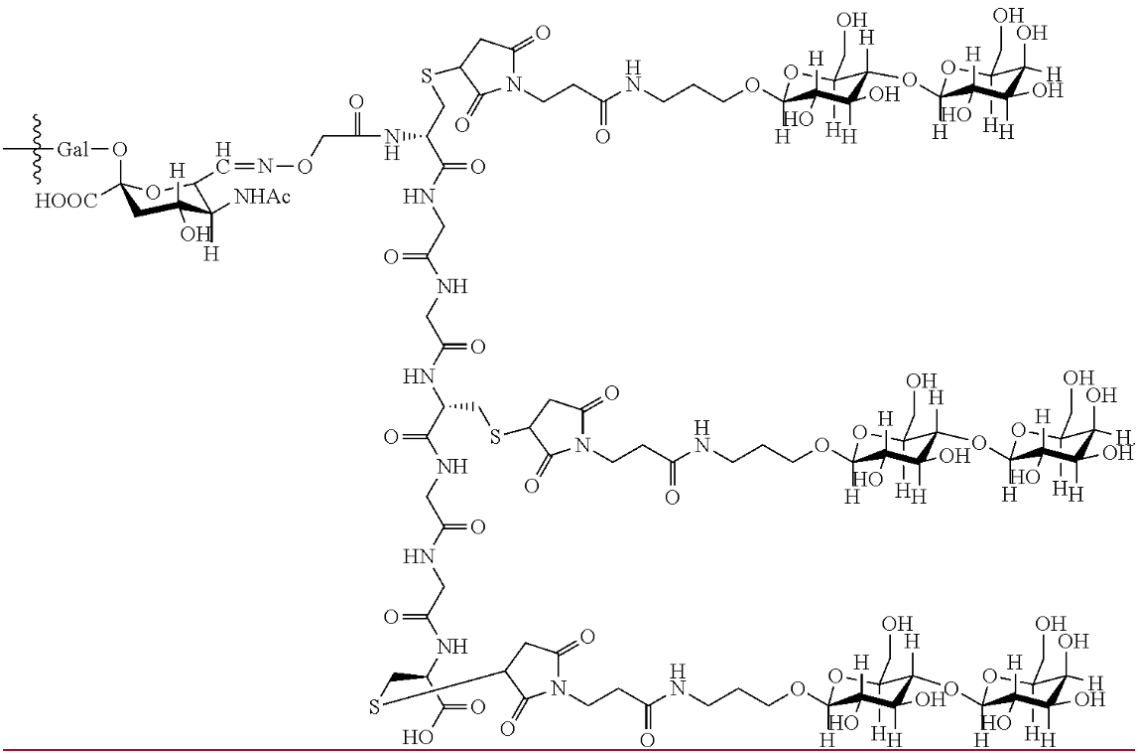
[Formula V]



8. The antibody, or antigen-binding fragment thereof, of claim 7, comprising at least one moiety selected from the following structural formulae:



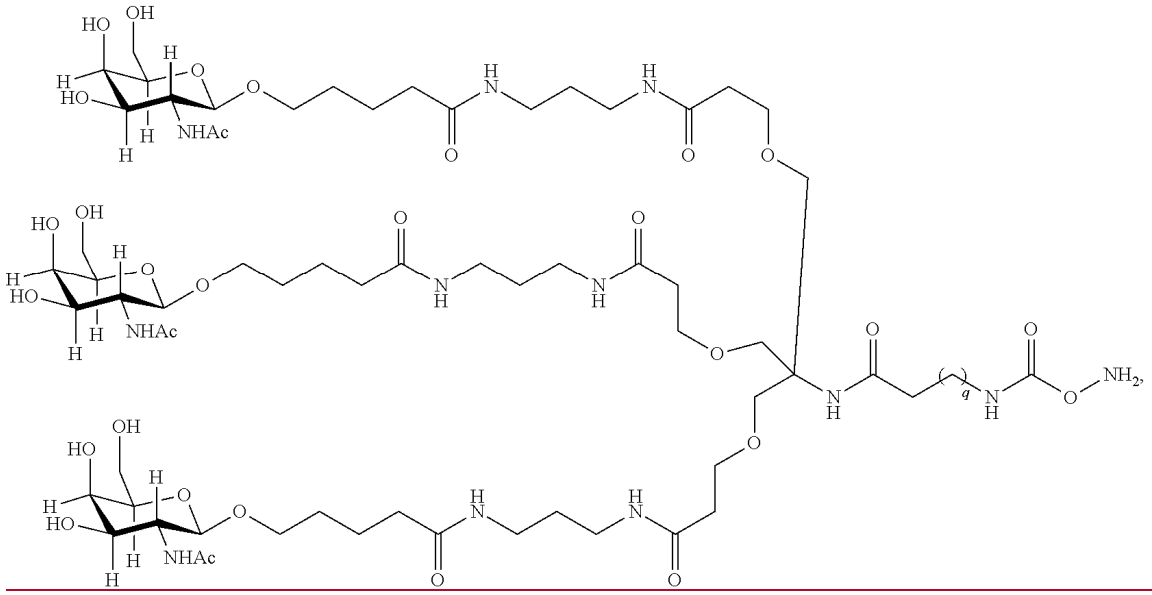
or



9. The antibody, or antigen-binding fragment thereof, of claim 1, wherein the targeting moiety is a trivalent GalNAc glycan moiety.

10. The antibody, or antigen-binding fragment thereof, of claim 9, wherein the trivalent GalNAc glycan moiety is represented by Formula VIII:

[Formula VIII]



wherein q is an integer between 1 and 29 inclusive.

11. The antibody, or antigen-binding fragment thereof, of claim 1, wherein the antibody, or antigen-binding fragment thereof, comprises an Fc domain.

12. The antibody, or antigen-binding fragment thereof, of claim 1, wherein the modified glycan is N-linked to the antibody, or antigen-binding fragment thereof, via an asparagine residue at amino acid position 297 of the Fc domain, according to EU numbering.

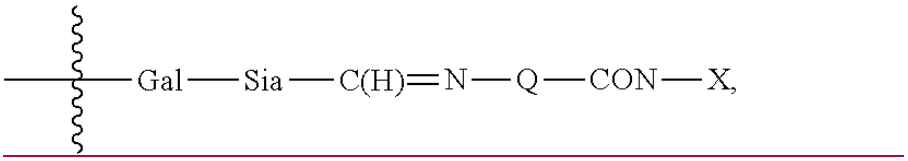
13. A composition comprising the antibody, or antigen-binding fragment thereof, of claim 1 and a pharmaceutically acceptable carrier or excipient.

14. The composition of claim 13, wherein the ratio of targeting moiety to antibody, or antigen-binding fragment thereof, is equal to or more than about 4.

15. The composition of claim 13, wherein the ratio of targeting moiety to antibody, or antigen-binding fragment thereof, is at least about 2.

16. An antibody, or antigen-binding fragment thereof, comprising at least one modified glycan comprising at least one moiety of Formula (IV):

Formula (IV)



wherein:

A) Q is NH or O;

B) CON is a connector moiety;

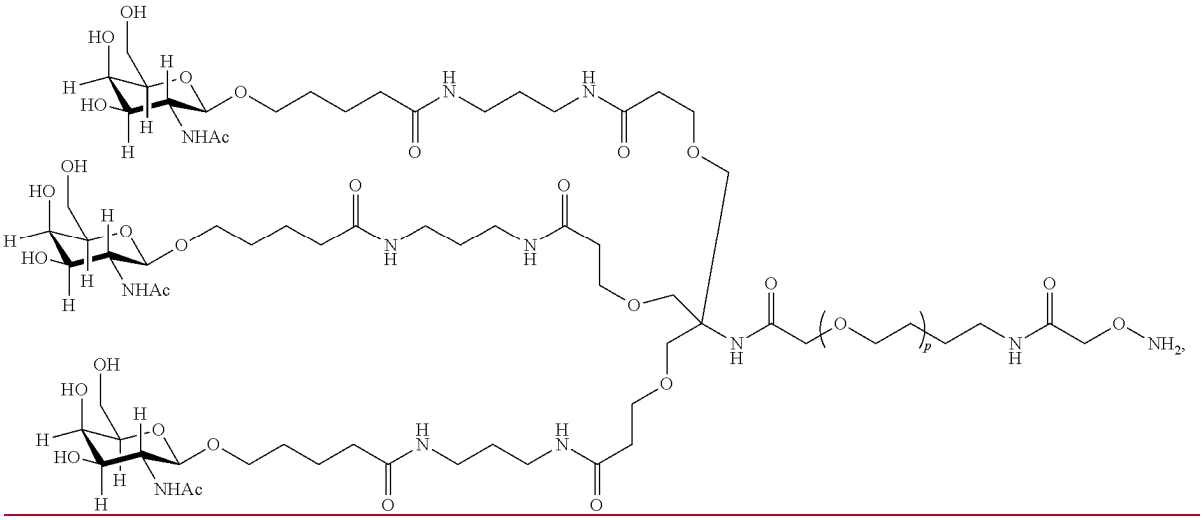
C) X is a targeting moiety comprising PEG that binds to a cell;

D) Gal is a galactose moiety; and

E) Sia is a sialic acid moiety.

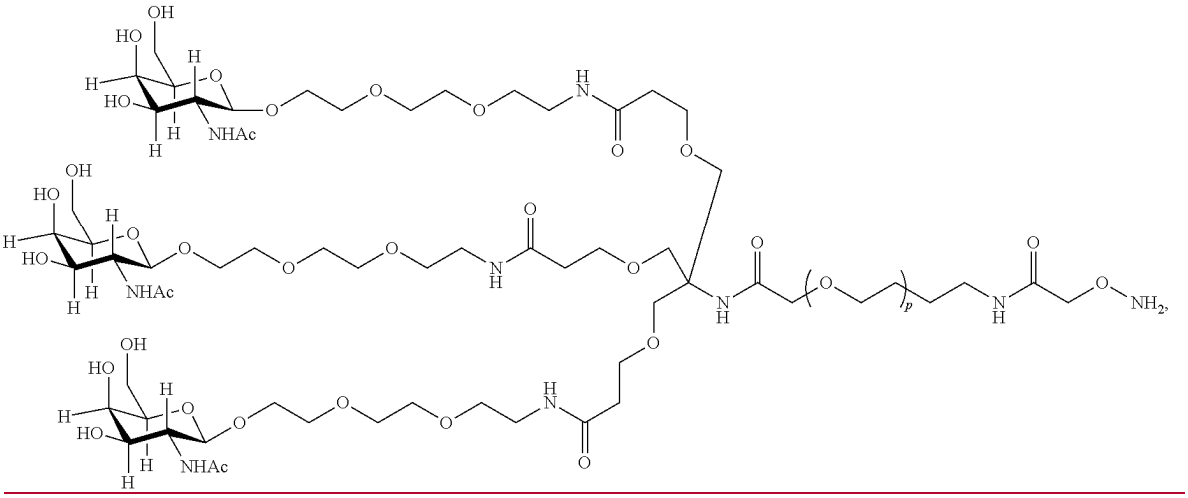
17. The antibody, or antigen-binding fragment thereof, of claim 16, wherein the glycan comprises at least one moiety represented by Formula IX or Formula XI:

[Formula IX]



wherein p has a value of 1 to 32; or

[Formula XI]



wherein p has a value of 1 to 32.

18. The antibody, or antigen-binding fragment thereof, of claim 16, wherein the PEG comprises 3 to 3.5 PEG.

19. The antibody, or antigen-binding fragment thereof, of claim 1, wherein targeting moiety binds to a mannose 6 phosphate ~~moiety;~~receptor on the cell.

