CLAIMS

We claim:

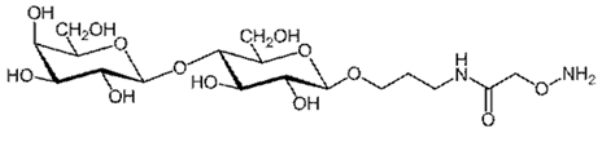
1. A method of making an effector moiety conjugated binding polypeptide comprising the steps of:

(a) reacting a cytidine monophosphate-sialic acid (CMP-sialic acid) derivative with a glycan of a binding polypeptide to form a sialic acid derivative-conjugated binding polypeptide;

(b) reacting the sialic acid derivative-conjugated binding polypeptide with an effector moiety to form the effector moiety conjugated binding polypeptide, wherein an imine bond is formed, and wherein neither the binding polypeptide nor the sialic acid derivative-conjugated binding polypeptide are treated with an oxidizing agent.

2. The method of claim 1, wherein the sialic acid derivative-conjugated binding polypeptide comprises a terminal keto or aldehyde moiety and/or wherein the effector moiety comprises a terminal aminooxy moiety, a terminal hydrazine, or is bound to a moiety comprising an aminooxy derivative .

3. The method of claim 1 or 2, wherein the effector moiety is selected from one or more of the following structural formulas:



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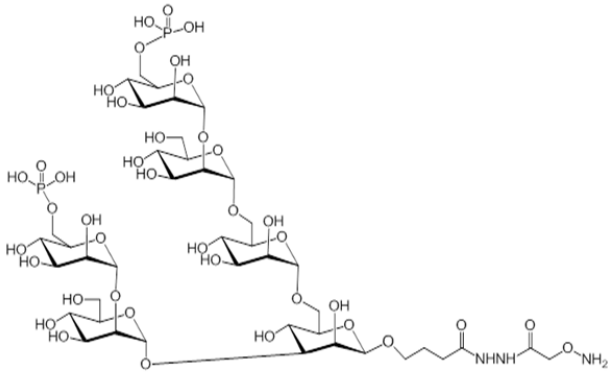

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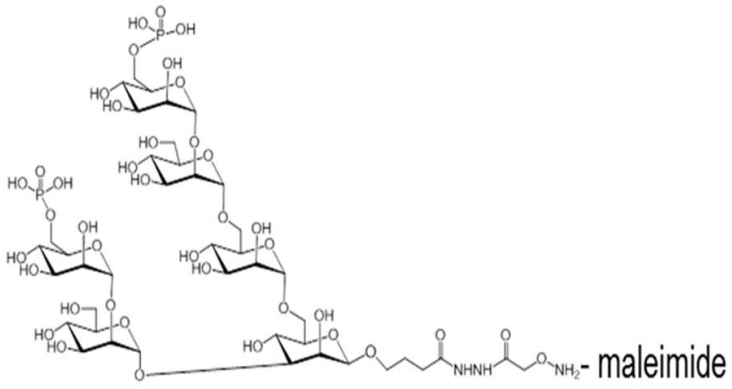

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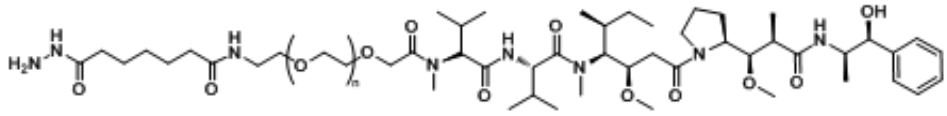
4. The method of any one of claims 1, 2 or 3, wherein step (b) results in the formation of an oxime bond, or wherein step (b) results in the formation of a hydrazone linkage.

