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(54) Title: IMMUNOGENIC COMPOSITIONS COMPRISING CONJUGATED CAPSULAR SACCHARIDE ANTIGENS AND USES THEREOF

(57) Abstract: The present invention relates to new conjugated capsular saccharide antigens (glycoconjugates), immunogenic compositions comprising said glycoconjugates and uses thereof.

Immunogenic compositions comprising conjugated capsular saccharide antigens and uses thereof

Field of the Invention

5 The present invention relates to new conjugated capsular saccharide antigens (glycoconjugates), immunogenic compositions comprising said glycoconjugates and uses thereof. Immunogenic compositions of the present invention will typically comprise glycoconjugates, wherein the saccharides are derived from bacterial capsular polysaccharide antigens, in particular a capsular polysaccharide derived from pathogenic
10 bacteria. The invention also relates to vaccination of human subjects, in particular infants and elderly, against infections using said glycoconjugates.

Background of the Invention

Both Gram-positive and Gram-negative bacteria may produce an extracellular
15 compartment, the capsule, which covers the bacterial cell and often prevents the reaction of underlying cell surface antigens with their homologous antibodies. Capsules are found in several bacteria of medical importance, especially in extraintestinal and invasive strains. Bacterial capsules are largely composed of polysaccharides. They form a gelatinous mass around the cell. Capsular polysaccharides are important immunogens
20 and has led to them being an important component in the design of vaccines. They have proved useful in eliciting immune responses especially when linked to carrier proteins.

The approach to increasing immunogenicity of poorly immunogenic molecules by conjugating these molecules to “carrier” molecules has been utilized successfully for decades (see, e.g., Goebel et al. (1939) J. Exp. Med. 69: 53). For example, many
25 immunogenic compositions have been described in which purified capsular polymers have been conjugated to carrier proteins to create more effective immunogenic compositions by exploiting this “carrier effect.” Schneerson et al. (1984) Infect. Immun. 45: 582-591). Conjugation has also been shown to bypass the poor antibody response usually observed in infants when immunized with a free polysaccharide (Anderson et al.
30 (1985) J. Pediatr. 107: 346; Insel et al. (1986) J. Exp. Med. 158: 294).

Conjugates have been successfully generated using various cross-linking or coupling reagents, such as homobifunctional, heterobifunctional, or zero-length crosslinkers. Many methods are currently available for coupling immunogenic molecules, such as saccharides, proteins, and peptides, to peptide or protein carriers. Most methods
35 create amine, amide, urethane, isothioureia, or disulfide bonds, or in some cases

thioethers. A disadvantage to the use of cross-linking or coupling reagents which introduce reactive sites into the side chains of reactive amino acid molecules on carrier and/or immunogenic molecules is that the reactive sites, if not neutralized, are free to react with any unwanted molecule either *in vitro* (thus potentially adversely affecting the functionality or stability of the conjugates) or *in vivo* (thus posing a potential risk of adverse events in persons or animals immunized with the preparations). Such excess reactive sites can be reacted or “capped”, so as to inactivate these sites, utilizing various known chemical reactions, but these reactions may be otherwise disruptive to the functionality of the conjugates.

Thus, there remains a need for new glycoconjugates appropriately capped and methods to prepare said conjugates, such that the functionality is preserved, and the conjugate retains the ability to elicit the desired immune response.

The present inventors have found a new and efficacious method to generate glycoconjugates. The method allows for generating conjugates with very low free saccharide and a good yield. Furthermore, the present inventors have found that some *Streptococcus pneumoniae* serotypes (e.g. 35B and 29 polysaccharides) pose particular challenges to generate conjugates due to their unique structure. There is a need for immunogenic *Streptococcus pneumoniae* serotypes polysaccharide-carrier protein conjugates and an improved process for making the same.

Summary of the Invention

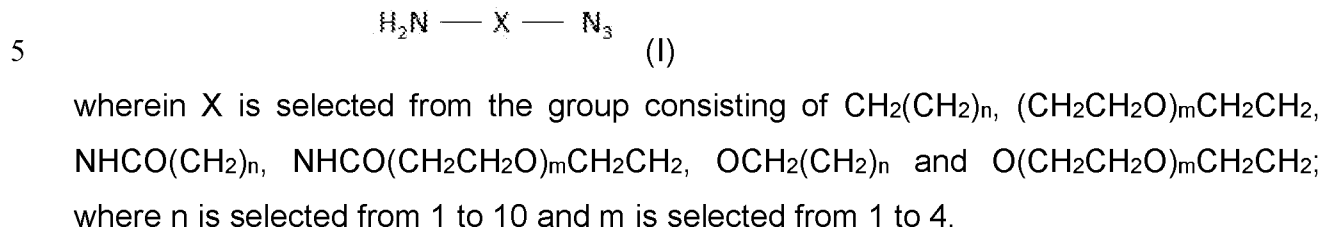
In an aspect, the present invention pertains to a method of making a capsular saccharide glycoconjugate, comprising the steps of:

- (a) reacting an isolated capsular saccharide with a carbonic acid derivative and an azido linker in an aprotic solvent to produce an activated azido saccharide,
- (b) reacting a carrier protein with an agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group where the NHS moiety reacts with the amino groups to form an amide linkage thereby obtaining an alkyne functionalized carrier protein,
- (c) reacting the activated azido saccharide of step (a) with the activated alkyne-carrier protein of step (b) by Cu^{+1} mediated azide-alkyne cycloaddition reaction to form a glycoconjugate.

In an aspect, the isolated saccharide is sized before the activation step (a).

In an aspect, the carbonic acid derivative is selected from the group consisting of 1,1'-carbonyldiimidazole (CDI), 1,1'-carbonyl-di-(1,2,4-triazole) (CDT), disuccinimidyl carbonate (DSC) and N-hydroxysuccinimidyl chloroformate.

In an aspect, said azido linker is a compound of formula (I),



10 In an aspect, 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.

In an aspect, step a) comprises reacting the capsular saccharide with a carbonic acid derivative followed by reacting the carbonic acid derivative-activated capsular saccharide with an azido linker in an aprotic solvent to produce an activated azido capsular saccharide.

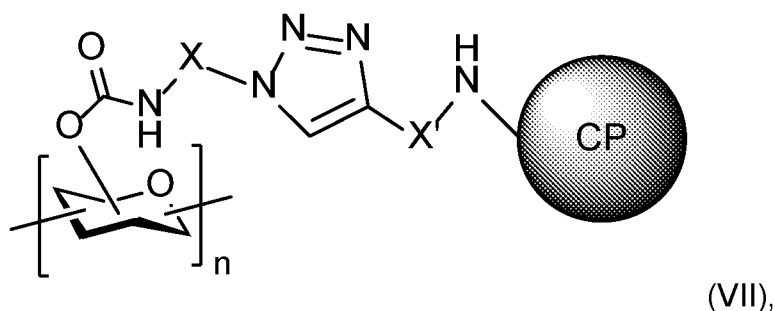
15 In an aspect, the conjugation reaction c) is carried out in aqueous buffer in the presence of copper (I) as catalyst.

In an aspect, following step c), the method further comprises a step of capping the unreacted azido groups remained in the conjugate with an azido group capping agent.

