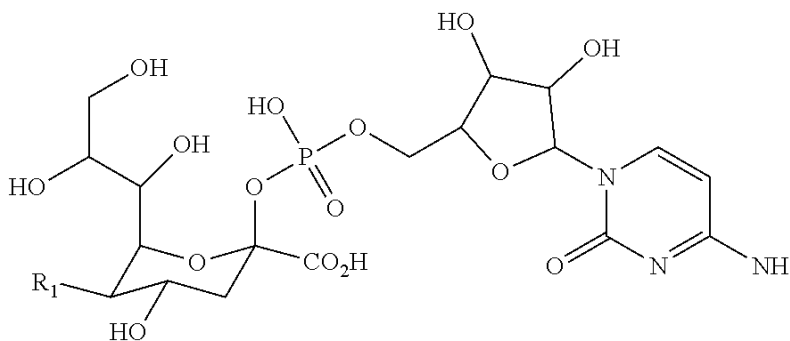


~~We claim:~~

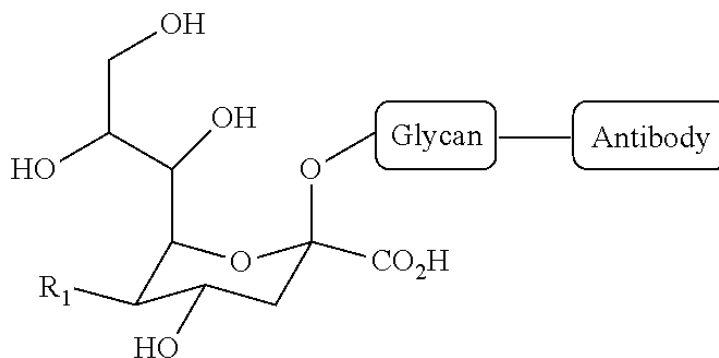
CLAIMS

1.- A method of making an effector moiety conjugated antibody or antigen-binding polypeptide fragment thereof comprising the steps of:

(a) reacting a cytidine monophosphate-sialic acid (CMP-sialic acid) derivative having the structure:



with a glycan attached to a glycosylation site of a-an antibody or antigen-binding polypeptide fragment thereof to form a sialic acid derivative-conjugated antibody having the structure:

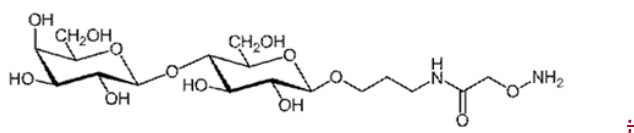
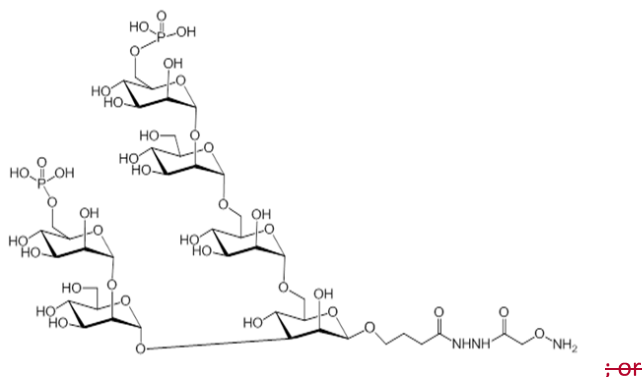
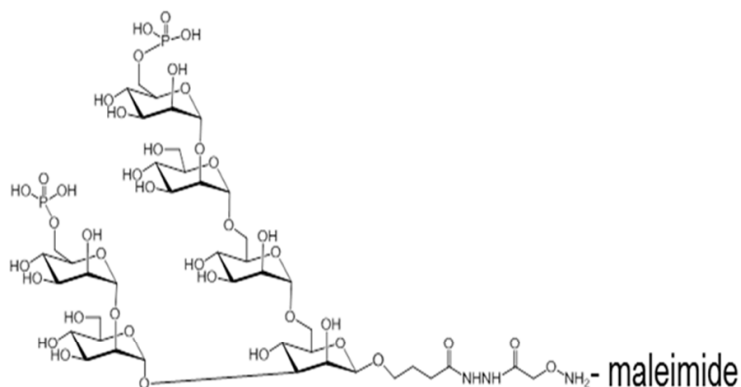