5. The method of claim 4, wherein the effector moiety has one or more of the following structural formulas: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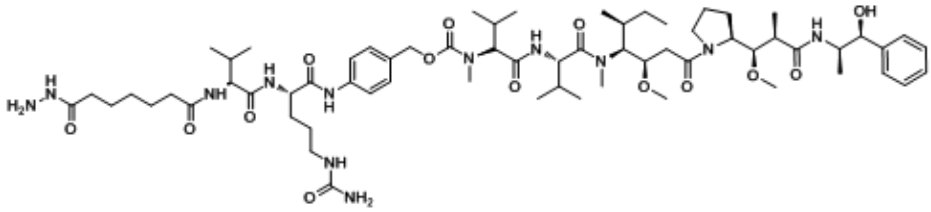
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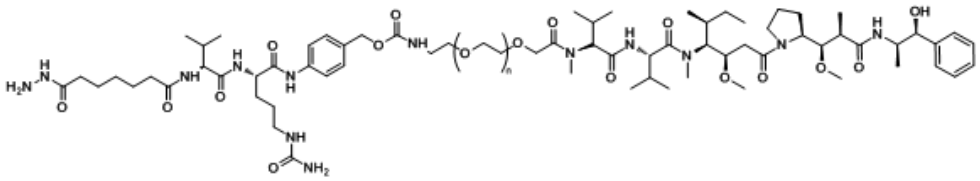
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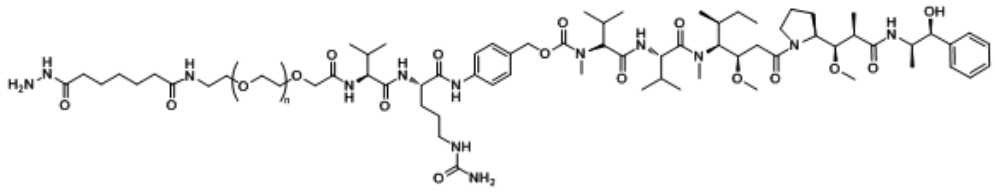
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6. A method of making an effector moiety conjugated binding polypeptide comprising the steps of:

(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;

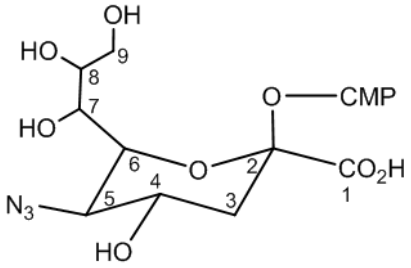
(b) reacting the sialic acid derivative-conjugated binding polypeptide with an effector moiety to form the effector moiety conjugated binding polypeptide using click chemistry.

7. The method of claim 6,

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, and/or

wherein neither the binding polypeptide nor the sialic acid derivative-conjugated binding polypeptide are treated with an oxidizing agent.

8. The method of any one of claims 6 or 7, wherein the CMP-sialic acid derivative has a structural formula selected from the following:



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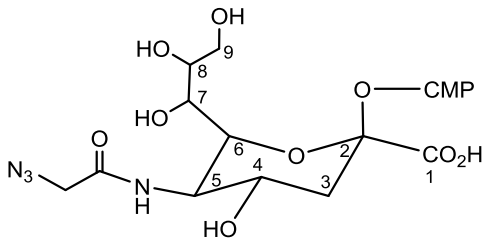

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9. The method of any one of claims 7 or 8, wherein the effector moiety comprises or is bound to a cyclooctyne.

10. The method of claim 9, wherein the cyclooctyne is an azadibenzocyclooctyne.

11. The method of any one of claims 7 or 8-10, wherein step (b) occurs at ambient temperatures.

12. The method of any one of claims 7 or 8-11, wherein step (b) is performed in the absence of copper.

13. 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;

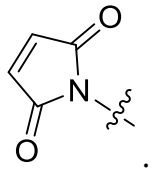
(b) reacting the sialic acid derivative-conjugated binding polypeptide with an effector moiety to form the effector moiety conjugated binding polypeptide, wherein a thioether bond is formed.

14. The method of claim 13, wherein neither the binding polypeptide nor the sialic acid derivative-conjugated binding polypeptide are treated with an oxidizing agent.

15. The method of claim 13 or 14, wherein the sialic acid derivative comprises a terminal thiol moiety.

16. The method of any one of claims 13 to 15, wherein the effector moiety comprises a maleimide moiety.

17. The method of any one of claims 13 to 16, wherein the effector moiety is bis-mannose-6-phosphate hexamannose maleimide, lactose maleimide, or any other component comprising at least one maleimide moiety of the following structural formula:



18. The method of any one of the preceding claims, wherein the effector moiety comprises one or more proteins, nucleic acids, lipids, carbohydrates, or combinations thereof.

19. The method of any one of the preceding claims, wherein the effector moiety comprises a glycan.

20. The method of any one of the preceding claims, wherein the effector moiety comprises one or more glycoproteins, glycopeptides, or glycolipids.

21. The method of any one of the preceding claims, wherein the binding protein has one or more native or engineered glycosylation sites.

22. The method of any one of the preceding claims, further comprising achieving or modifying the glycosylation of the binding protein using one or more glycosyltransferases, one or more glycosidases, or a combination thereof.

23. The method of claim 22, wherein step (a) occurs in a reaction with sialyltransferase.

24. The method of claim 23, wherein the sialyltransferase is a mammalian sialyltransferase.

25. The method of claim 24, wherein the sialyltransferase is beta-galactoside alpha-2,6-sialyltransferase 1.