20 In an aspect, following step c), the method further comprises a step of capping the unreacted alkyne groups remained in the conjugate with an alkyne group capping agent.

In an aspect, the present invention pertains to a capsular saccharide glycoconjugate produced according said methods.

25 In a further aspect, the present invention pertains to a capsular saccharide glycoconjugate comprising a capsular saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (VII):

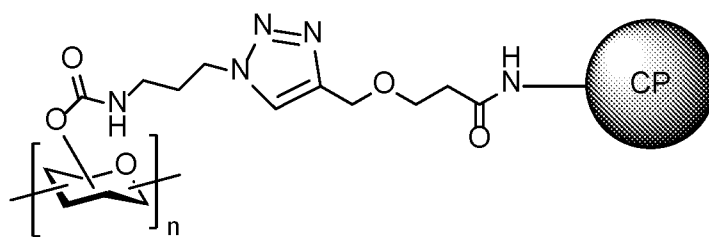


wherein X is selected from the group consisting of $\text{CH}_2(\text{CH}_2)_{n'}$, $(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$, $\text{NHCO}(\text{CH}_2)_{n'}$, $\text{NHCO}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$, $\text{OCH}_2(\text{CH}_2)_{n'}$ and $\text{O}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$; 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 $\text{CH}_2\text{O}(\text{CH}_2)_{n''}\text{CH}_2\text{C}=\text{O}$, $\text{CH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_{m'}(\text{CH}_2)_{n''}\text{CH}_2\text{C}=\text{O}$, where n'' is selected from 0 to 10 and m' is selected from 0 to 4.

In a particular aspect, the invention is directed to a capsular saccharide glycoconjugate comprising a capsular saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (VII), wherein X is $\text{CH}_2(\text{CH}_2)_{n'}$, where n' is 2 and wherein X' is $\text{CH}_2\text{O}(\text{CH}_2)_{n''}\text{CH}_2\text{C}=\text{O}$ where n'' is 1.

In a particular aspect, the invention pertains to a capsular saccharide glycoconjugate comprising a capsular saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (VIII),



(VIII).

Figures

Figure 1 shows a repeating polysaccharide structure of the *S. pneumoniae* serotype 35B capsular polysaccharide.

Figure 2 shows a repeating polysaccharide structure of the *S. pneumoniae* serotype 29 capsular polysaccharide.

Figure 3 shows a general scheme for the preparation of glycoconjugate of the invention prepared using click chemistry and using 3-Azido-1-propylamine as azido linker. CP = Carrier Protein, CDI = 1,1'-carbonyldiimidazole.

Detailed description of the Invention

The present inventors have developed new polysaccharide-carrier protein conjugates and processes for making these conjugates. The method to generate glycoconjugates has been found to allow for producing glycoconjugates with very low free saccharide and a good yield.

Furthermore, the present inventors have found that *Streptococcus pneumoniae* serotypes 35B and 29 polysaccharides pose particular challenges to generate conjugates. *Streptococcus pneumoniae* serotypes 35B and 29 polysaccharides have been found to be cleaved during activation with periodate, which is the classical oxidant used in the commonly used reductive amination process. It appears that periodate oxidation occurs on the backbone of serotypes 35B and 29 polysaccharides and cleaves the mannitol or ribitol, leading to size reduction. This leads to several issues, including difficulties to obtain activated serotypes 35B and 29 polysaccharides of a certain size. The activation with periodate results in a decrease in polysaccharide Mw drastically limiting the effective activation range.

WO 2020/247299 suggests decreasing the amount of periodate and using a certain temperature range for the conjugation reaction in order to allegedly improve serotype 35B conjugates attributes. However, such a solution has been found to lead to low yield and/or high free saccharide. The present inventors have developed new *Streptococcus pneumoniae* serotypes 35B and 29 polysaccharide-carrier protein conjugates and improved processes for making these conjugates which does not suffer from these deficiencies.

1. Glycoconjugates of the invention

The present invention is directed in part to conjugated bacterial capsular saccharide antigens (also named glycoconjugates). For the purpose of the invention the term 'glycoconjugate' indicates a capsular saccharide (in particular a bacterial capsular saccharide) linked covalently to a carrier protein.

1.1 Capsular saccharide of the invention

The term "saccharide" throughout this specification may indicate polysaccharide or oligosaccharide and includes both. In an embodiment, saccharide of the invention may be oligosaccharides. Oligosaccharides have a low number of repeat units (typically 5-15 repeat units) and are typically derived synthetically or by hydrolysis of polysaccharides. Preferably though, all of the saccharides of the present invention and in the immunogenic compositions of the present invention are polysaccharides. High molecular weight polysaccharides are able to induce certain antibody immune responses due to the epitopes present on the antigenic surface. The isolation and purification of high molecular weight capsular polysaccharides is preferably contemplated for use in the conjugates,

compositions and methods of the present invention. Therefore, in a preferred embodiment of the present invention, the saccharide is a polysaccharide.

Preferably, the saccharide used in the present invention is a bacterial capsular saccharide (also named 'capsular saccharide' herein). Capsules are found in several bacteria of medical importance. Bacterial capsules are largely composed of polysaccharides. Capsular saccharides are prepared by standard techniques known to those of ordinary skill in the art.

In a most preferred embodiment of the present invention, the saccharide is a *S. pneumoniae* capsular polysaccharide.

In an embodiment, the capsular saccharide used in the present invention is a synthetic carbohydrate.

In a preferred embodiment though, the source of bacterial capsular saccharide according to this invention can be bacterial cells. Bacterial strains which can be used as source of capsular saccharide may be obtained from established culture collections (such as for example from the American Type Culture Collection (ATCC, Manassas, VA USA) or the Streptococcal Reference Laboratory (Centers for Disease Control and Prevention, Atlanta, GA USA)) or clinical specimens.

Bacterial capsular saccharides can be obtained directly from bacteria using isolation procedures known to one of ordinary skill in the art (see for example methods disclosed in US2006/0228380, US2006/0228381, US2007/0184071, US2007/0184072, US2007/0231340, and US2008/0102498 and WO2008/118752). They can also be produced using synthetic protocols known to the man skilled in the art.

In case the bacterial capsular saccharide is obtained directly from bacteria, the bacterial cells can be grown in a medium. Following fermentation of bacterial cells that produce the capsular saccharide, the bacterial cells can be lysed to produce a cell lysate. The capsular saccharide may then be isolated from the cell lysate using purification techniques known in the art, including the use of centrifugation, depth filtration, precipitation, ultra-filtration, treatment with activate carbon, diafiltration and/or column chromatography (see, for example, US2006/0228380, US2006/0228381 and WO2008/118752). The purified capsular saccharide can then be used for the preparation of immunogenic conjugates.

The isolated capsular saccharide obtained by purification can be characterized by different parameters including, for example the weight average molecular weight (Mw). The molecular weight of the polysaccharide can be measured by Size Exclusion

Chromatography (SEC) combined with Multiangle Laser Light Scattering detector (MALLS).