or antigen-binding polypeptide fragment thereof through a sialylation reaction, wherein R₁ is selected from the group consisting of —NHC(O)CH₃, —NHC(O)CH₂OH, —NHC(O)CH₂CH₂C(O)CH₃, —NHC(O)CH₂N₃, and —NHC(O)SH; and

(b) reacting the R₁ group of the sialic acid derivative-conjugated antibody or antigen-binding polypeptide fragment thereof from step (a) with an effector moiety selected from the group consisting of: a drug moiety, a cytotoxic agent, a targeting agent, a diagnostic agent, an anti-cancer agent, an anti-inflammatory agent, an anti-cancer agent, an anti-infective agent, and an anesthetic agent, to form the effector moiety conjugated antibody or antigen-binding polypeptide fragment thereof, wherein an imine bond is formed, and wherein neither the antibody or antigen-binding polypeptide fragment thereof nor the sialic acid derivative-

conjugated antibody or antigen-binding polypeptide fragment thereof are treated with an oxidizing agent during step (a) and step (b), resulting in a hydrazone or oxime linkage.

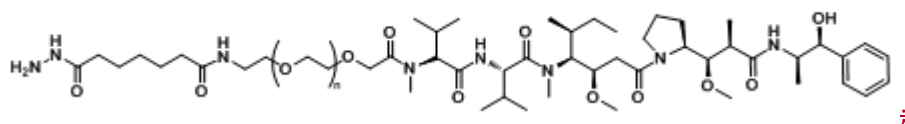
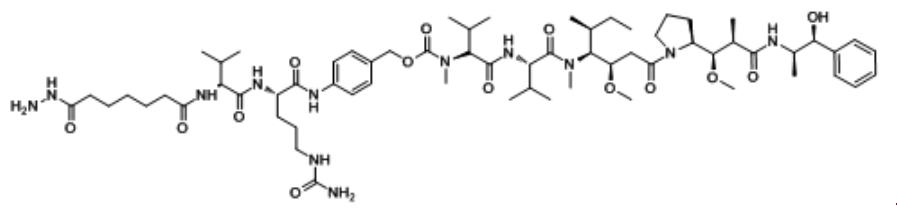
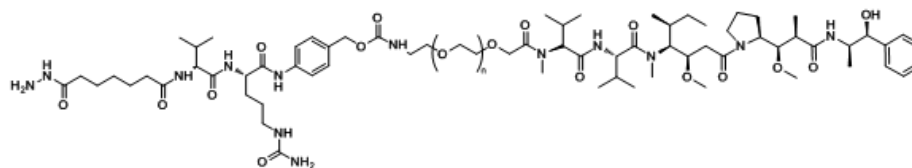
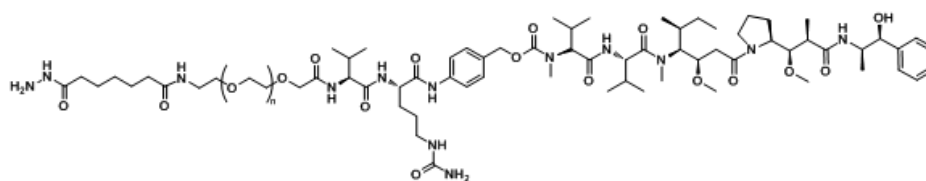
2. The method of claim 1, wherein the sialic acid derivative-conjugated antibody or antigen-binding polypeptide fragment thereof comprises a terminal keto or aldehyde moiety.
3. The method of claim 1 or 2, wherein the effector moiety comprises a terminal aminooxy moiety or is bound to a moiety comprising an aminooxy derivative.
4. — The method of any one of claims 1 to 3, wherein the effector moiety is selected from one or more of the following structural formulas:



5. The method of any one of claims 1 to 4 claim 1, wherein step (b) results in the formation of an oxime bond.
6. — 5. The method of claim 1 or 2, wherein the effector moiety comprises a terminal hydrazine.