~~d)~~

20. The antibody, or antigen-binding fragment thereof, of claim 1, wherein the targeting moiety binds to a sialic acid-binding immunoglobulin-type lectin (Siglec) on the cell.

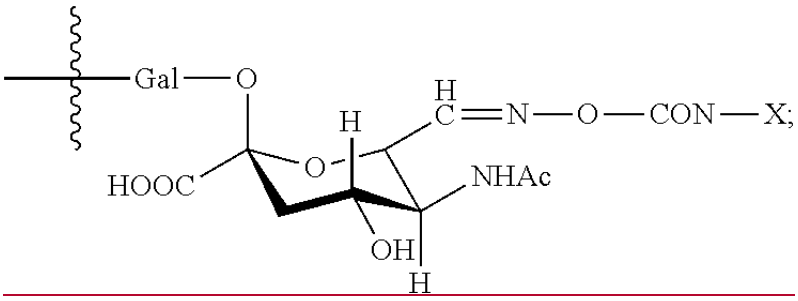
21. The antibody, or antigen-binding fragment thereof, of claim 20, wherein the Siglec is sialoadhesin (Siglec-1), CD22 (Siglec-2), CD33 (Siglec-3), myelin-associated glycoprotein

(MAG (Siglec-4)), Siglec-5, Siglec-6, Siglec-7, Siglec-8, Siglec-9, Siglec-10, Siglec-11, Siglec-12, Siglec-14, or Siglec-15~~;~~.

~~e)~~22. The antibody, or antigen-binding fragment thereof, of claim 1, wherein the targeting moiety binds to a C-type lectin receptor, a galectin, or a L-type lectin receptor.

23. The antibody, or antigen-binding fragment thereof, of claim 1, wherein Q is O.

24. The antibody, or antigen-binding fragment thereof, of claim 1, wherein the glycan comprises a moiety of the following structural formula:



25. The antibody, or antigen-binding fragment thereof, of claim 1, wherein the targeting moiety is a glycopeptide capable of binding an ~~α2,3-, α2,6-, or α2,8-linked sialic acid residue;~~asialoglycoprotein receptor (ASGPR) on a cell.

~~f)~~

26. The antibody, or antigen-binding fragment thereof, of claim 1, wherein the amount of the antibody, or antigen-binding fragment thereof, internalized by the cell is greater than the amount of a reference antibody, or antigen-binding fragment thereof, lacking a targeting moiety internalized by the cell.

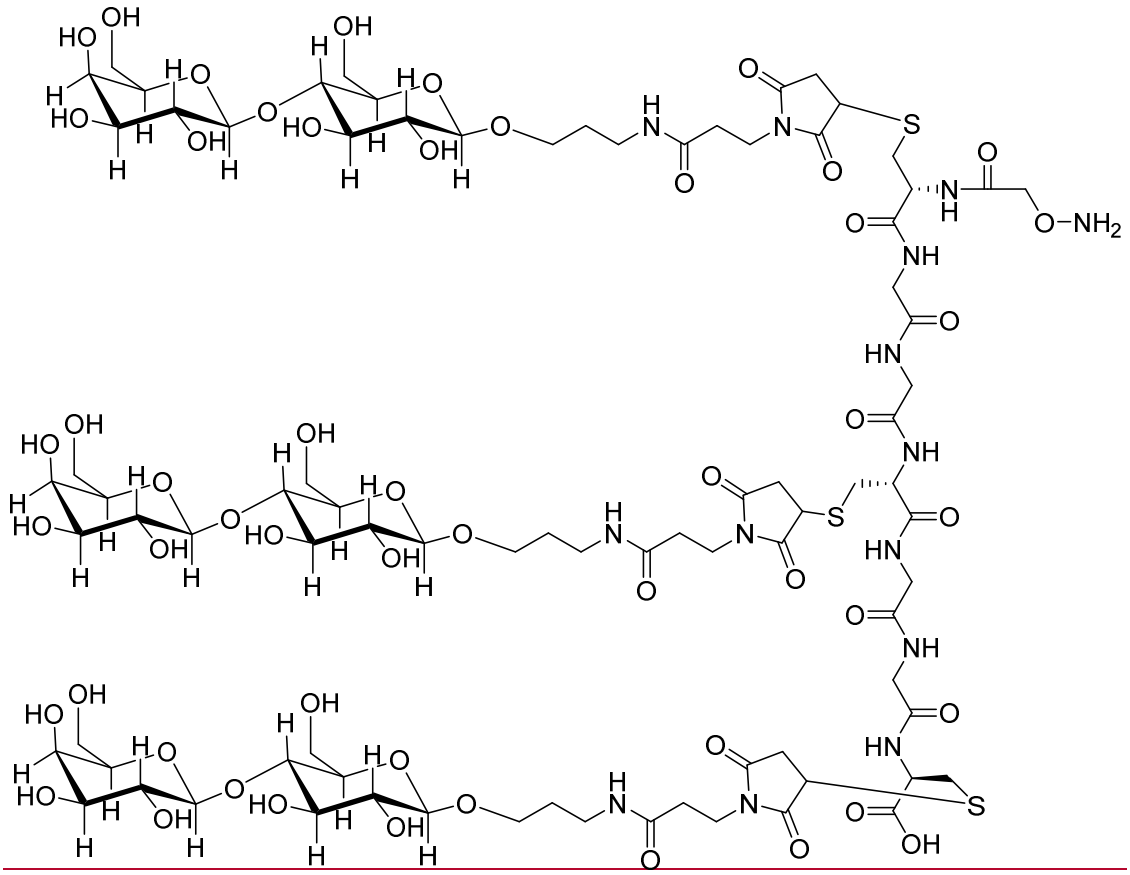
27. The antibody, or antigen-binding fragment thereof, of claim 1, wherein the targeting moiety binds to DEC-205 (CD205; lymphocyte antigen 75), macrophage mannose receptor (MMR; CD206), Dectin-1, Dectin-2, macrophage-inducible C-type lectin (Mincle), dendritic cell-specific ICAM3-grabbing nonintegrin (DC-SIGN, CD209), DC NK lectin group receptor-1 (DNGR-1), Langerin (CD207), a lectican, an asialoglycoprotein receptor (ASGPR), C-lectin receptor dendritic cell immunoreceptor (CLEC4A; CLECSF6; DCIR), macrophage galactose-type lectin (MGL), a DC receptor, a collectin, a selectin, an NK-cell receptor, a multi-~~-~~C-type lectin domain (CTLD) endocytic receptor, a Reg group (type VII) lectin, chondrolectin, tetranectin, polycystin, attractin (ATRN), eosinophil major basic protein (EMBP), DiGeorge Syndrome Critical Region Gene 2 (DGCR2), Thrombomodulin, Bimlec, a group XVI lectin (SEEC), or a group XVII lectin (CBCP/Frem1/QBRICK~~); and~~).

~~g) the~~

28. The antibody, or antigen-binding fragment thereof, of claim 1, wherein the targeting moiety is a trivalent GalNAc glycan moiety.

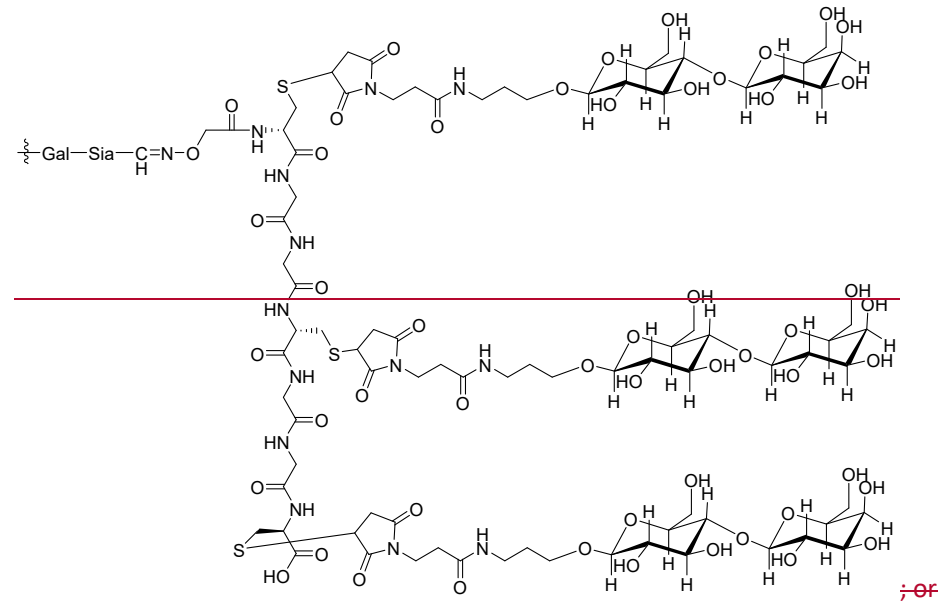
~~4. The binding polypeptide of claim 2, wherein the mammalian cell is selected from a B cell, a T cell, a dendritic cell, a natural killer (NK) cell, a macrophage, a neutrophil, a hepatocyte, a liver sinusoidal endothelial cell, or a hepatoma cell.~~

~~5.~~ 29. The antibody, or antigen-binding ~~polypeptide~~fragment thereof, of ~~claim 3,~~ ~~wherein the targeting moiety comprises an α2,3-siallylactose moiety or an α2,6-siallylactose moiety~~1, wherein the antibody, or ~~wherein the targeting moiety is a tri-galactosylated glycopeptide or lactose3-Cys3Gly4, optionally wherein Sia is present and the lactose$_3$-Cys$_3$Gly$_4$ moiety is represented by Formula V:~~

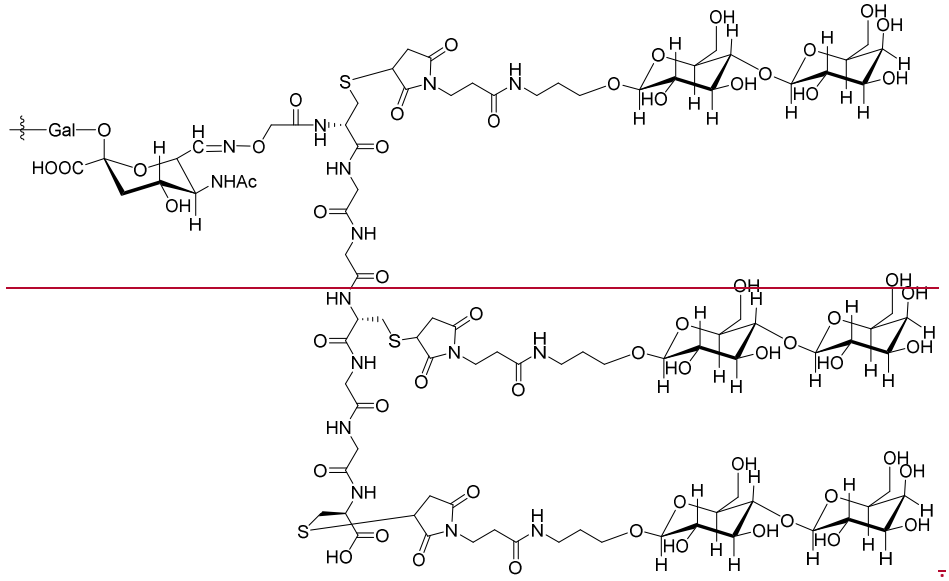


~~Formula V~~

~~6. The~~ antigen-binding ~~polypeptide of claim 5, wherein the glycan comprises at least one moiety selected from the following structural formulae:~~