In an embodiment, the capsular saccharide used in the method of making or part of the glycoconjugate of the present invention is a capsular saccharide from a pathogenic bacteria. Preferably, the capsular saccharide used in the present invention is a capsular saccharide from a pathogenic *Streptococcus*, a pathogenic *Staphylococcus*, a pathogenic *Enterococcus*, a pathogenic *Bacillus*, a pathogenic *Corynebacterium*, a pathogenic *Listeria*, a pathogenic *Erysipelothrix*, a pathogenic *Clostridium*, a pathogenic *Haemophilus*, a pathogenic *Neisseria* or a pathogenic *Escherichia*. More preferably, the capsular saccharide used in the present invention is a capsular saccharide from a pathogenic *Streptococcus*, a pathogenic *Neisseria* or a pathogenic *Escherichia*.

In an embodiment, the capsular saccharide used in the present invention is a capsular saccharide from *Aeromonas hydrophila* and other species (spp.); *Bacillus anthracis*; *Bacillus cereus*; *Botulinum* neurotoxin producing species of *Clostridium*; *Brucella abortus*; *Brucella melitensis*; *Brucella suis*; *Burkholderia mallei* (formally *Pseudomonas mallei*); *Burkholderia pseudomallei* (formerly *Pseudomonas pseudomallei*); *Campylobacter jejuni*; *Chlamydia psittaci*; *Chlamydia trachomatis*; *Clostridium botulinum*; *Clostridium difficile*; *Clostridium perfringens*; *Coccidioides immitis*; *Coccidioides posadasii*; *Cowdria ruminantium* (Heartwater); *Coxiella burnetii*; *Enterococcus faecalis*; *Enterovirulent Escherichia coli* group (EEC Group) such as *Escherichia coli* - enterotoxigenic (ETEC), *Escherichia coli* - enteropathogenic (EPEC), *Escherichia coli* - O157:H7 enterohemorrhagic (EHEC), and *Escherichia coli* - enteroinvasive (EIEC); *Ehrlichia* spp. such as *Ehrlichia chaffeensis*; *Francisella tularensis*; *Legionella pneumophila*; *Liberobacter africanus*; *Liberobacter asiaticus*; *Listeria monocytogenes*; miscellaneous enterics such as *Klebsiella*, *Enterobacter*, *Proteus*, *Citrobacter*, *Aerobacter*, *Providencia*, and *Serratia*; *Mycobacterium bovis*; *Mycobacterium tuberculosis*; *Mycoplasma capricolum*; *Mycoplasma mycoides* ssp *mycoides*; *Peronosclerosporaphilippinensis*; *Phakopsora pachyrhizi*; *Plesiomonas shigelloides*; *Ralstonia solanacearum* race 3, biovar 2; *Rickettsia prowazekii*; *Rickettsia rickettsii*; *Salmonella* spp.; *Schlerophthora rayssiae* var *zeae*; *Shigella* spp.; *Staphylococcus aureus*; *Streptococcus*; *Synchytrium endobioticum*; *Vibrio cholerae* non-01; *Vibrio cholerae* 01; *Vibrio parahaemolyticus* and other *Vibrios*; *Vibrio vulnificus*; *Xanthomonas oryzae*; *Xylella fastidiosa* (citrus variegated chlorosis strain); *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*; or *Yersinia pestis*. Preferably, the

capsular saccharide used in the present invention is a capsular saccharide from *Enterococcus faecalis*, *Escherichia coli*, *Staphylococcus aureus* or *Streptococcus*.

In an embodiment, the capsular saccharide used in the present invention is a capsular saccharide from *Haemophilus influenzae*, *Neisseria meningitidis*, *S. pneumoniae*, *S. pyogenes*, *S. agalactiae*, Group C & G *Streptococci* or *Escherichia coli*. More preferably, the capsular saccharide used in the present invention is a capsular saccharide from *Neisseria meningitidis*, *S. pneumoniae*, *S. agalactiae* or *Escherichia coli*. Even more preferably, the capsular saccharide used in the present invention is a capsular saccharide from *S. pneumoniae* or *S. agalactiae*. Even more preferably, the capsular saccharide used in the present invention is a capsular saccharide from *S. pneumoniae*.

In an embodiment, the capsular saccharide used in the present invention is a capsular saccharide from *Staphylococcus aureus*. In an embodiment, the capsular saccharide used in the present invention is a capsular saccharide from *Staphylococcus aureus* type 5 or *Staphylococcus aureus* type 8.

In an embodiment, the capsular saccharide used in the present invention is a capsular saccharide from *Enterococcus faecalis*. In yet a further embodiment, the capsular saccharide used in the present invention is a capsular saccharide from *Haemophilus influenzae* type b.

In a further embodiment, the capsular saccharide used in the present invention is a capsular saccharide from *Neisseria meningitidis*. In an embodiment the capsular saccharide used in the present invention is a capsular saccharide from *N. meningitidis* serogroup A (MenA), *N. meningitidis* serogroup W135 (MenW135), *N. meningitidis* serogroup Y (MenY), *N. meningitidis* serogroup X (MenX) or *N. meningitidis* serogroup C (MenC). In an embodiment the capsular saccharide used in the present invention is a capsular saccharide from *N. meningitidis* serogroup A (MenA). In an embodiment the capsular saccharide used in the present invention is a capsular saccharide from *N. meningitidis* serogroup W135 (MenW135). In an embodiment the capsular saccharide used in the present invention is a capsular saccharide from *N. meningitidis* serogroup Y (MenY). In an embodiment the capsular saccharide used in the present invention is a capsular saccharide from *N. meningitidis* serogroup C (MenC). In an embodiment the capsular saccharide used in the present invention is a capsular saccharide from *N. meningitidis* serogroup X (MenX).

In a further embodiment, the capsular saccharide used in the present invention is a capsular saccharide from *Escherichia coli*. In a further embodiment, the capsular

saccharide used in the present invention is a capsular saccharide from *Enterococcus faecalis*.

In a further embodiment, the capsular saccharide used in the present invention is a capsular saccharide from *Streptococcus agalactiae* (Group B streptococcus (GBS)). In some embodiments, the capsular saccharide used in the present invention is a capsular saccharide from GBS type Ia, Ib, II, III, IV, V, VI, VII or VIII. In some embodiments, the capsular saccharide used in the present invention is a capsular saccharide from GBS types Ia, Ib, II, III or V.

In a further embodiment, the capsular saccharide used in the present invention is a capsular saccharide from *Escherichia coli*. In an embodiment, the capsular saccharide used in the present invention is a capsular saccharide from an *Escherichia coli* part of the Enterovirulent *Escherichia coli* group (EEC Group) such as *Escherichia coli* - enterotoxigenic (ETEC), *Escherichia coli* - enteropathogenic (EPEC), *Escherichia coli* - O157:H7 enterohemorrhagic (EHEC), or *Escherichia coli* - enteroinvasive (EIEC). In an embodiment, the capsular saccharide used in the present invention is a capsular saccharide from an Uropathogenic *Escherichia coli* (UPEC).