~~7. The method of claim 6~~1, wherein step (b) results in the formation of a hydrazone linkage.

~~8. — 6. The method of claim 7~~1, wherein the ~~effector moiety has one or more of the following structural formulas:~~

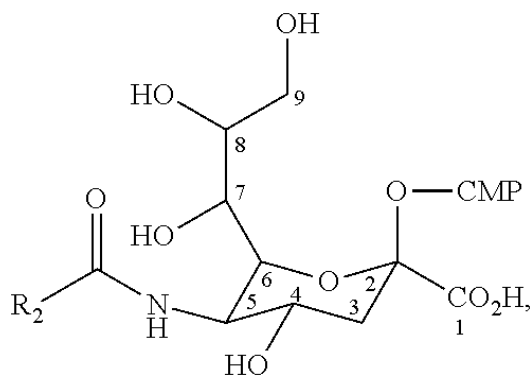


~~9. — A method of making an effector moiety conjugated binding polypeptide comprising the steps of:~~

~~(a) reacting a CMP-sialic acid derivative comprising a terminal reactive moiety at the C5 position with a glycan of a binding polypeptide to form a sialic acid derivative conjugated binding polypeptide;has the structure of:~~

~~— (b) reacting the sialic acid derivative conjugated binding polypeptide with an effector moiety to form the effector moiety conjugated binding polypeptide using click chemistry.~~

~~10. —~~

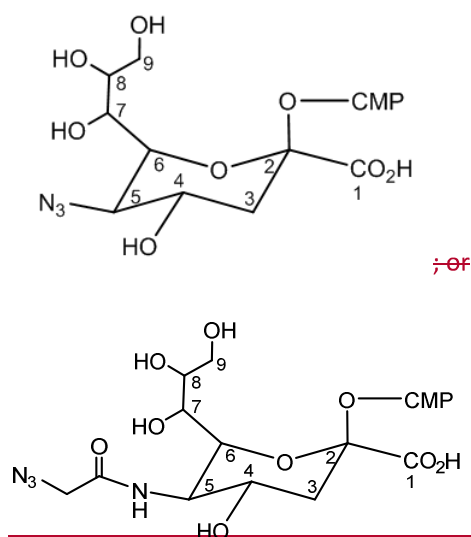


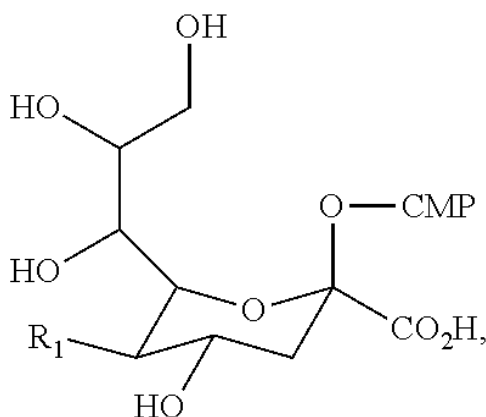
wherein R_2 is CH_3 , $CH_2CH_2(C=O)CH_3$, CH_2OH , OH or H .