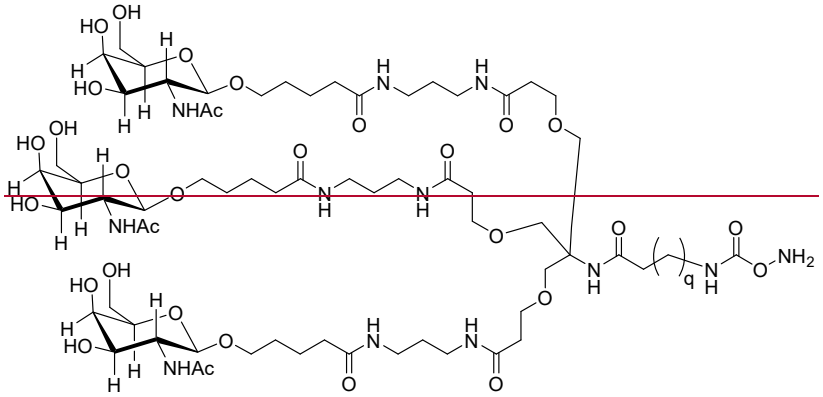
; or



7. The binding polypeptide of claim 2, wherein the targeting moiety is a trivalent GalNAc glycan moiety.

8. The binding polypeptide of claim 7, wherein one or more of the following conditions are met:

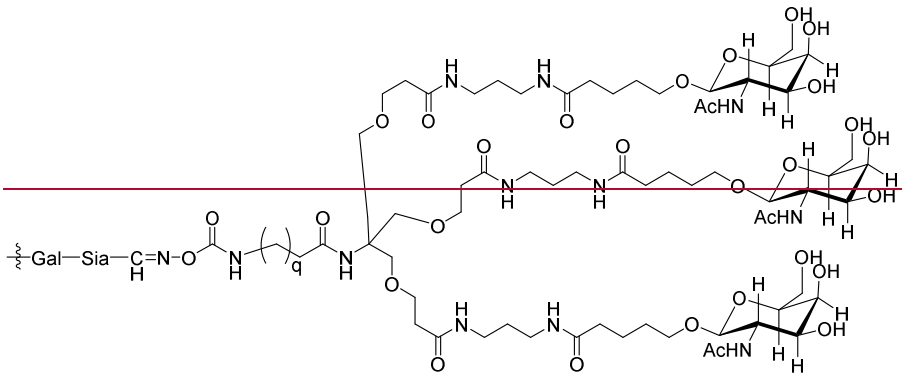
a) Sia is present and the trivalent GalNAc glycan moiety is represented by Formula VIII:



~~Formula VIII~~

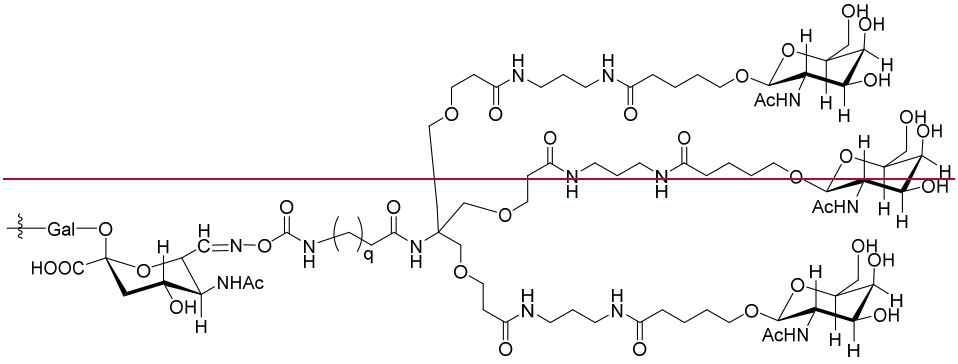
~~wherein q is an integer between 1 and 29 inclusive;~~

~~b) the glycan comprises at least one moiety having the following structural formula:~~



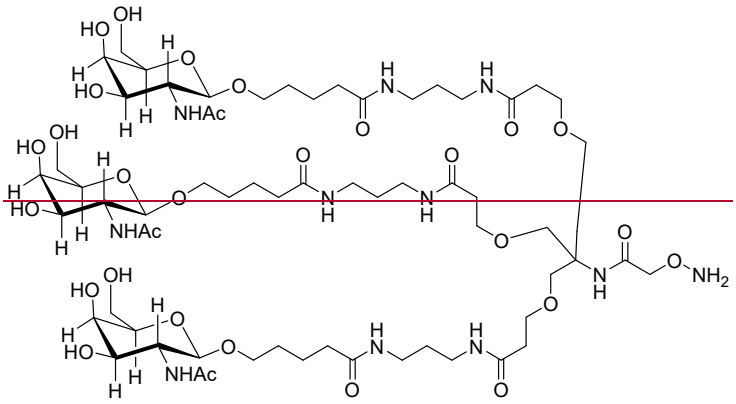
~~wherein q is an integer between 1 and 29 inclusive;~~

~~c) the glycan comprises at least one moiety having the following structural formula:~~

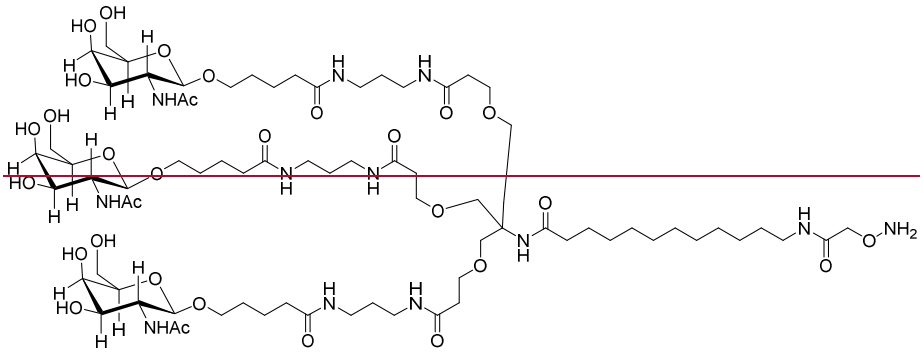


~~wherein q is an integer between 1 and 29 inclusive;~~

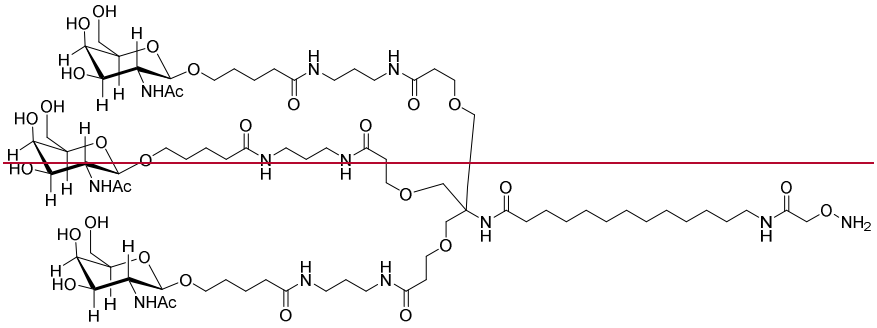
~~d) the trivalent GalNAc glycan moiety is represented by Formula VII, Formula XIII, or Formula XIV:~~



~~Formula VII~~

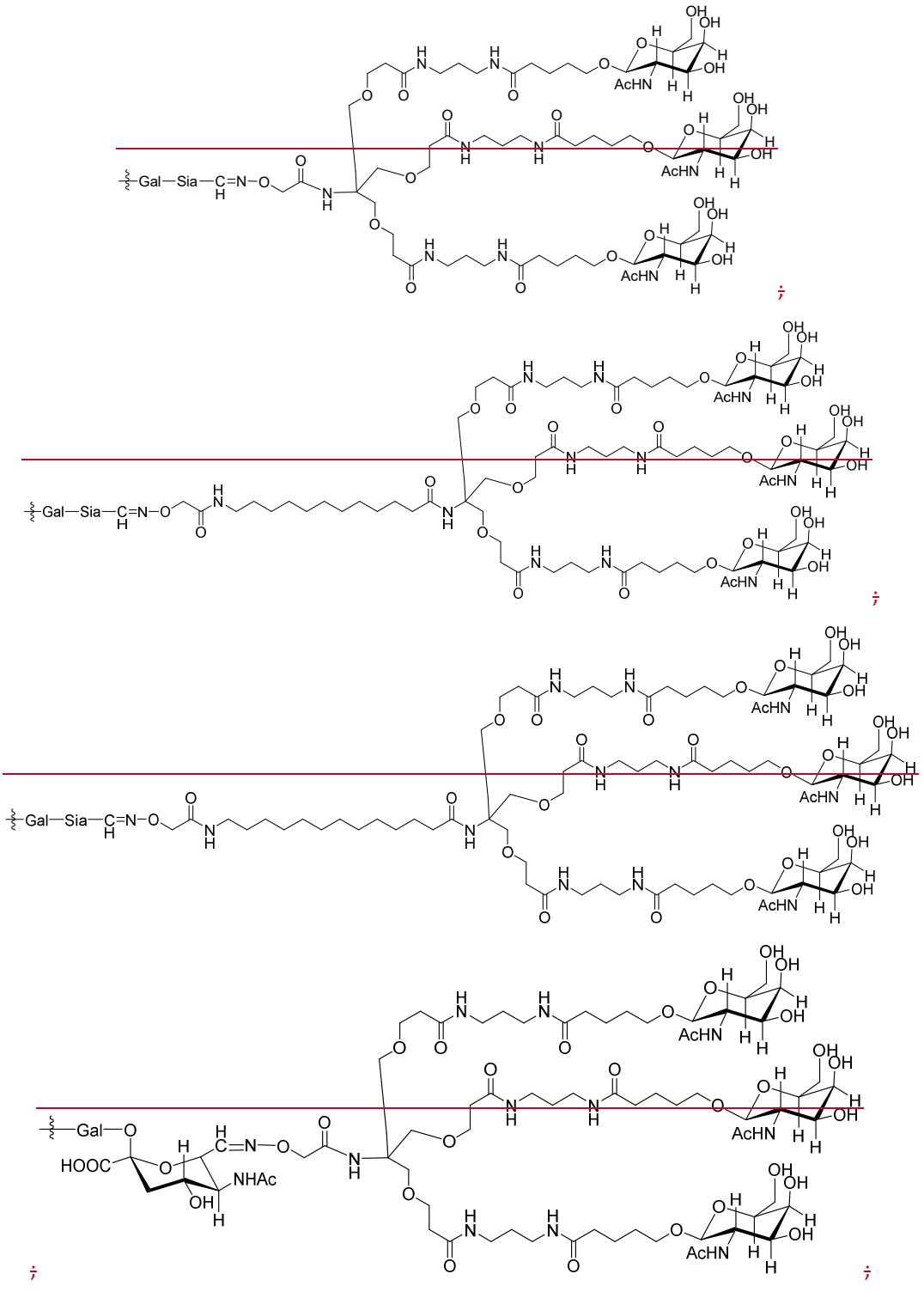


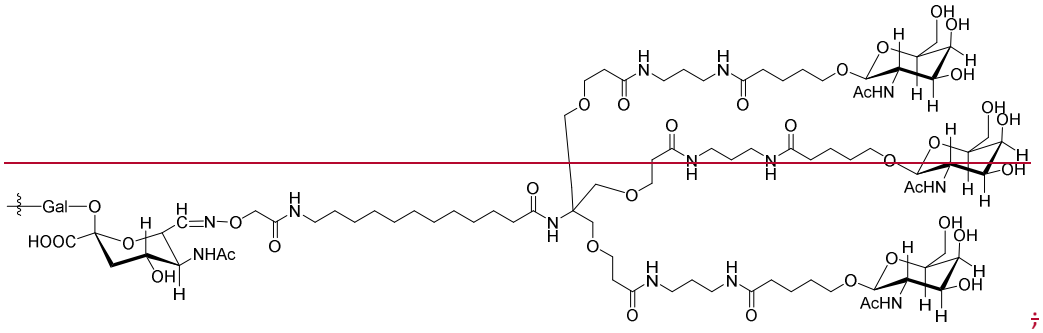
~~Formula XIII or~~



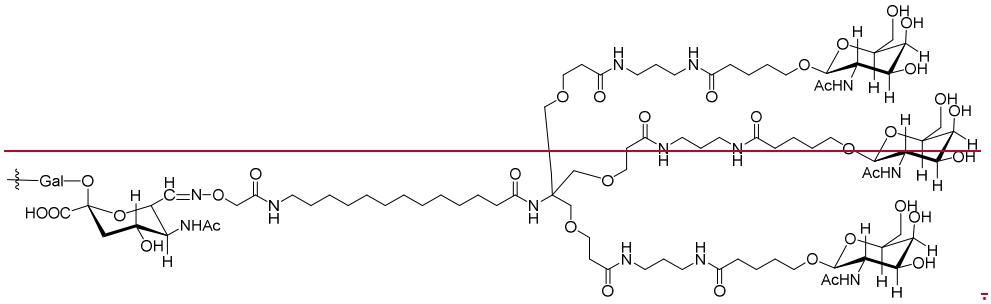
~~Formula XIV; and~~

~~e) the glycan comprises at least one moiety selected from the following structural formulae:~~