In an embodiment, the capsular saccharide used in the present invention is a capsular saccharide from an *Escherichia coli* serotype selected from the group consisting of serotypes O157:H7, O26:H11, O111:H- and O103:H2. In an embodiment, the capsular saccharide used in the present invention is a capsular saccharide from an *Escherichia coli* serotype selected from the group consisting of serotypes O6:K2:H1 and O18:K1:H7. In an embodiment, the capsular saccharide used in the present invention is a capsular saccharide from an *Escherichia coli* serotype selected from the group consisting of serotypes O45:K1, O17:K52:H18, O19:H34 and O7:K1. In an embodiment, the capsular saccharide used in the present invention is a capsular saccharide from an *Escherichia coli* serotype O104:H4. In an embodiment, the capsular saccharide used in the present invention is a capsular saccharide from an *Escherichia coli* serotype O1:K12:H7. In an embodiment, the capsular saccharide used in the present invention is a capsular saccharide from an *Escherichia coli* serotype O127:H6. In an embodiment, the capsular saccharide used in the present invention is a capsular saccharide from an *Escherichia coli* serotype O139:H28. In an embodiment, the capsular saccharide used in the present invention is a capsular saccharide from an *Escherichia coli* serotype O128:H2.

In a preferred embodiment, the capsular saccharide used in the present invention is a capsular saccharide from *Streptococcus pneumoniae*. Preferably, the capsular saccharide used in the present invention is a capsular saccharide from a *Streptococcus*

pneumoniae serotype selected from the group consisting of serotypes 1, 2, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9V, 9N, 10A, 10B, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 19A, 19F, 20, 21, 22A, 22F, 23A, 23B, 23F, 24B, 24F, 27, 29, 31, 33B, 33F, 34, 35B, 35F, 38, 72 and 73.

5 In a preferred embodiment, the capsular saccharide used in the present invention is not a capsular saccharide from *Streptococcus pneumoniae* serotype 3.

In an embodiment, the capsular saccharide used in the present invention is a capsular saccharide from *Streptococcus pneumoniae* serotype 1. In an embodiment, the capsular saccharide used in the present invention is a capsular saccharide from
10 *Streptococcus pneumoniae* serotype 2. In an embodiment, the capsular saccharide used in the present invention is a capsular saccharide from *Streptococcus pneumoniae* serotype 4. In an embodiment, the capsular saccharide used in the present invention is a capsular saccharide from *Streptococcus pneumoniae* serotype 5. In an embodiment, the capsular saccharide used in the present invention is a capsular saccharide from
15 *Streptococcus pneumoniae* serotype 6A. In an embodiment, the capsular saccharide used in the present invention is a capsular saccharide from *Streptococcus pneumoniae* serotype 6B. In an embodiment, the capsular saccharide used in the present invention is a capsular saccharide from *Streptococcus pneumoniae* serotype 7C. In an embodiment, the capsular saccharide used in the present invention is a capsular saccharide from
20 *Streptococcus pneumoniae* serotype 7F. In an embodiment, the capsular saccharide used in the present invention is a capsular saccharide from *Streptococcus pneumoniae* serotype 8. In an embodiment, the capsular saccharide used in the present invention is a capsular saccharide from *Streptococcus pneumoniae* serotype 9V. In an embodiment, the capsular saccharide used in the present invention is a capsular saccharide from
25 *Streptococcus pneumoniae* serotype 9N. In an embodiment, the capsular saccharide used in the present invention is a capsular saccharide from *Streptococcus pneumoniae* serotype 10A. In an embodiment, the capsular saccharide used in the present invention is a capsular saccharide from *Streptococcus pneumoniae* serotype 10B. In an embodiment, the capsular saccharide used in the present invention is a capsular
30 saccharide from *Streptococcus pneumoniae* serotype 11A. In an embodiment, the capsular saccharide used in the present invention is a capsular saccharide from *Streptococcus pneumoniae* serotype 12F. In an embodiment, the capsular saccharide used in the present invention is a capsular saccharide from *Streptococcus pneumoniae* serotype 14. In an embodiment, the capsular saccharide used in the present invention is
35 a capsular saccharide from *Streptococcus pneumoniae* serotype 15A. In an embodiment,

[illegible]

is a capsular saccharide from *Streptococcus pneumoniae* serotype 33F. In an embodiment, the capsular saccharide used in the present invention is a capsular saccharide from *Streptococcus pneumoniae* serotype 34. In an embodiment, the capsular saccharide used in the present invention is a capsular saccharide from *Streptococcus pneumoniae* serotype 35B. In an embodiment, the capsular saccharide used in the present invention is a capsular saccharide from *Streptococcus pneumoniae* serotype 35F. In an embodiment, the capsular saccharide used in the present invention is a capsular saccharide from *Streptococcus pneumoniae* serotype 38. In an embodiment, the capsular saccharide used in the present invention is a capsular saccharide from *Streptococcus pneumoniae* serotype 72. In an embodiment, the capsular saccharide used in the present invention is a capsular saccharide from *Streptococcus pneumoniae* serotype 73.

In a preferred embodiment, the capsular saccharide used in the present invention is a capsular saccharide from *Streptococcus pneumoniae* serotype 35B. In another preferred embodiment, the capsular saccharide used in the present invention is a capsular saccharide from *Streptococcus pneumoniae* serotype 29.

In a preferred embodiment, the capsular saccharide used in the present invention (purified before further treatment) has a weight average molecular weight between 50 kDa and 5000 kDa. In a preferred embodiment, the capsular saccharide used in the present invention has a weight average molecular weight between 500 kDa and 5000 kDa. In another preferred embodiment, the capsular saccharide used in the present invention has a weight average molecular weight between 1000 kDa and 5000 kDa.

Any whole number integer within any of the above ranges is contemplated as an embodiment of the disclosure.

Preferably, in order to generate glycoconjugates with advantageous filterability characteristics, immunogenicity and/or yields, sizing of the saccharide to a target molecular weight range is performed prior to the conjugation to a carrier protein.

Advantageously, the size of the purified capsular saccharide is reduced while preserving critical features of the structure of the polysaccharide. Mechanical or chemical sizing maybe employed.

In an embodiment, the size of the purified capsular saccharide is reduced by chemical hydrolysis. Chemical hydrolysis maybe conducted using a mild acid (e.g acetic acid, formic acid, propanoic acid). In an embodiment, chemical hydrolysis is conducted using formic acid. In an embodiment, chemical hydrolysis is conducted using propanoic acid. In a preferred embodiment, chemical hydrolysis is conducted using acetic acid.

Chemical hydrolysis may also be conducted using a diluted strong acid (such as diluted hydrochloric acid, diluted sulfuric acid, diluted phosphoric acid, diluted nitric acid or diluted perchloric acid). In an embodiment, chemical hydrolysis is conducted using diluted hydrochloric acid. In an embodiment, chemical hydrolysis is conducted using diluted sulfuric acid. In an embodiment, chemical hydrolysis is conducted using diluted phosphoric acid. In an embodiment, chemical hydrolysis is conducted using diluted nitric acid. In an embodiment, chemical hydrolysis is conducted using diluted perchloric acid.

The size of the purified capsular saccharide can also be reduced by mechanical homogenization. In an embodiment, the size of the purified capsular saccharide is reduced by high pressure homogenization. High pressure homogenization achieves high shear rates by pumping the process stream through a flow path with sufficiently small dimensions. The shear rate is increased by using a larger applied homogenization pressure, and exposure time can be increased by recirculating the feed stream through the homogenizer.