~~7. The method of claim 9, wherein the terminal reactive moiety is an azide, wherein the effector moiety comprises an alkyne or is bound to a moiety comprising an alkyne, and wherein step (b) forms a triazole ring at or linked to the C5 position of the sialic acid derivative.~~

~~11. The method of claim 9 or 10, wherein neither the binding polypeptide nor the sialic acid derivative-conjugated binding polypeptide are treated with an oxidizing agent.~~

~~12. The method of any one of claims 9 to 11, wherein the CMP-sialic acid derivative has a structural formula selected from the following the structure of:~~





wherein R_1 is $\text{NH}(\text{C}=\text{O})\text{CH}_3$, $\text{NH}(\text{C}=\text{O})\text{CH}_2\text{CH}_2(\text{C}=\text{O})\text{CH}_3$, $\text{NH}(\text{C}=\text{O})\text{CH}_2\text{OH}$, $\text{NH}(\text{C}=\text{O})\text{CH}_2\text{N}_3$, or $\text{NH}(\text{C}=\text{O})\text{SH}$.

~~8. The method of any one of claims 10 to 12 claim 1, wherein the effector moiety comprises or is bound to a cyclooctyne.~~

~~14. The method of claim 13, wherein the cyclooctyne is an azadibenzocyclooctyne.~~

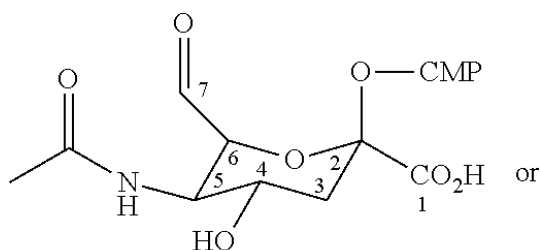
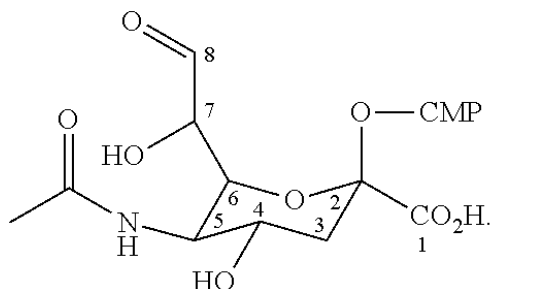
~~15. The method of any one of claims 10 to 14, wherein step (b) occurs at ambient temperatures.~~

~~16. The method of any one of claims 10 to 15, wherein step (b) is performed in the absence of copper.~~

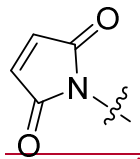
~~17. A method of making an effector moiety conjugated binding polypeptide comprising the steps of:~~

~~—(a) reacting a CMP-sialic acid derivative with a glycan of a binding polypeptide to form a sialic acid derivative-conjugated binding polypeptide; has one of the following structures:~~

~~——(b) reacting the sialic acid derivative-conjugated binding polypeptide with an effector moiety to form the effector moiety conjugated binding polypeptide, wherein a thioether bond is formed.~~



9. The method of ~~claim 17~~, wherein neither the binding polypeptide nor the sialic acid derivative-conjugated binding polypeptide are treated with an oxidizing agent.
- ~~19. The method of claim 17~~1, wherein the antibody or ~~18~~, wherein the sialic acid derivative antigen-binding fragment comprises a ~~terminal thiol moiety~~ S298N mutant.
- ~~20~~10. The method of claim 1, wherein the antibody or antigen-binding fragment comprises an A114N mutant in the CH1 domain.
- ~~11. The method of any one claim 1~~1, wherein the antibody or antigen-binding fragment comprises a Y300S mutant.
- ~~12. The method of claims 17 to 19~~12, wherein the antibody comprises a heavy chain of SEQ ID NO:14 and a light chain of SEQ ID NO:12.
- ~~13. The method of claim 1~~13, wherein step (a) is catalyzed by α 2,6 sialyltransferase.
- ~~14. The method of claim 1~~14, wherein the effector moiety comprises a ~~maleimide moiety~~-poly(ethylene glycol).
- ~~21~~15. The method of ~~any one of claims 17 to 20~~ claim 1, wherein the effector moiety is ~~bis-mannose-6-phosphate hexamannose~~ maleimide, lactose maleimide, or any other component comprising at least one maleimide moiety of selected from the following structural formula:



~~22. The method of any one of claims 1 to 21, wherein the effector moiety comprises one or more proteins, nucleic acids, lipids, carbohydrates, or combinations thereof.~~

~~23. The method of any one of claims 1 to 22, wherein the effector moiety comprises a glycan.~~

~~24. The method of any one of claims 1 to 23, wherein the effector moiety comprises one or more glycoproteins, glycopeptides, or glycolipids.~~

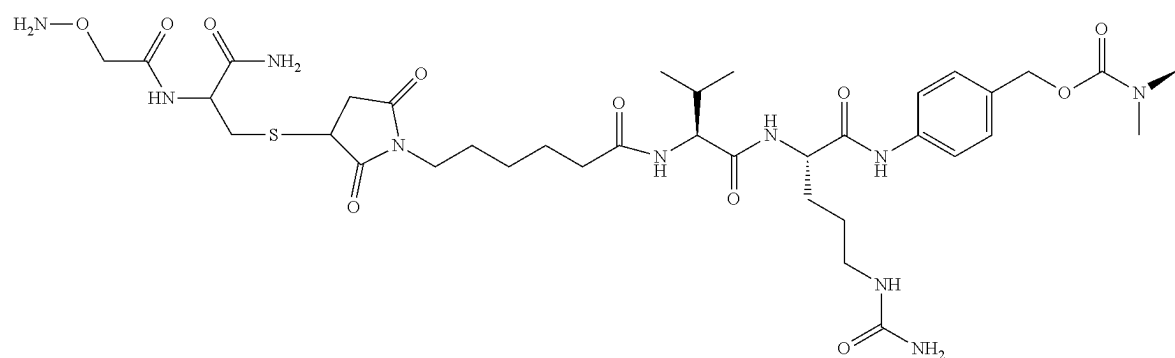
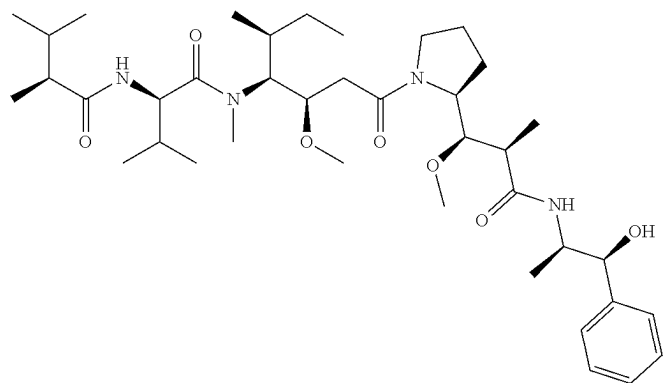
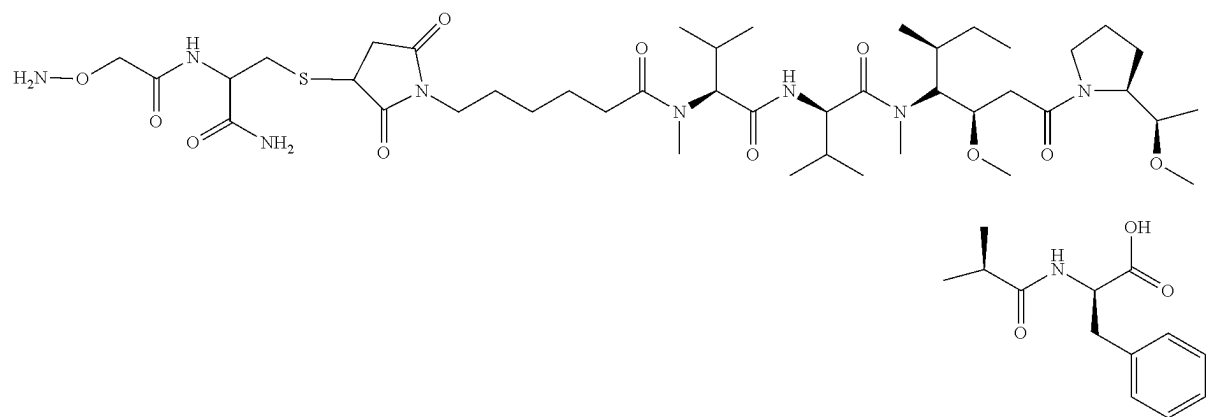
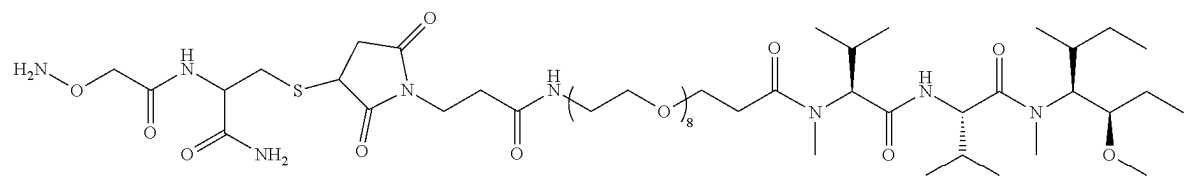
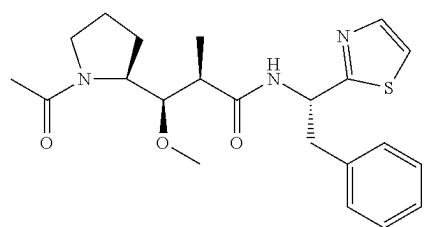
~~25. The method of any one of claims 1 to 24, wherein the binding protein has one or more native or engineered glycosylation sites.~~