Gal—Sia—C=N—O
AcHN
OH
HOOC
NHAc



or



9. The binding polypeptide of any one of the preceding claims, wherein one or more of the following conditions are met:

a) the binding polypeptide comprises an Fc domain, wherein the Fc domain is optionally human;

b) the binding polypeptidefragment thereof, comprises a CH1 domain;.

c)

30. The antibody, or antigen-binding fragment thereof, of claim 1, wherein the connector moiety comprises a pH-sensitive linker, disulfide linker, enzyme-sensitive linker or other cleavable linker moiety;.

d) the connector moiety comprises a linker moiety selected from the group consisting of linker moieties depicted in Table 2 and 14;

e) the binding polypeptide is an

31. The antibody, or immunoadhesin;

f) the modified glycan is N-linked to the antigen-binding fragment thereof, of claim 9, wherein the antibody, or antigen-binding polypeptide via an asparagine residue at amino acid position 297 of the Fc domain, according to EU numbering;

g) the modified glycan is N-linked to the binding polypeptide via an asparagine residue at amino acid position 298 of the Fc domain, according to EU numbering; and

h) the modified glycan is N-linked to the binding polypeptide via an asparagine

~~residue at amino acid position 114 of the CH1 domain, according to Kabat numbering.~~

~~10. A method of making the binding polypeptide of any one of the preceding claims, the method comprising reacting an effector moiety of Formula (I):~~

~~NH_2-Q-CON-X~~

~~Formula (I),~~

~~wherein:~~

~~A) Q is NH or O;~~

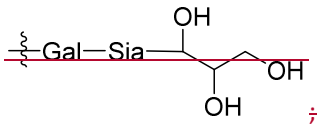
~~B) CON is a connector moiety; and~~

~~C) X is a targeting moiety,~~

~~with a precursor binding polypeptide comprising an oxidized glycan.~~

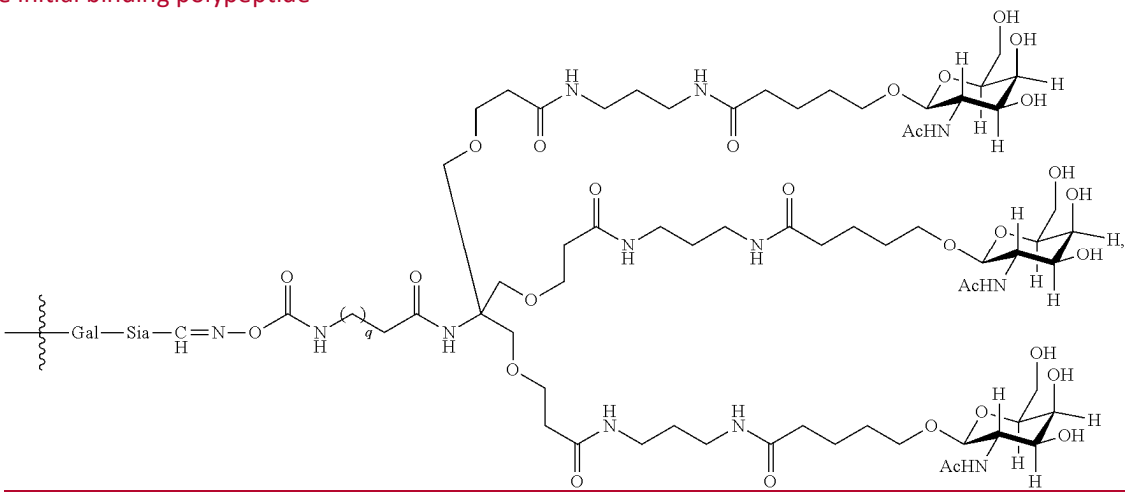
~~11. The method of claim 10, wherein one or more of the following conditions are met:~~

~~a) the initial binding polypeptide~~fragment thereof, comprises at least one moiety ~~of~~having the following structural formula:



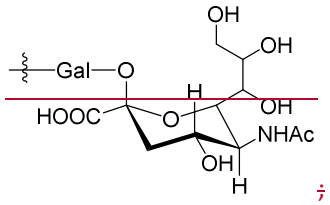
~~;~~

~~b) the initial binding polypeptide~~



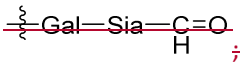
wherein q is an integer between 1 and 29 inclusive.

32. The antibody, or antigen-binding fragment thereof, of claim 9, wherein the antibody, or antigen-binding fragment thereof, comprises at least one moiety ~~of~~having the following structural formula:



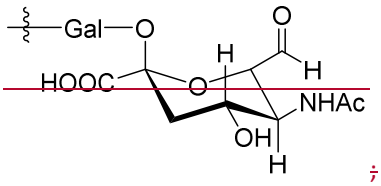
~~;~~

~~c) the precursor binding polypeptide comprises an oxidized glycan comprises at least one moiety of the following structural formula:~~



~~;~~

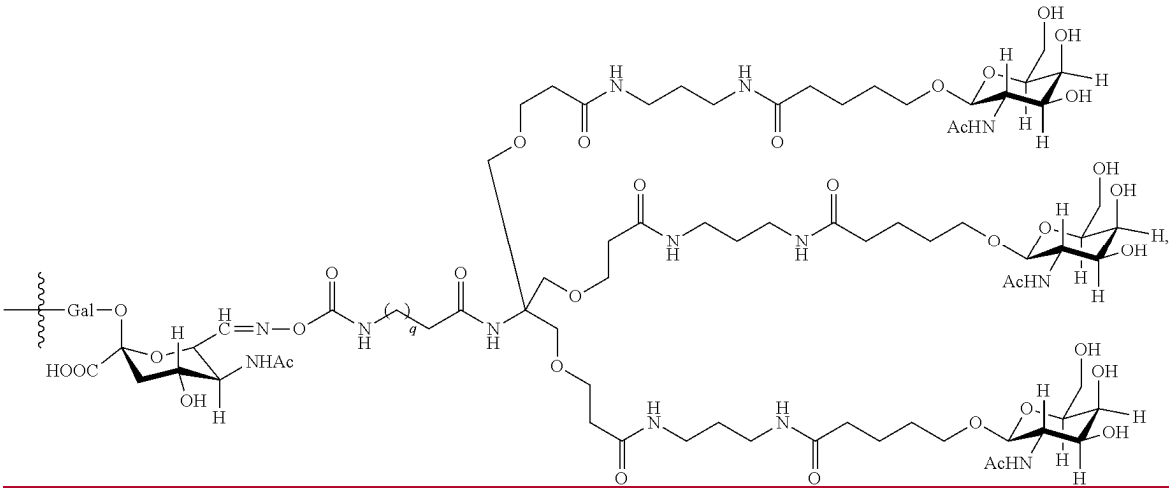
~~d) the precursor binding polypeptide comprises an oxidized glycan comprises at least one moiety of the following structural formula: and~~



~~;~~

~~e) the precursor binding polypeptide comprising an oxidized glycan is generated by reacting an initial binding polypeptide comprising a glycan with a mildly oxidizing agent,~~

~~optionally wherein the mildly oxidizing agent is sodium periodate,~~



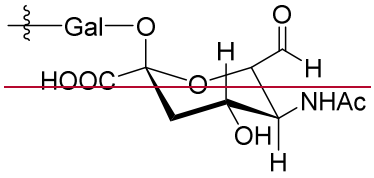
wherein ~~less than 1mM sodium periodate is employed, or~~ q is an integer between 1 and 29 inclusive.

33. The antibody, or antigen-binding fragment thereof, of claim 9, wherein the ~~mildly oxidizing agent is periodate or galactose oxidase.~~

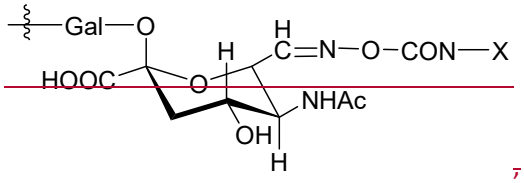
~~12. The method of claim 10 comprising reacting an effector moiety of the following structural formula:~~

~~NH_2-O-CON-X~~

~~with the precursor binding polypeptide comprising an oxidized glycan comprising at least one moiety of the following structural formula:~~



~~to form a binding polypeptide of the following structural formula:~~



~~,~~

~~wherein the targeting moiety is optionally a~~ trivalent GalNAc glycan moiety~~.~~

~~13. The method of claim 11, wherein the reacting step is conducted in the presence of a salt comprising a metal ion, optionally wherein~~

~~the metal ion is a copper ion,~~

~~the salt is copper acetate, or~~

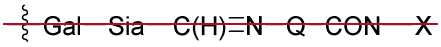
~~the salt comprises a metal ion present at a concentration of at least 0.1 mM.~~

14. The method of any of any one of the preceding claims wherein the binding polypeptide comprising the glycan comprises one or two terminal sialic acid residues, optionally wherein the one or two terminal sialic acid residues are introduced by treatment of the binding polypeptide with a sialyltransferase or combination of sialyltransferase and galactosyltransferase.

15. A composition comprising a binding polypeptide of any one of the preceding claims and a pharmaceutically acceptable carrier or excipient, optionally wherein the ratio of targeting moiety to binding polypeptide is at least about 2 or equal to or more than about 4.

16. Use of an effective amount of the composition of claim 15 in the manufacture of a medicament for the treatment of neoplastic disorders in a patient in need thereof.

