Claims

1. A method of making a capsular saccharide glycoconjugate, comprising the steps of:

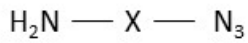
(a) reacting an isolated capsular saccharide, wherein said capsular saccharide is not a capsular saccharide from *Streptococcus pneumoniae* serotype 3, with 1,1'-carbonyldiimidazole (CDI), 1,1'-carbonyl-di-(1,2,4-triazole) (CDT), disuccinimidyl carbonate (DSC) or N-hydroxysuccinimidyl chloroformate and an azido linker in an aprotic solvent comprising 0.1% to 1% (v/v) water, to produce an activated azido saccharide,

(b) reacting a carrier protein with an agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group wherein the NHS moiety reacts with the amino groups of the carrier protein to form an amide linkage thereby obtaining an alkyne functionalized carrier protein,

(c) reacting the activated azido saccharide of step (a) with the alkyne functionalized carrier protein of step (b) by Cu^{+1} mediated azide-alkyne cycloaddition reaction to form a glycoconjugate.

2. The method of claim 1, wherein the isolated capsular saccharide is sized to a weight average molecular weight (Mw) between 200 kDa and 800 kDa before the activation step (a).

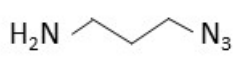
3. The method of claim 1 or claim 2, wherein said azido linker is a compound of formula (I),



(I)

wherein X is selected from the group consisting of $CH_2(CH_2)_n$, $(CH_2CH_2O)_mCH_2CH_2$, $NHCO(CH_2)_n$, $NHCO(CH_2CH_2O)_mCH_2CH_2$, $OCH_2(CH_2)_n$ and $O(CH_2CH_2O)_mCH_2CH_2$; where n is selected from 1 to 10 and m is selected from 1 to 4.

4. The method of claim 1 or claim 2, wherein said azido linker is a compound of formula (II),

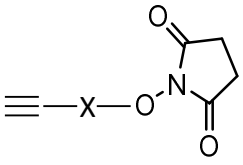


(II).

5. The method of any one of claims 1-4, wherein said agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group is an agent bearing an N-Hydroxysuccinimide (NHS) moiety and a terminal alkyne.

6. The method of any one of claims 1-4, wherein said agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group is an agent bearing an N-Hydroxysuccinimide (NHS) moiety and a cycloalkyne.

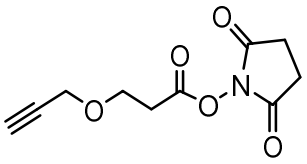
7. The method of any one of claims 1-4, wherein said agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group is a compound of formula (III),



(III)

where X is selected from the group consisting of $CH_2O(CH_2)_nCH_2C=O$ and $CH_2O(CH_2CH_2O)_m(CH_2)_nCH_2C=O$, where n is selected from 0 to 10 and m is selected from 0 to 4.

8. The method of any one of claims 1-4, wherein said agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group is a compound of formula (IV):



(IV).

9. The method of any one of claims 1-8, wherein at step a) the isolated capsular saccharide is reacted with CDI in an aprotic solvent comprising 0.1% to 1% (v/v) water.

10. The method of any one of claims 1-9, wherein at step a) 1,1'-carbonyldiimidazole (CDI), 1,1'-carbonyl-di-(1,2,4-triazole) (CDT), disuccinimidyl carbonate (DSC) or N-hydroxysuccinimidyl chloroformate activation is followed by the addition of water.

11. The method of any one of claims 1-10, wherein step a) further comprises reacting said 1,1'-carbonyldiimidazole (CDI), 1,1'-carbonyl-di-(1,2,4-triazole) (CDT), disuccinimidyl carbonate (DSC) or N-hydroxysuccinimidyl chloroformate activated

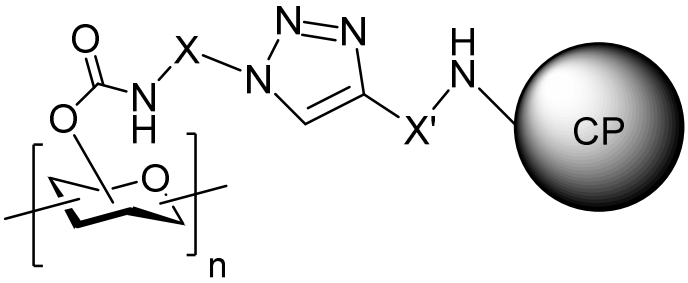
saccharide with an amount of azido linker that is between 0.01-10 molar equivalent to the amount of saccharide Repeat Unit of the capsular saccharide.