The high-pressure homogenization process can be appropriate for reducing the size of the purified capsular saccharide while preserving the structural features of the saccharide.

In a preferred embodiment, the isolated capsular saccharide is sized to a weight average molecular weight between 10 kDa and 1000 kDa. In an embodiment, the isolated capsular saccharide is sized to a weight average molecular weight between 50 kDa and 500 kDa. In an embodiment, the isolated capsular saccharide is sized to a weight average molecular weight between 50 kDa and 400 kDa. In an embodiment, the isolated capsular saccharide is sized to a weight average molecular weight between 50 kDa and 250 kDa.

In an embodiment, the isolated capsular saccharide is sized to a weight average molecular weight between 250 kDa and 1000 kDa. In an embodiment, the isolated capsular saccharide is sized to a weight average molecular weight between 250 kDa and 500 kDa. In an embodiment, the isolated capsular saccharide is sized to a weight average molecular weight between 250 kDa and 400 kDa. In a preferred embodiment, the isolated capsular saccharide is sized to a weight average molecular weight between 200 kDa and 800 kDa.

In an embodiment, the isolated capsular saccharide is sized to a weight average molecular weight of about 250 kDa. In an embodiment, the isolated capsular saccharide is sized to a weight average molecular weight of about 300 kDa. In an embodiment, the isolated capsular saccharide is sized to a weight average molecular weight of about 350 kDa. In an embodiment, the isolated capsular saccharide is sized to a weight average

molecular weight of about 400 kDa. In an embodiment, the isolated capsular saccharide is sized to a weight average molecular weight of about 450 kDa. In an embodiment, the isolated capsular saccharide is sized to a weight average molecular weight of about 500 kDa. In an embodiment, the isolated capsular saccharide is sized to a weight average molecular weight of about 550 kDa. In an embodiment, the isolated capsular saccharide is sized to a weight average molecular weight of about 600 kDa. In an embodiment, the isolated capsular saccharide is sized to a weight average molecular weight of about 700 kDa. In an embodiment, the isolated capsular saccharide is sized to a weight average molecular weight of about 800 kDa. In an embodiment, the isolated capsular saccharide is sized to a weight average molecular weight of about 900 kDa. In an embodiment, the isolated capsular saccharide is sized to a weight average molecular weight of about 1000 kDa.

In an embodiment, the isolated capsular saccharide is not sized.

The isolated capsular saccharide described above may be activated (e.g., chemically activated) to make them capable of reacting (e.g. with a linker) and then incorporated into glycoconjugates, as further described herein.

For the purposes of the invention the term 'glycoconjugate' indicates a saccharide covalently linked to a carrier protein.

In general, covalent conjugation of saccharides to carriers enhances the immunogenicity of saccharides as it converts them from T-independent antigens to T-dependent antigens, thus allowing priming for immunological memory. Conjugation is particularly useful for pediatric vaccines.

1.2 Capsular saccharide glycoconjugates of the invention

In some embodiments, the glycoconjugate of the present invention comprises a capsular saccharide wherein the weight average molecular weight (Mw) of said polysaccharide before conjugation is between 10 kDa and 2,000 kDa.

The weight average molecular weight (Mw) of the saccharide before conjugation refers to the Mw before the activation of the saccharide (i.e. after an eventual sizing step but before reacting the saccharide with an activating agent). In the context of the present invention the Mw of the saccharide is not substantially modified by the activation step and the Mw of the saccharide incorporated in the conjugate is similar to the Mw of the saccharide as measured before activation. In an embodiment, the saccharide is activated with a carbonic acid derivative (e.g. CDI or CDT) in combination with an azido linker (see

sections 1.3 below). In an embodiment, the saccharide is activated with CDI in combination with an azido linker (see sections 1.3 below). In an embodiment, the saccharide is activated with CDT in combination with an azido linker (see sections 1.3 below).

5 In an embodiment, the glycoconjugate of the present invention comprises a capsular saccharide wherein the weight average molecular weight (Mw) of said saccharide before conjugation is between 50 kDa and 1,000 kDa. In an embodiment, the weight average molecular weight (Mw) is between 50 kDa and 750 kDa. In an embodiment, the weight average molecular weight (Mw) is between 50 kDa and 500 kDa.
10 In an embodiment, the weight average molecular weight (Mw) is between 50 kDa and 250 kDa. In an embodiment, the weight average molecular weight (Mw) is between 50 kDa and 200 kDa. In an embodiment, the weight average molecular weight (Mw) is between 50 kDa and 150 kDa. In an embodiment, the weight average molecular weight (Mw) is between 50 kDa and 100 kDa.

15 In an embodiment, the glycoconjugate of the present invention comprises a capsular saccharide wherein the weight average molecular weight (Mw) of said saccharide before conjugation is between 75 kDa and 1,000 kDa. In an embodiment, the weight average molecular weight (Mw) is between 75 kDa and 750 kDa. In an embodiment, the weight average molecular weight (Mw) is between 75 kDa and 500 kDa.
20 In an embodiment, the weight average molecular weight (Mw) is between 75 kDa and 250 kDa. In an embodiment, the weight average molecular weight (Mw) is between 75 kDa and 200 kDa. In an embodiment, the weight average molecular weight (Mw) is between 75 kDa and 150 kDa. In an embodiment, the weight average molecular weight (Mw) is between 75 kDa and 100 kDa.

25 In an embodiment, the glycoconjugate of the present invention comprises a capsular saccharide wherein the weight average molecular weight (Mw) of said saccharide before conjugation is between 100 kDa and 1,000 kDa. In an embodiment, the weight average molecular weight (Mw) is between 100 kDa and 750 kDa. In an embodiment, the weight average molecular weight (Mw) is between 100 kDa and 500
30 kDa. In an embodiment, the weight average molecular weight (Mw) is between 100 kDa and 250 kDa. In an embodiment, the weight average molecular weight (Mw) is between 100 kDa and 200 kDa. In an embodiment, the weight average molecular weight (Mw) is between 100 kDa and 150 kDa.

In an embodiment, the glycoconjugate of the present invention comprises a
35 capsular saccharide wherein the weight average molecular weight (Mw) of said

saccharide before conjugation is between 150 kDa and 1,000 kDa. In an embodiment, the weight average molecular weight (Mw) is between 150 kDa and 750 kDa. In an embodiment, the weight average molecular weight (Mw) is between 150 kDa and 500 kDa. In an embodiment, the weight average molecular weight (Mw) is between 150 kDa and 250 kDa. In an embodiment, the weight average molecular weight (Mw) is between 150 kDa and 200 kDa.

In an embodiment, the glycoconjugate of the present invention comprises a capsular saccharide wherein the weight average molecular weight (Mw) of said saccharide before conjugation is between 200 kDa and 1,000 kDa. In an embodiment, the weight average molecular weight (Mw) is between 200 kDa and 750 kDa. In an embodiment, the weight average molecular weight (Mw) is between 200 kDa and 500 kDa. In an embodiment, the weight average molecular weight (Mw) is between 200 kDa and 300 kDa. In an embodiment, the weight average molecular weight (Mw) is between 200 kDa and 250 kDa.