17. A binding polypeptide comprising at least one modified glycan comprising at least one moiety of Formula (IV):



Formula (IV)

wherein:

A) Q is NH or O;

B) CON is a connector moiety;

C) X is a moiety comprising PEG;

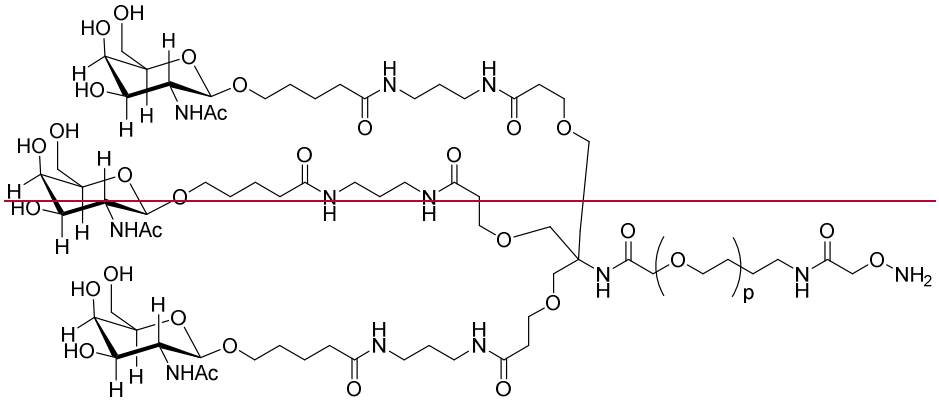
D) Gal is a component derived from galactose; and

E) Sia is a component derived from sialic acid,

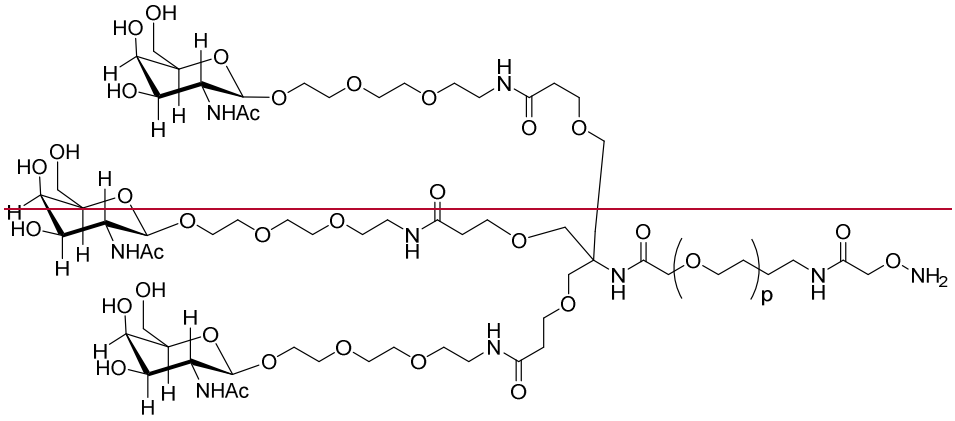
wherein Sia is present or absent.

18. The binding polypeptide of claim 17, wherein one or more of the following conditions are met:

~~a) the glycan comprises at least one moiety~~ is represented by Formula ~~IX~~VII, Formula XIII, or Formula ~~XI~~XIV:



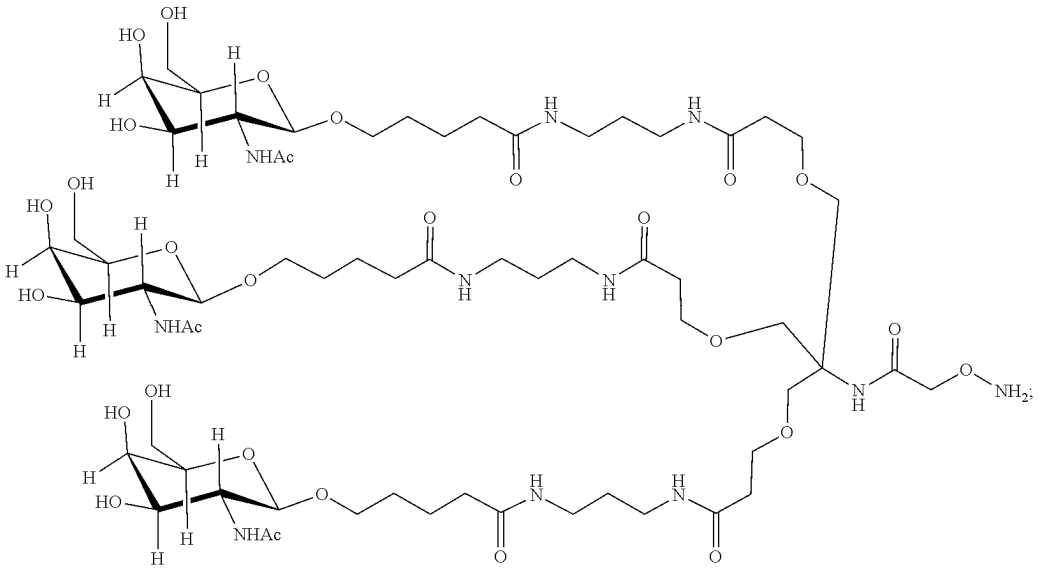
~~[Formula IX], wherein p has a value of 1 to 32; or~~



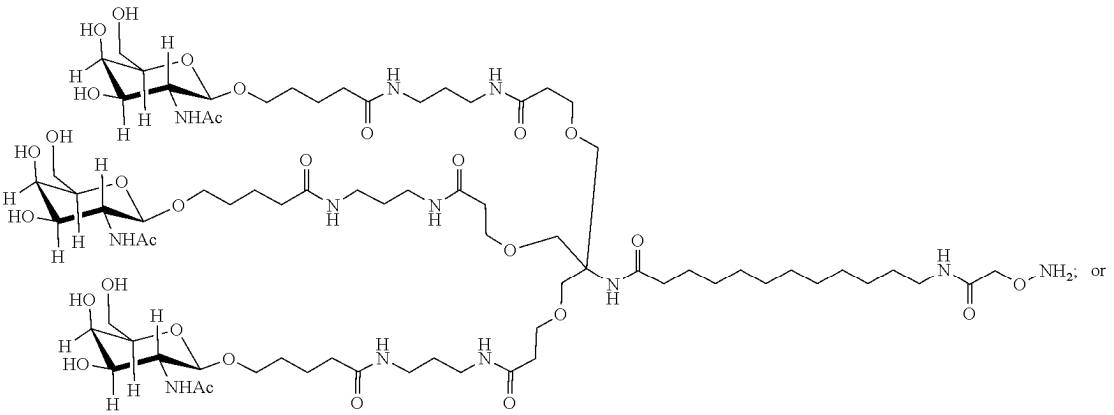
~~[Formula XI], wherein p has a value of 1 to 32;~~

~~b) the glycan~~

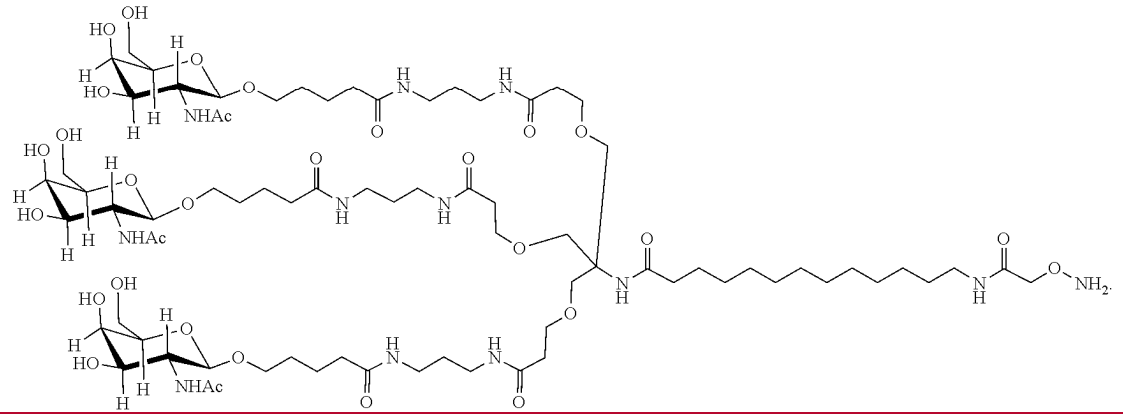
[Formula VII]



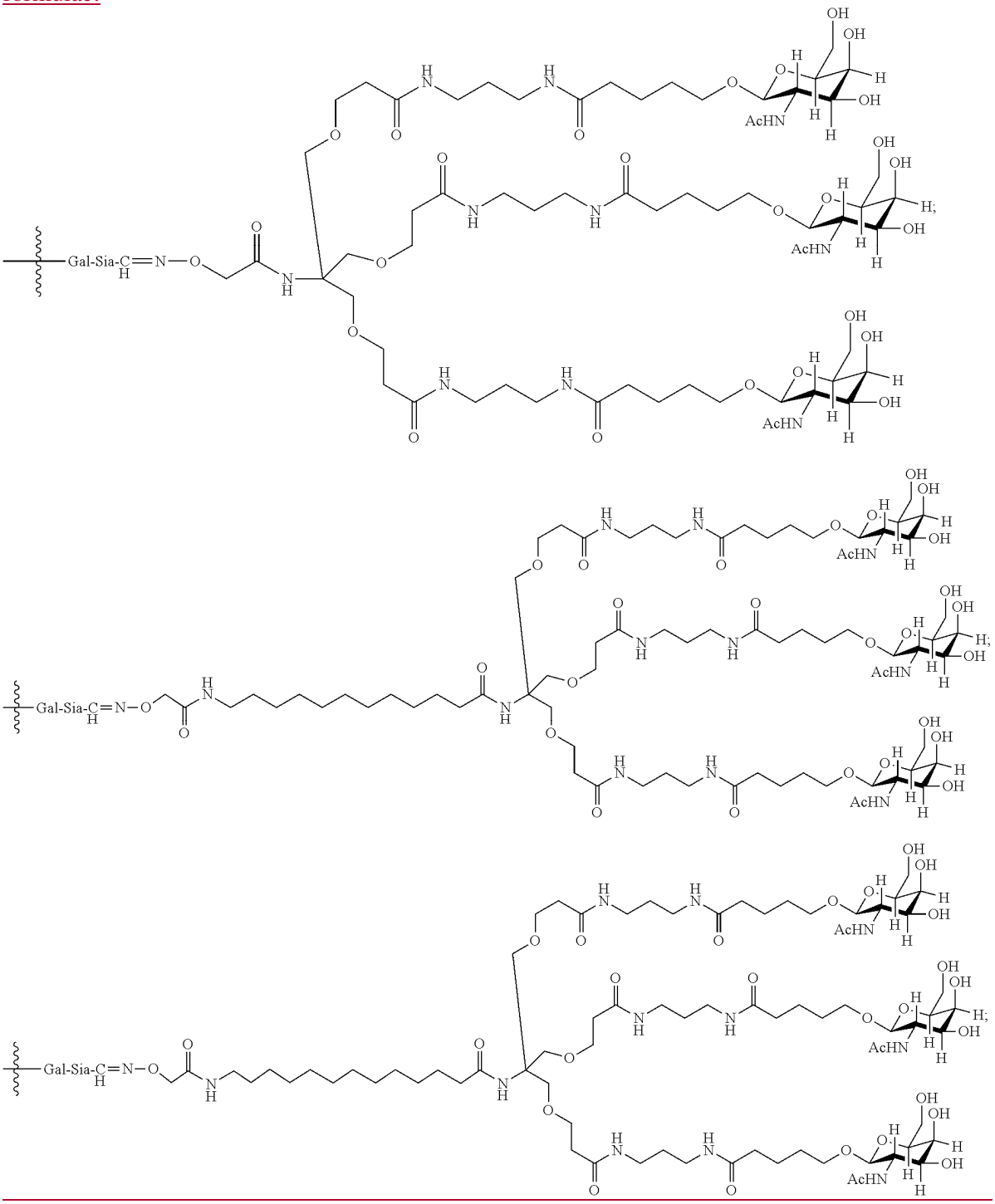
[Formula XIII]