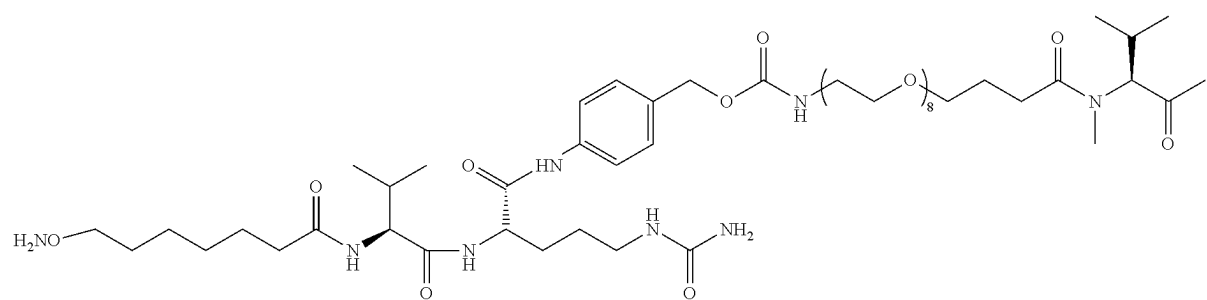
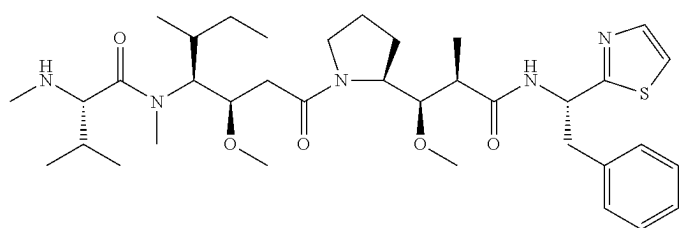
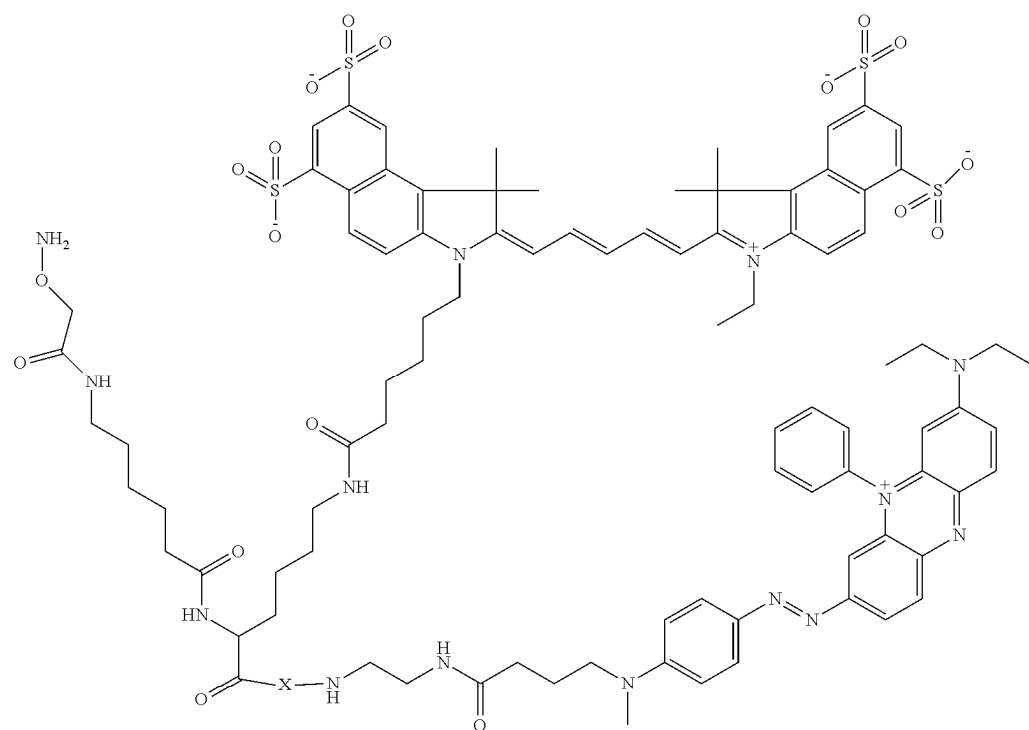
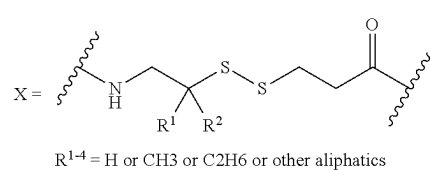
~~26. The method of any one of claims 1 to 25, further comprising achieving or modifying the glycosylation of the binding protein using one or more glycosyltransferases, one or more glycosidases, or a combination thereof.~~