In an embodiment, the glycoconjugate of the present invention comprises a capsular saccharide wherein the weight average molecular weight (Mw) of said saccharide before conjugation is between 500 kDa and 1,000 kDa. In an embodiment, the weight average molecular weight (Mw) is between 500 kDa and 750 kDa. In an embodiment, the weight average molecular weight (Mw) is between 500 kDa and 700 kDa. In an embodiment, the weight average molecular weight (Mw) is between 500 kDa and 600 kDa.

Any whole number integer within any of the above ranges is contemplated as an embodiment of the disclosure.

In an embodiment, the glycoconjugate of the present invention comprises a capsular saccharide wherein the weight average molecular weight (Mw) of said saccharide before conjugation is about 1,000 kDa. In an embodiment, the weight average molecular weight (Mw) is about 750 kDa. In an embodiment, the weight average molecular weight (Mw) is about 700 kDa. In an embodiment, the weight average molecular weight (Mw) is about 600 kDa. In an embodiment, the weight average molecular weight (Mw) is about 500 kDa.

In an embodiment, the weight average molecular weight (Mw) is about 400 kDa. In an embodiment, the weight average molecular weight (Mw) is about 300 kDa. In an embodiment, the weight average molecular weight (Mw) is about 200 kDa. In an embodiment, the weight average molecular weight (Mw) is about 150 kDa. In an embodiment, the weight average molecular weight (Mw) is about 125 kDa. In an embodiment, the weight average molecular weight (Mw) is about 100 kDa.

In some embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of between 250 kDa and 20,000 kDa. In other embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of between 500 kDa and 15,000 kDa. In yet other embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of between 500 kDa and 10,000 kDa.

In other embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of between 500 kDa and 10,000 kDa. In other embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of between 500 kDa and 7,500 kDa. In still other embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of between 500 kDa and 5,000 kDa. In still other embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of between 500 kDa and 2,500 kDa. In still other embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of between 500 kDa and 2,000 kDa. In still other embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of between 500 kDa and 1,500 kDa. In still other embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of between 500 kDa and 1,000 kDa. In still other embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of between 500 kDa and 750 kDa.

In other embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of between 750 kDa and 10,000 kDa. In other embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of between 750 kDa and 7,500 kDa. In still other embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of between 750 kDa and 5,000 kDa. In still other embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of between 750 kDa and 2,500 kDa. In still other embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of between 750 kDa and 2,000 kDa. In still other embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of between 750 kDa and 1,500 kDa. In still other embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of between 750 kDa and 1,000 kDa.

In other embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of between 1,000 kDa and 10,000 kDa. In other embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of between 1,000 kDa and 7,500 kDa. In still other embodiments, the glycoconjugate of the

invention has a weight average molecular weight (Mw) of between 1,000 kDa and 5,000 kDa. In still other embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of between 1,000 kDa and 2,500 kDa. In still other embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of between 1,000 kDa and 2,000 kDa. In still other embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of between 1,000 kDa and 1,500 kDa.

In other embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of between 2,000 kDa and 10,000 kDa. In other embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of between 2,000 kDa and 7,500 kDa. In still other embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of between 2,000 kDa and 5,000 kDa. In still other embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of between 2,000 kDa and 4,000 kDa. In still other embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of between 2,000 kDa and 3,000 kDa. In still other embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of between 2,000 kDa and 3,500 kDa.

In still other embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of between 2,250 kDa and 3,500 kDa.

In preferred embodiment, the glycoconjugate of the invention has a weight average molecular weight (Mw) of between 1,000 kDa and 2,500 kDa.

Any whole number integer within any of the above ranges is contemplated as an embodiment of the disclosure.

In other embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of about 10,000 kDa. In other embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of about 9,000 kDa. In other embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of about 8,000 kDa. In still other embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of about 7,500 kDa. In still other embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of about 6,000 kDa. In still other embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of about 5,000 kDa. In still other embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of about 4,000 kDa. In still other embodiments, the glycoconjugate of the invention

has a weight average molecular weight (Mw) of about 3,500 kDa. In still other embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of about 3,250 kDa. In still other embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of about 3,000 kDa. In still other
5 embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of about 2,500 kDa. In still other embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of about 2,000 kDa. In still other embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of about 1,500 kDa. In still other embodiments, the glycoconjugate of the invention
10 has a weight average molecular weight (Mw) of about 1,000 kDa. In still other embodiments, the glycoconjugate of the invention has a weight average molecular weight (Mw) of about 750 kDa.

Another way to characterize the glycoconjugates of the invention is by the number of lysine residues in the carrier protein (e.g., CRM₁₉₇ or SCP) that become conjugated to
15 the saccharide which can be characterized as a range of conjugated lysines (degree of conjugation). The evidence for lysine modification of the carrier protein, due to covalent linkages to the saccharides, can be obtained by amino acid analysis using routine methods known to those of skill in the art. Conjugation results in a reduction in the number of lysine residues recovered compared to the carrier protein starting material used to
20 generate the conjugate materials. In a preferred embodiment, the degree of conjugation of the glycoconjugate of the invention is between 2 and 15. In an embodiment, the degree of conjugation of the glycoconjugate of the invention is between 2 and 13. In an embodiment, the degree of conjugation of the glycoconjugate of the invention is between 2 and 10. In an embodiment, the degree of conjugation of the glycoconjugate of the
25 invention is between 2 and 8. In an embodiment, the degree of conjugation of the glycoconjugate of the invention is between 2 and 6. In an embodiment, the degree of conjugation of the glycoconjugate of the invention is between 2 and 5. In an embodiment, the degree of conjugation of the glycoconjugate of the invention is between 2 and 4. In an embodiment, the degree of conjugation of the glycoconjugate of the invention is
30 between 3 and 15. In an embodiment, the degree of conjugation of the glycoconjugate of the invention is between 3 and 13. In an embodiment, the degree of conjugation of the glycoconjugate of the invention is between 3 and 10. In an embodiment, the degree of conjugation of the glycoconjugate of the invention is between 3 and 8. In an embodiment, the degree of conjugation of the glycoconjugate of the invention is between 3 and 6. In
35 an embodiment, the degree of conjugation of the glycoconjugate of the invention is

between 3 and 5. In an embodiment, the degree of conjugation of the glycoconjugate of the invention is between 3 and 4. In an embodiment, the degree of conjugation of the glycoconjugate of the invention is between 5 and 15. In an embodiment, the degree of conjugation of the glycoconjugate of the invention is between 5 and 10. In an embodiment, the degree of conjugation of the glycoconjugate of the invention is between 8 and 15. In an embodiment, the degree of conjugation of the glycoconjugate of the invention is between 8 and 12. In an embodiment, the degree of conjugation of the glycoconjugate of the invention is between 10 and 15. In an embodiment, the degree of conjugation of the glycoconjugate of the invention is between 10 and 12.