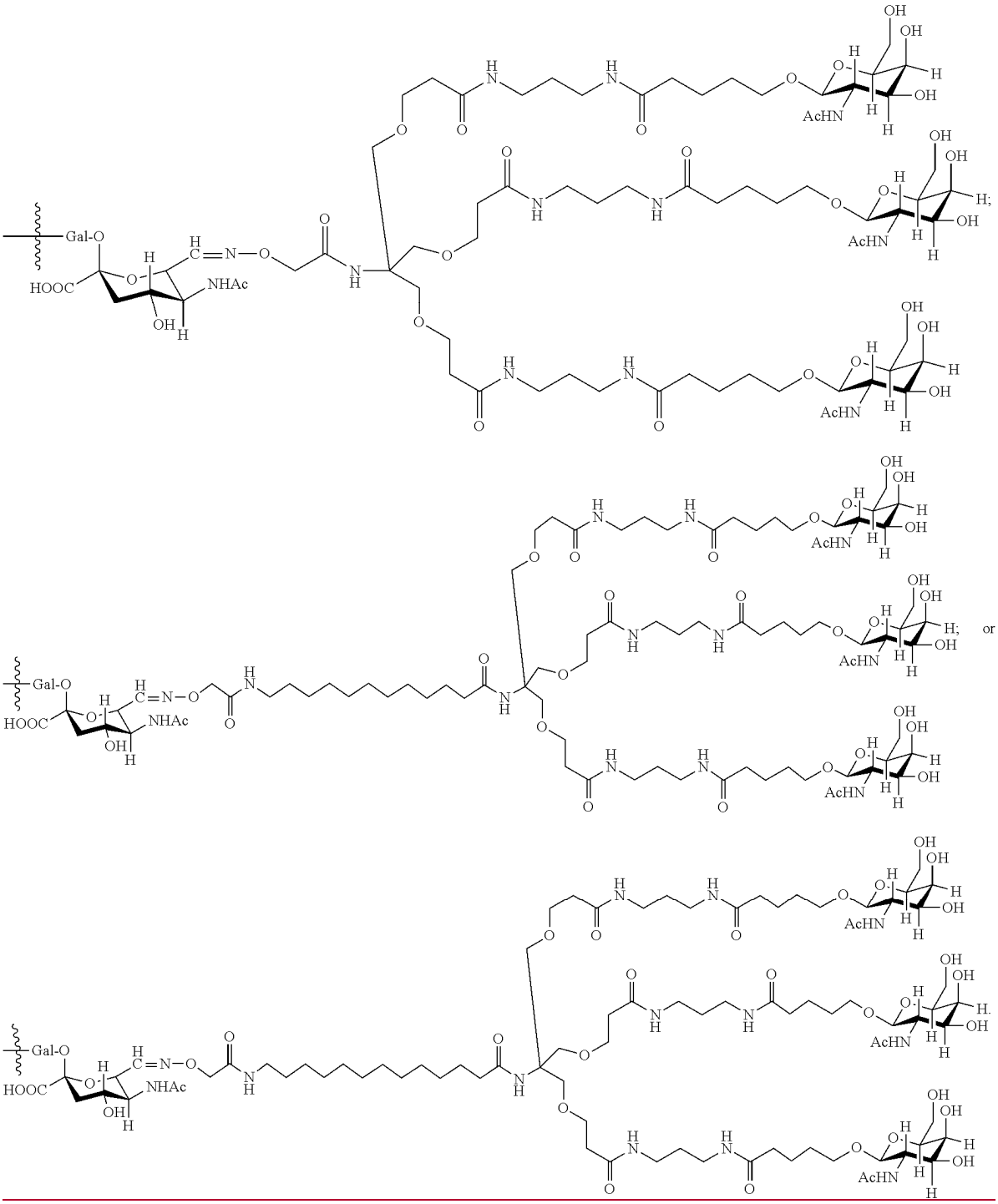


[Formula XIV]



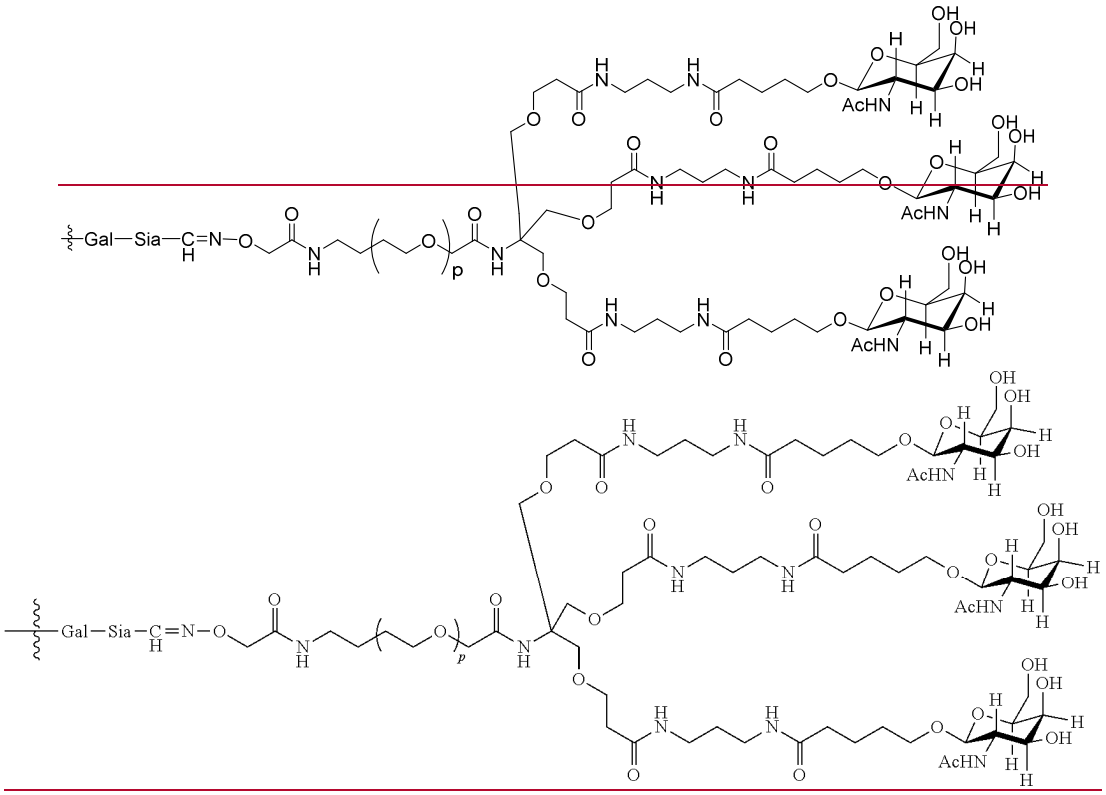
34. The antibody, or antigen-binding fragment thereof, of claim 9, wherein the antibody, or antigen-binding fragment thereof, comprises at least one moiety selected from the following structural formulae:



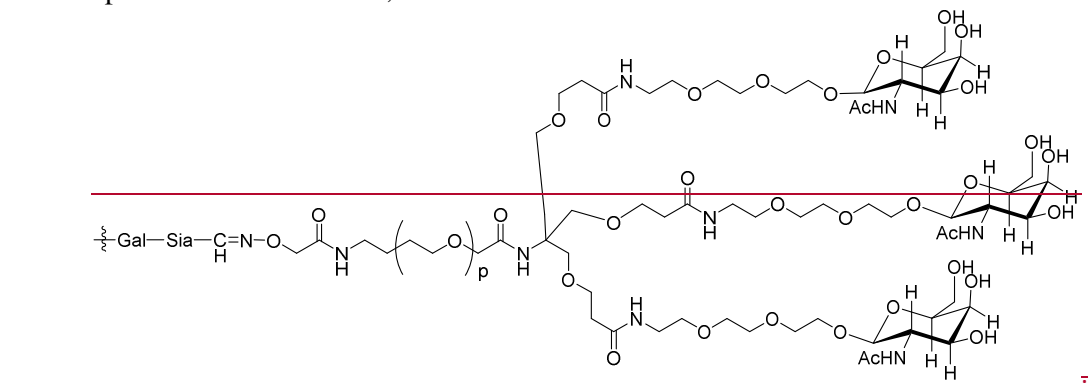


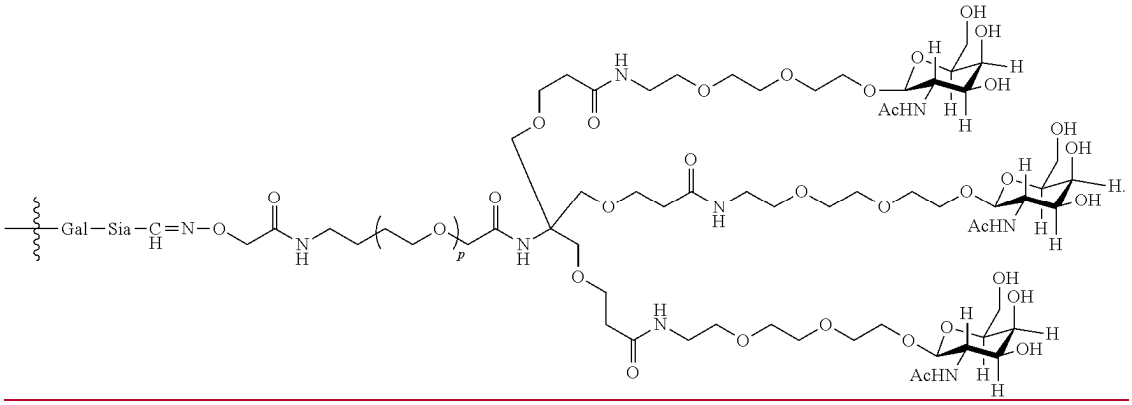
35. The antibody, or antigen-binding fragment thereof, of claim 9, wherein the component comprising galactose residue is GalNAc.