~~27. The method of claim 26, wherein step (a) occurs in a reaction with sialyltransferase.~~

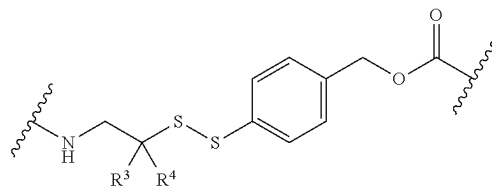
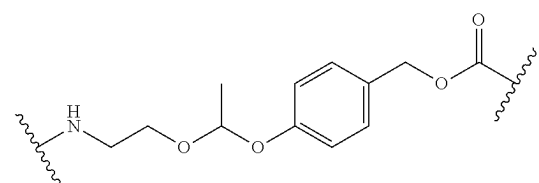
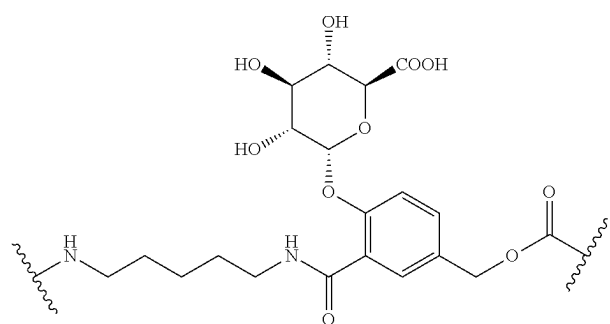
~~28. The method of claim 27, wherein the sialyltransferase is a mammalian sialyltransferase.~~