In an embodiment, the degree of conjugation of the glycoconjugate of the invention is about 2. In an embodiment, the degree of conjugation of the glycoconjugate of the invention is about 3. In an embodiment, the degree of conjugation of the glycoconjugate of the invention is about 4. In an embodiment, the degree of conjugation of the glycoconjugate of the invention is about 5. In an embodiment, the degree of conjugation of the glycoconjugate of the invention is about 6. In an embodiment, the degree of conjugation of the glycoconjugate of the invention is about 7. In an embodiment, the degree of conjugation of the glycoconjugate of the invention is about 8. In an embodiment, the degree of conjugation of the glycoconjugate of the invention is about 9. In an embodiment, the degree of conjugation of the glycoconjugate of the invention is about 10, about 11. In an embodiment, the degree of conjugation of the glycoconjugate of the invention is about 12. In an embodiment, the degree of conjugation of the glycoconjugate of the invention is about 13. In an embodiment, the degree of conjugation of the glycoconjugate of the invention is about 14. In an embodiment, the degree of conjugation of the glycoconjugate of the invention is about 15. In a preferred embodiment, the degree of conjugation of the glycoconjugate of the invention is between 4 and 7. In some such embodiments, the carrier protein is CRM₁₉₇. In other such embodiments, the carrier protein is SCP.

The glycoconjugates of the invention may also be characterized by the ratio (weight/weight) of saccharide to carrier protein. In some embodiments, the ratio of saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 3.0. In other embodiments, the saccharide to carrier protein ratio (w/w) is between 0.5 and 2.0. In other embodiments, the saccharide to carrier protein ratio (w/w) is between 0.5 and 1.5. In other embodiments, the saccharide to carrier protein ratio (w/w) is between 0.8 and 1.2. In other embodiments, the saccharide to carrier protein ratio (w/w) is between 0.5 and 1.0. In other embodiments, the saccharide to carrier protein ratio (w/w) is between 1.0 and 1.5. In other

embodiments, the saccharide to carrier protein ratio (w/w) is between 1.0 and 2.0. In further embodiments, the saccharide to carrier protein ratio (w/w) is between 0.8 and 1.2. In a preferred embodiment, the ratio of saccharide to carrier protein in the conjugate is between 0.9 and 1.1.

5 In an embodiment, the saccharide to carrier protein ratio (w/w) is about 0.5. In other embodiments, the saccharide to carrier protein ratio (w/w) is about 0.6. In other embodiments, the saccharide to carrier protein ratio (w/w) is about 0.7. In other embodiments, the saccharide to carrier protein ratio (w/w) is about 0.8. In other
10 embodiments, the saccharide to carrier protein ratio (w/w) is about 0.9. In other embodiments, the saccharide to carrier protein ratio (w/w) is about 1.0. In other embodiments, the saccharide to carrier protein ratio (w/w) is about 1.1. In other embodiments, the saccharide to carrier protein ratio (w/w) is about 1.2. In other
15 embodiments, the saccharide to carrier protein ratio (w/w) is about 1.3. In other embodiments, the saccharide to carrier protein ratio (w/w) is about 1.4. In other embodiments, the saccharide to carrier protein ratio (w/w) is about 1.5. In other
20 embodiments, the saccharide to carrier protein ratio (w/w) is about 1.6. In other embodiments, the saccharide to carrier protein ratio (w/w) is about 1.7. In other embodiments, the saccharide to carrier protein ratio (w/w) is about 1.8. In other
25 embodiments, the saccharide to carrier protein ratio (w/w) is about 1.9. In other embodiments, the saccharide to carrier protein ratio (w/w) is about 2.0. In other embodiments, the saccharide to carrier protein ratio (w/w) is about 2.1. In other
embodiments, the saccharide to carrier protein ratio (w/w) is about 2.2. In other
embodiments, the saccharide to carrier protein ratio (w/w) is about 2.5. In other
embodiments, the saccharide to carrier protein ratio (w/w) is about 2.8. In other
embodiments, the saccharide to carrier protein ratio (w/w) is about 3.0. In some such
embodiments, the carrier protein is CRM₁₉₇. In other such embodiments, the carrier
protein is SCP.

The glycoconjugates of the invention may also be characterized by the number of covalent linkages between the carrier protein and the saccharide as a function of repeat
30 units of the saccharide. In one embodiment, the glycoconjugate of the invention comprises at least one covalent linkage between the carrier protein and the saccharide for every 4 saccharide repeat units of the saccharide. In another embodiment, the covalent linkage between the carrier protein and the saccharide occurs at least once in every 10 saccharide repeat units of the saccharide. In another embodiment, the covalent
35 linkage between the carrier protein and the saccharide occurs at least once in every 15

saccharide repeat units of the saccharide. In a further embodiment, the covalent linkage between the carrier protein and the saccharide occurs at least once in every 25 saccharide repeat units of the saccharide. In a further embodiment, the covalent linkage between the carrier protein and the saccharide occurs at least once in every 50
5 saccharide repeat units of the saccharide. In yet a further embodiment, the covalent linkage between the carrier protein and the saccharide occurs at least once in every 100 saccharide repeat units of the saccharide.

In other embodiments, the glycoconjugate of the invention comprises at least one covalent linkage between the carrier protein and the saccharide for every 5 to 10
10 saccharide repeat units of the saccharide.

In other embodiments, the glycoconjugate of the invention comprises at least one covalent linkage between the carrier protein and the saccharide for every 2 to 7 saccharide repeat units of the saccharide.

In other embodiments, the glycoconjugate of the invention comprises at least one
15 covalent linkage between the carrier protein and the saccharide for every 6 to 11 saccharide repeat units of the saccharide.

In other embodiments, the glycoconjugate of the invention comprises at least one covalent linkage between the carrier protein and the saccharide for every 9 to 14 saccharide repeat units of the saccharide.

20 In other embodiments, the glycoconjugate of the invention comprises at least one covalent linkage between the carrier protein and the saccharide for every 10 to 20 saccharide repeat units of the saccharide.

In other embodiments, the glycoconjugate of the invention comprises at least one covalent linkage between the carrier protein and the saccharide for every 4 to 25
25 saccharide repeat units of the saccharide.

In frequent embodiments, the carrier protein is CRM₁₉₇. In other embodiments, the carrier protein is SCP.

In some embodiments, the carrier protein is CRM₁₉₇ and the covalent linkage between the CRM₁₉₇ and the saccharide occurs at least once in every 4, 10, 15 or 25
30 saccharide repeat units of the saccharide. In other embodiments, the carrier protein is SCP and the covalent linkage between the SCP and the saccharide occurs at least once in every 4, 10, 15 or 25 saccharide repeat units of the saccharide.

The glycoconjugates and immunogenic compositions of the invention may contain free saccharide that is not covalently conjugated to the carrier protein but is nevertheless
35 present in the glycoconjugate composition. The free saccharide may be noncovalently

associated with (i.e., noncovalently bound to, adsorbed to, or entrapped in or with) the glycoconjugate.

In a preferred embodiment, the glycoconjugate comprises less than about 50% of free saccharide compared to the total amount of said saccharide. In a preferred embodiment the glycoconjugate comprises less than about 40% of free saccharide compared to the total amount of said saccharide. In a yet preferred embodiment, the glycoconjugate comprises less than about 25% of free saccharide compared to the total amount of said saccharide. In an even preferred embodiment, the glycoconjugate comprises less than about 20% of free saccharide compared to the total amount of said saccharide. In a yet preferred embodiment, the glycoconjugate comprises less than about 15% of free saccharide compared to the total amount of said saccharide.