36. The antibody, or antigen-binding fragment thereof, of claim 16, wherein the antibody, or antigen-binding fragment thereof, comprises at least one moiety selected from the following structural formulae:

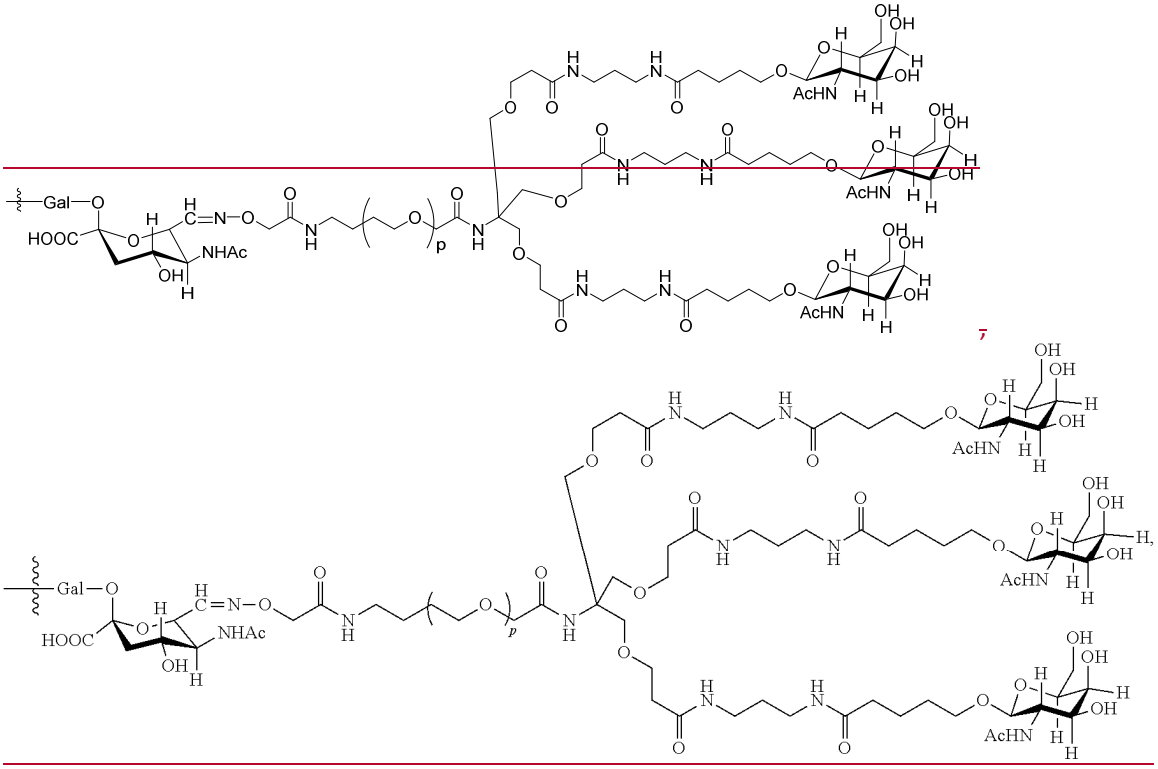


wherein p has a value of 1 to 32;

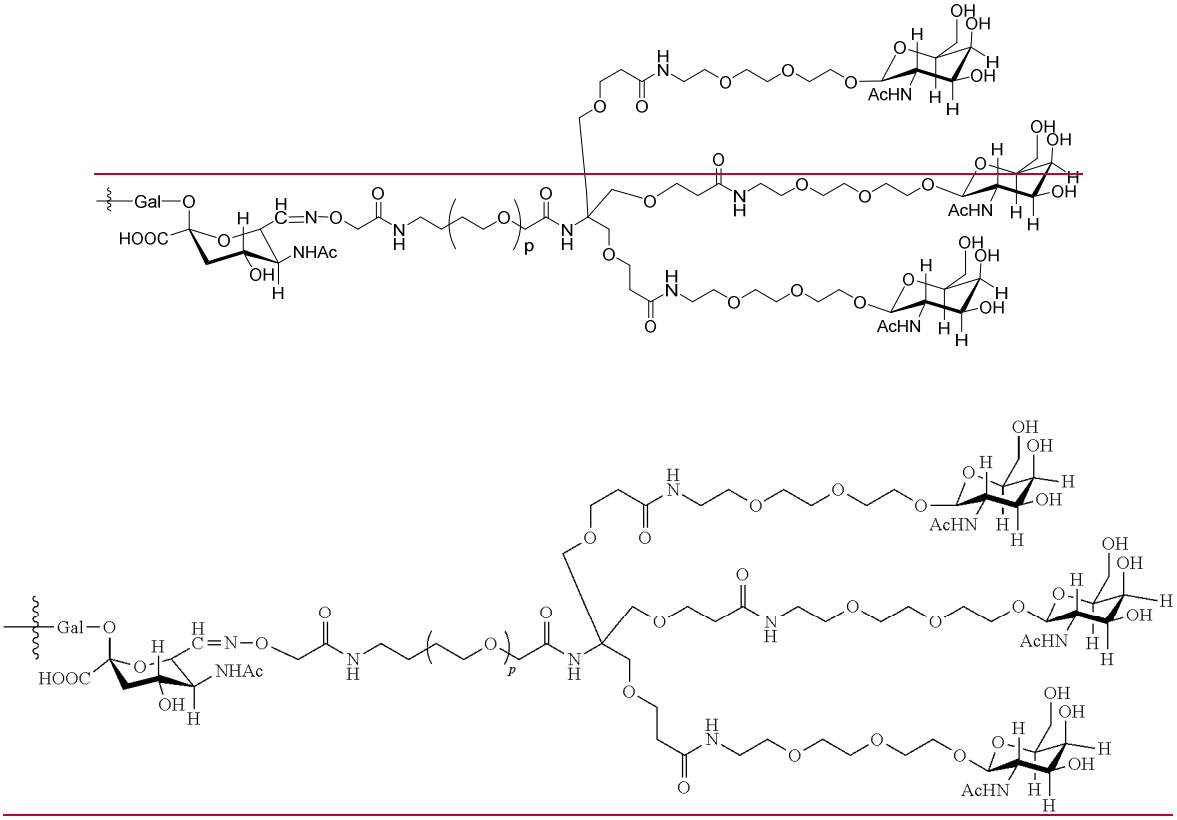




wherein p has a value of 1 to 32;



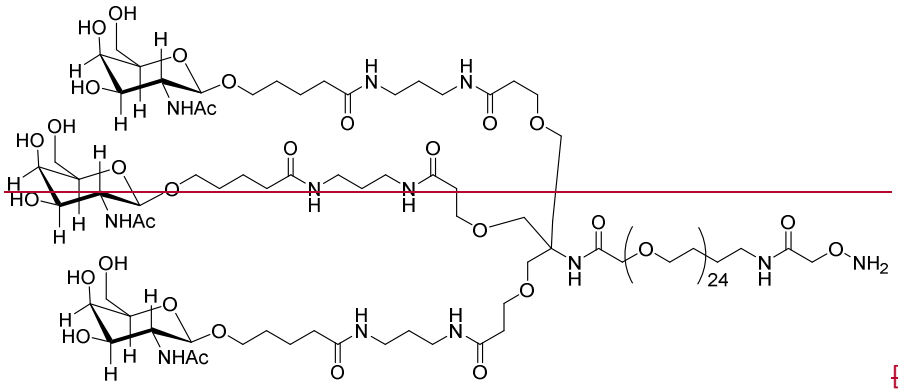
wherein p has a value of 1 to 32; or



wherein p has a value of 1 to 32~~;~~.

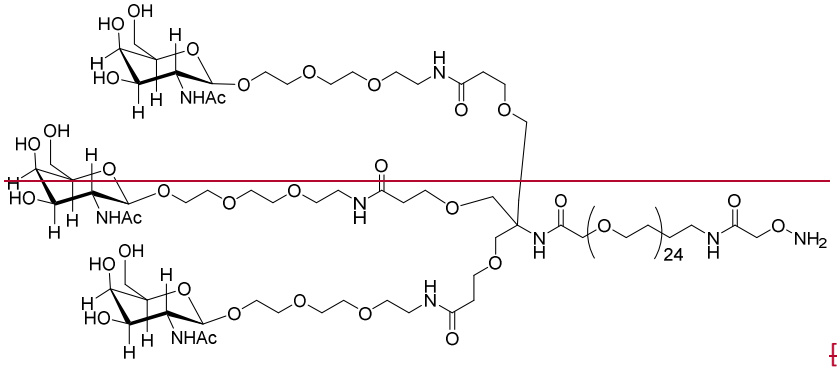
~~c)~~

37. The antibody, or antigen-binding fragment thereof, of claim 16, wherein the targeting moiety comprises PEG and is represented by Formula X or Formula XII:



~~[Formula X];~~

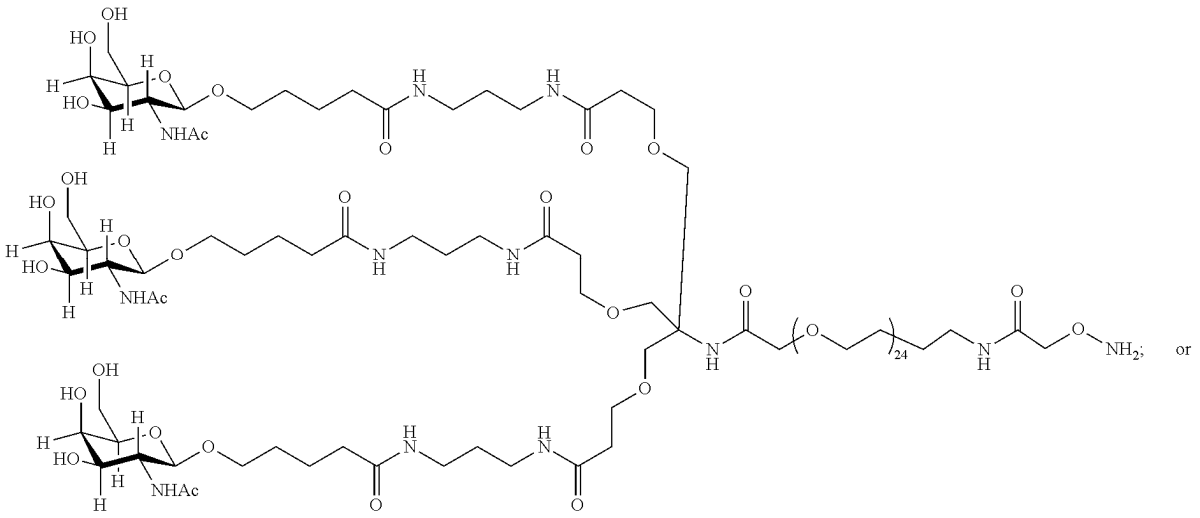
~~or~~



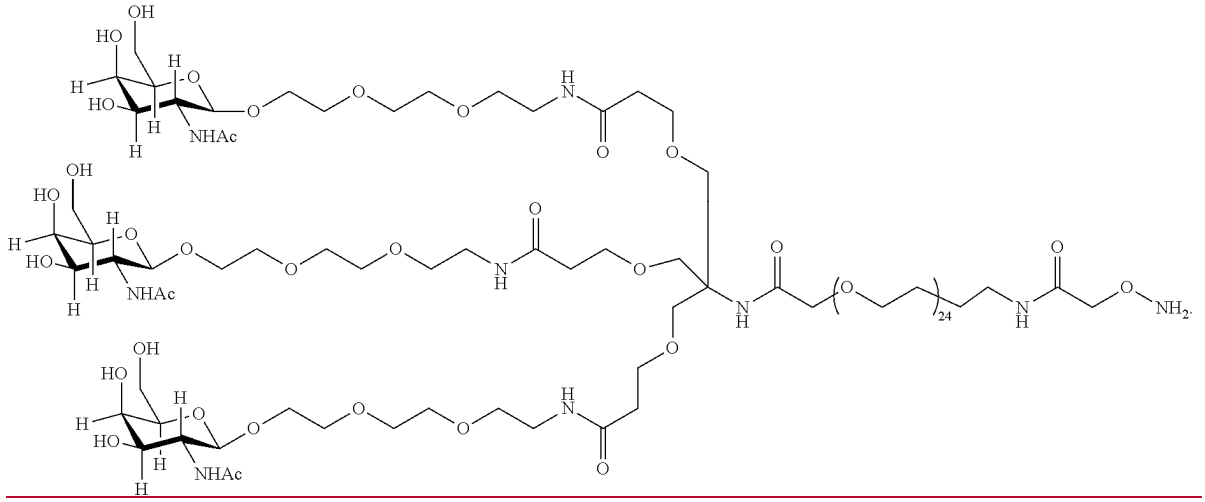
~~[Formula XII];~~

~~d) the glycan~~

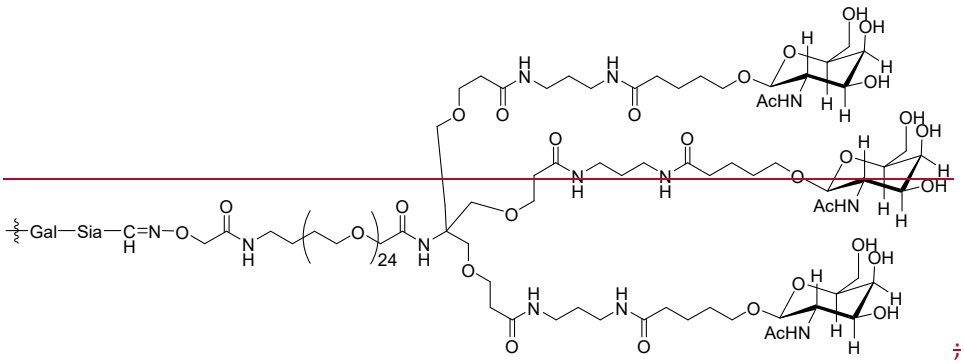
[Formula X]