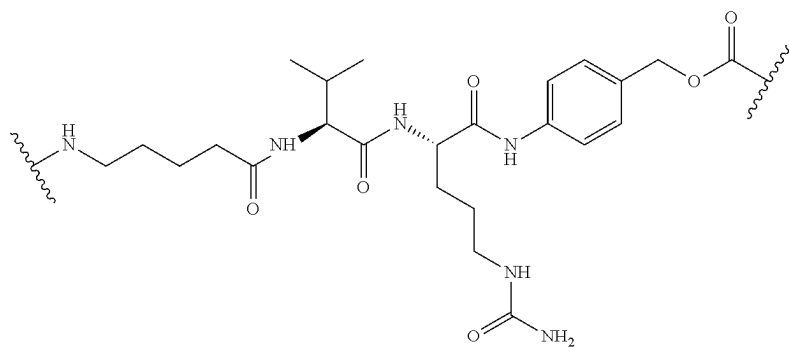
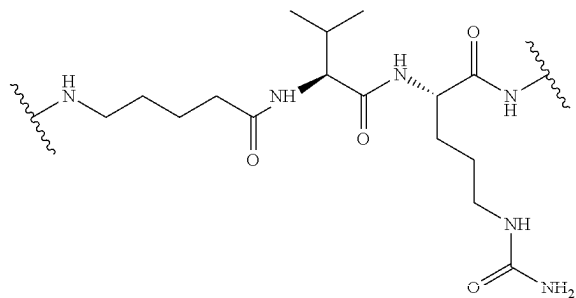
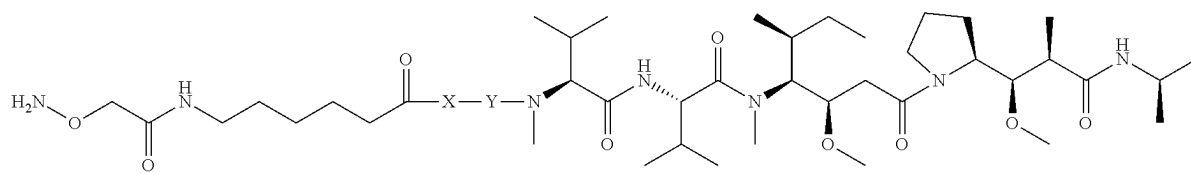
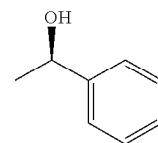
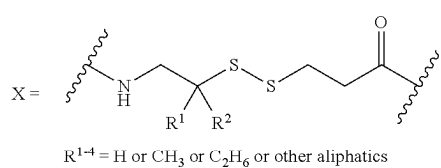
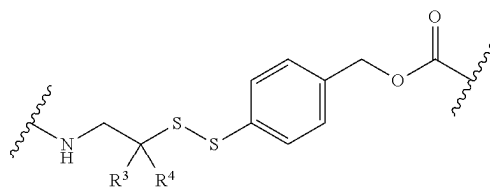
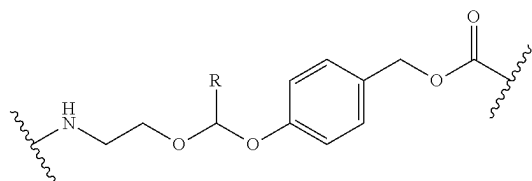
~~29. The method of claim 28, wherein the sialyltransferase is beta-galactoside alpha-2,6-sialyltransferase 1.~~





$\text{R}^{1-4} = \text{H or CH}_3 \text{ or C}_2\text{H}_6 \text{ or other aliphatics}$





$R = H \text{ or substituted or unsubstituted alkyl, alkylaryl groups}$