12. The method of any one of claims 1-11, wherein the conjugation reaction c) is carried out in aqueous buffer in the presence of copper (I) as catalyst.

13. The method of any one of claims 1-12, wherein the method further comprises the step of purifying the glycoconjugate after it is produced.

14. A capsular saccharide glycoconjugate produced according to the method of any one of claims 1 to 13.

15. A capsular saccharide glycoconjugate comprising a capsular saccharide, wherein said capsular saccharide is not a capsular saccharide from *Streptococcus pneumoniae* serotype 3, covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (VII):



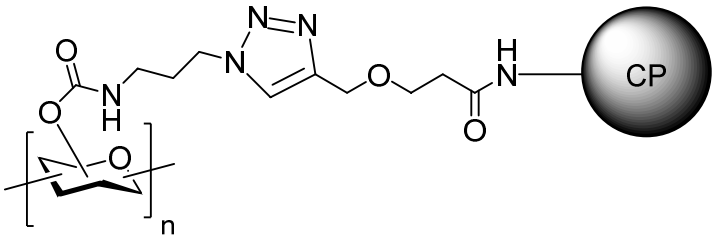
(VII),

wherein X is selected from the group consisting of $CH_2(CH_2)_{n'}$, $(CH_2CH_2O)_mCH_2CH_2$, $NHCO(CH_2)_{n'}$, $NHCO(CH_2CH_2O)_mCH_2CH_2$, $OCH_2(CH_2)_{n'}$ and $O(CH_2CH_2O)_mCH_2CH_2$; where n' is selected from 1 to 10 and m is selected from 1 to 4,

and wherein X' is selected from the group consisting of $CH_2O(CH_2)_{n''}CH_2C=O$ and $CH_2O(CH_2CH_2O)_{m'}(CH_2)_{n''}CH_2C=O$, where n'' is selected from 0 to 10 and m' is selected from 0 to 4.

16. The capsular saccharide glycoconjugate of claim 15, comprising a capsular saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (VII), wherein X is $CH_2(CH_2)_{n'}$, where n' is 2 and wherein X' is $CH_2O(CH_2)_{n''}CH_2C=O$ where n'' is 1.

17. A capsular saccharide glycoconjugate comprising a capsular saccharide, wherein said capsular saccharide is not a capsular saccharide from *Streptococcus pneumoniae* serotype 3, covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (VIII),



(VIII).

18. The capsular saccharide glycoconjugate of any one of claims 14 to 17, wherein the weight average molecular weight (Mw) of said capsular saccharide before conjugation is between 10 kDa and 2,000 kDa.

19. The capsular saccharide glycoconjugate of any one of claims 14 to 18, having a weight average molecular weight (Mw) of between 250 kDa and 20,000 kDa.

20. The capsular saccharide glycoconjugate of any one of claims 14 to 19, wherein the degree of conjugation of the capsular saccharide glycoconjugate is between 2 and 15.

21. The capsular saccharide glycoconjugate of any one of claims 14 to 20, wherein the ratio of capsular saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 3.0.

22. The capsular saccharide glycoconjugate of any one of claims 14 to 21, wherein the said capsular saccharide glycoconjugate comprises at least one covalent linkage between the carrier protein and the saccharide for every 5 to 10 saccharide repeat units of the saccharide.

23. The capsular saccharide glycoconjugate of any one of claims 14 to 22, wherein said carrier protein is CRM_{197}, SCP, DT (Diphtheria Toxoid), TT (Tetanus Toxoid), or PD (*H. influenzae* protein D).

24. The method of making a capsular saccharide glycoconjugate of any one of claims 1 to 13 or the capsular saccharide glycoconjugate of any one of claims 14 to 23, wherein said capsular saccharide is a capsular saccharide from a pathogenic bacteria.

25. The method of making a capsular saccharide glycoconjugate of any one of claims 1 to 13 or the capsular saccharide glycoconjugate of any one of claims 14 to 23, wherein said capsular saccharide is a capsular saccharide from *S. pneumoniae*.

26. An immunogenic composition comprising the capsular saccharide glycoconjugate of any one of claims 14 to 25.