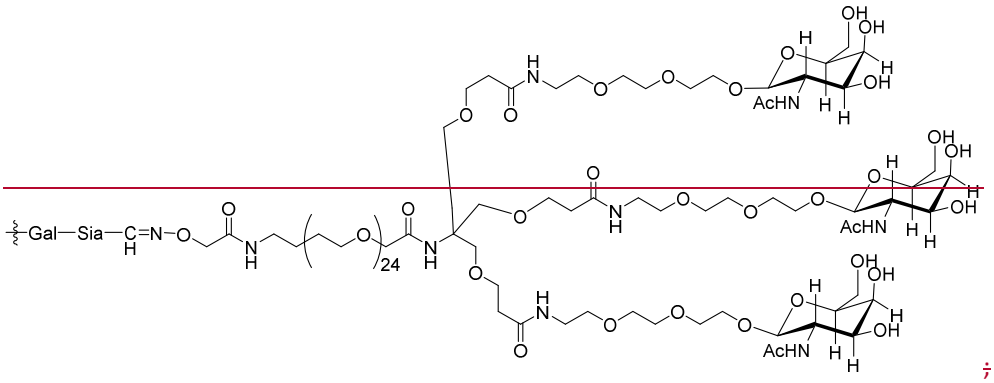
[Formula XII]



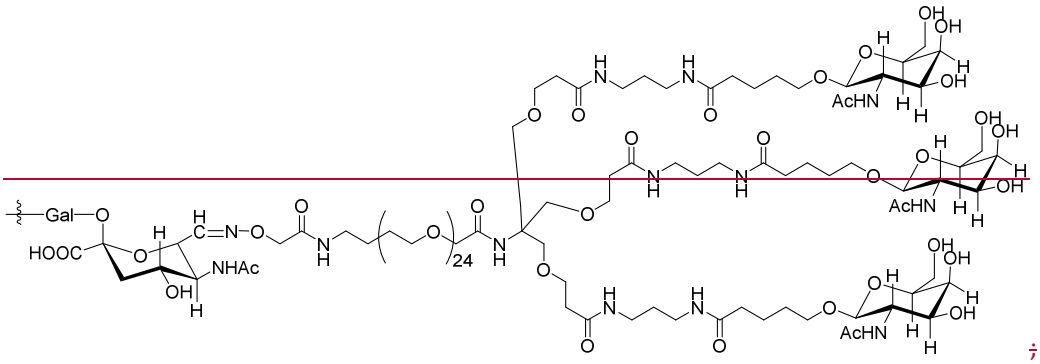
38. The antibody, or antigen-binding fragment thereof, of claim 16, wherein the antibody, or antigen-binding fragment thereof, comprises at least one moiety selected from the following structural formulae:



;

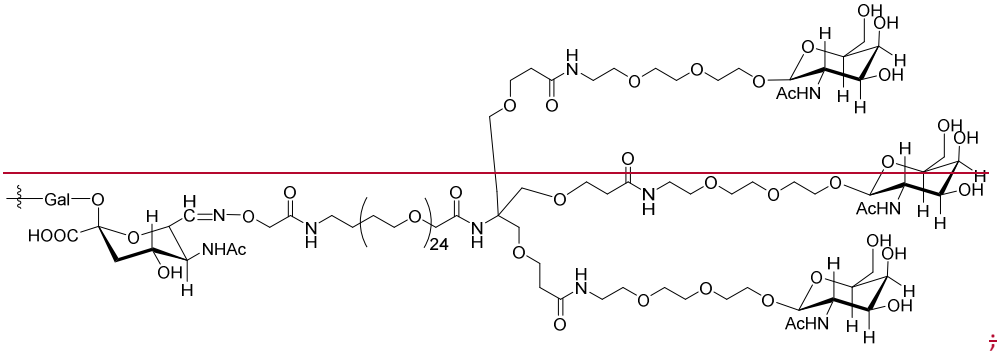


;



;

or

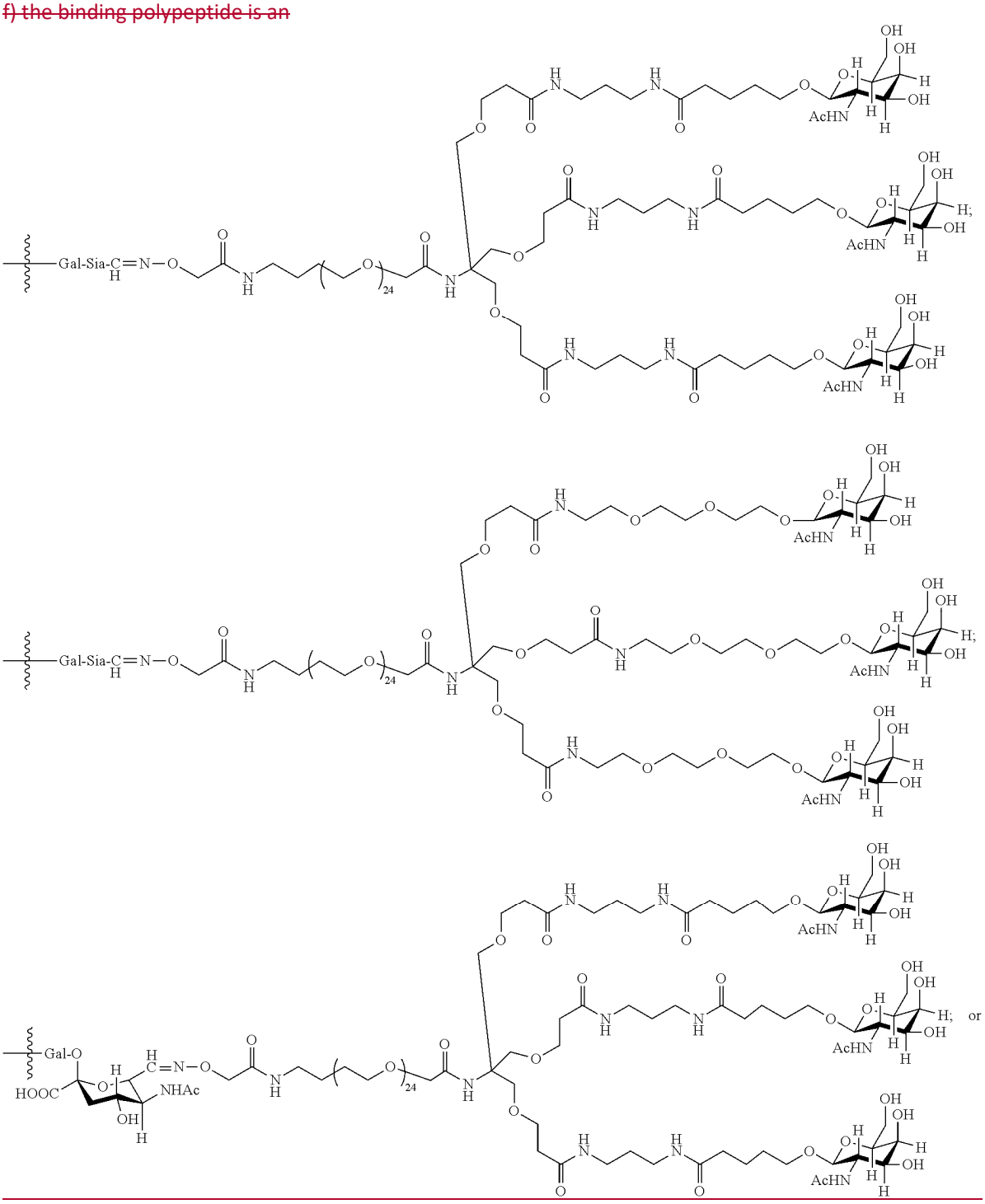


;

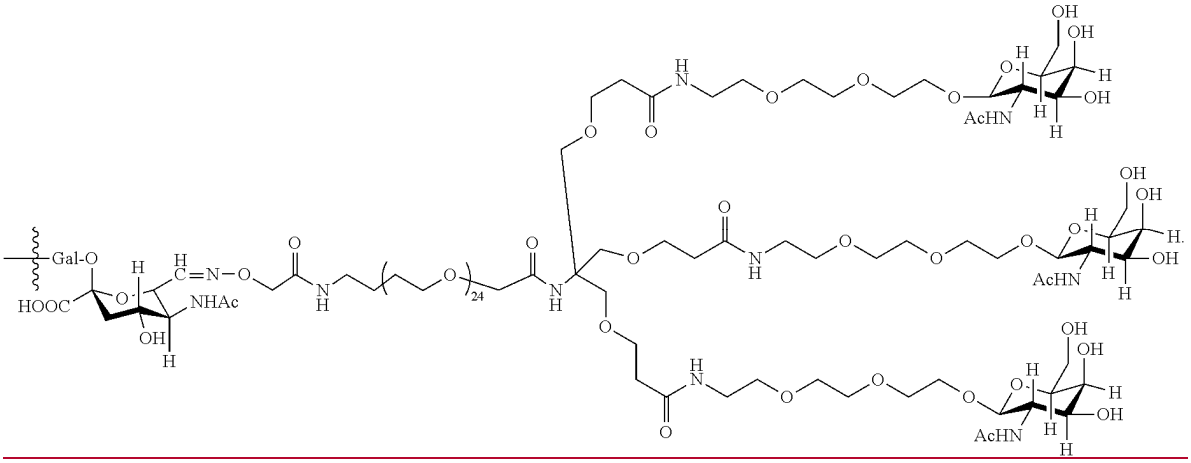
e) the PEG comprises mono-PEG, bi-PEG, or tri-PEG, wherein

~~the PEG optionally comprises 3 to 3.5 PEG; and~~

~~f) the binding polypeptide is an~~



; or



39. The antibody, or ~~immunoadhesin.~~

~~19. A method of making a PEGylated binding polypeptide, wherein the method comprises:~~

~~(a) reacting a binding polypeptide comprising at least one glycan with a mildly oxidizing agent in the presence of a salt comprising a metal ion to generate an oxidized~~ antigen-binding ~~polypeptide comprising at least one oxidized glycan, and~~

~~(b) conjugating the oxidized binding polypeptide with at least one moiety comprising PEG, thereby generating the PEGylated binding polypeptide.~~

~~20. The method~~fragment thereof, of ~~claim 18~~16, wherein ~~one or more of~~ the ~~following conditions are met:~~

~~a) the metal ion is a copper ion;~~

~~b) the salt is copper acetate;~~

~~c) the salt~~component comprising ~~a metal ion is present at a concentration of at least 0.1 mM;~~

~~d) the mildly oxidizing agent is periodate oxidase or~~ galactose ~~oxidase; and~~is GalNAc.

~~e) the at least one glycan is a modified glycan.~~