The glycoconjugates may also be characterized by their molecular size distribution (K_d). Size exclusion chromatography media (CL-4B) can be used to determine the relative molecular size distribution of the conjugate. Size Exclusion Chromatography (SEC) is used in gravity fed columns to profile the molecular size distribution of conjugates. Large molecules excluded from the pores in the media elute more quickly than small molecules. Fraction collectors are used to collect the column eluate. The fractions are tested colorimetrically by saccharide assay. For the determination of K_d , columns are calibrated to establish the fraction at which molecules are fully excluded (V_0), ($K_d=0$), and the fraction representing the maximum retention (V_i), ($K_d=1$). The fraction at which a specified sample attribute is reached (V_e), is related to K_d by the expression, $K_d = (V_e - V_0) / (V_i - V_0)$.

In a preferred embodiment, at least 30% of the glycoconjugate of the invention has a K_d below or equal to 0.3 in a CL-4B column. In a preferred embodiment, at least 40% of the glycoconjugate of the invention has a K_d below or equal to 0.3 in a CL-4B column. In a preferred embodiment, at least 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, or 85% of the glycoconjugate of the invention has a K_d below or equal to 0.3 in a CL-4B column. In a preferred embodiment, at least 60% of the glycoconjugate of the invention has a K_d below or equal to 0.3 in a CL-4B column. In a preferred embodiment, between 50% and 80% of the glycoconjugate of the invention has a K_d below or equal to 0.3 in a CL-4B column. In a preferred embodiment, between 65% and 80% of the glycoconjugate of the invention has a K_d below or equal to 0.3 in a CL-4B column.

1.3 Capsular saccharide glycoconjugates of the invention prepared using click chemistry

The glycoconjugates of the present invention are prepared using click chemistry. The invention also relates to a method of making a glycoconjugate, as disclosed herein.

According to the present invention, click chemistry comprises three steps, (a) reacting an isolated capsular saccharide with a carbonic acid derivative and an azido linker in an aprotic solvent to produce an activated azido saccharide (activation of the saccharide), (b) reacting a carrier protein with an agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group where the NHS moiety reacts with the amino groups to form an amide linkage thereby obtaining an alkyne functionalized carrier protein (activation of the carrier protein), (c) reacting the activated azido saccharide of step (a) with the activated alkyne-carrier protein of step (b) by Cu^{+1} mediated azide-alkyne cycloaddition reaction to form a glycoconjugate.

Following step (a) the saccharide is said to be activated and is referred to herein as “activated saccharide” or “activated azido saccharide”.

Following step (b) the carrier is said to be activated and is referred to as “activated carrier”.

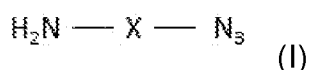
As mentioned above, before the activation (a), sizing of the saccharide to a target molecular weight (MW) range can be performed. Therefore, in an embodiment, the isolated saccharide is sized before activation with a carbonic acid derivative and an azido linker. In an embodiment, the isolated saccharide is sized to any of the target molecular weight (MW) range defined above.

In an embodiment, said carbonic acid derivative is selected from the group consisting of 1,1'-carbonyldiimidazole (CDI), 1,1'-carbonyl-di-(1,2,4-triazole) (CDT), disuccinimidyl carbonate (DSC) and N-hydroxysuccinimidyl chloroformate.

In an embodiment, said carbonic acid derivative is 1,1'-carbonyldiimidazole (CDI). In another embodiment, said carbonic acid derivative is 1,1'-Carbonyl-di-(1,2,4-triazole) (CDT). In another embodiment, said carbonic acid derivative is disuccinimidyl carbonate (DSC). In yet a further embodiment, said carbonic acid derivative is N-hydroxysuccinimidyl chloroformate.

In an embodiment, said carbonic acid derivative is 1,1'-carbonyldiimidazole (CDI) or 1,1'-Carbonyl-di-(1,2,4-triazole) (CDT). Preferably, said carbonic acid derivative is 1,1'-carbonyldiimidazole (CDI).

In an embodiment, said azido linker is a compound of formula (I),



wherein X is selected from the group consisting of $\text{CH}_2(\text{CH}_2)_n$, $(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$, $\text{NHCO}(\text{CH}_2)_n$, $\text{NHCO}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$, $\text{OCH}_2(\text{CH}_2)_n$ and $\text{O}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$; where n is selected from 1 to 10 and m is selected from 1 to 4.

In an embodiment, said azido linker is a compound of formula (I), wherein X is $\text{CH}_2(\text{CH}_2)_n$, and n is selected from 1 to 10. In an embodiment, n is selected from 1 to 5. In an embodiment, n is selected from 1 to 4. In an embodiment, n is selected from 1 to 3. In an embodiment, n is selected from 1 to 2. In a particular embodiment, n is 1. In another embodiment, n is 2. In yet another embodiment, n is 3. In yet a further embodiment, n is 4. In yet a further embodiment, n is 5. In yet a further embodiment, n is 6. In yet a further embodiment, n is 7. In yet a further embodiment, n is 8. In yet a further embodiment, n is 9. In yet a further embodiment, n is 10.

In an embodiment, said azido linker is a compound of formula (I), wherein X is $(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$, wherein m is selected from 1 to 4. In an embodiment, m is selected from 1 to 3. In an embodiment, m is selected from 1 to 2. In a particular embodiment, m is 1. In another embodiment, m is 2. In yet another embodiment, m is 3. In yet a further embodiment, m is 4.

In an embodiment, said azido linker is a compound of formula (I), wherein X is $\text{NHCO}(\text{CH}_2)_n$, and n is selected from 1 to 10. In an embodiment, n is selected from 1 to 5. In an embodiment, n is selected from 1 to 4. In an embodiment, n is selected from 1 to 3. In an embodiment, n is selected from 1 to 2. In a particular embodiment, n is 1. In another embodiment, n is 2. In yet another embodiment, n is 3. In yet a further embodiment, n is 4. In yet a further embodiment, n is 5. In yet a further embodiment, n is 6. In yet a further embodiment, n is 7. In yet a further embodiment, n is 8. In yet a further embodiment, n is 9. In yet a further embodiment, n is 10.

In an embodiment, said azido linker is a compound of formula (I), wherein X is $\text{NHCO}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$, where m is selected from 1 to 4. In an embodiment, m is selected from 1 to 3. In an embodiment, m is selected from 1 to 2. In a particular embodiment, m is 1. In another embodiment, m is 2. In yet another embodiment, m is 3. In yet a further embodiment, m is 4.

In an embodiment, said azido linker is a compound of formula (I), wherein X is $\text{OCH}_2(\text{CH}_2)_n$, and n is selected from 1 to 10. In an embodiment, n is selected from 1 to 5. In an embodiment, n is selected from 1 to 4. In an embodiment, n is selected from 1 to 3. In an embodiment, n is selected from 1 to 2. In a particular embodiment, n is 1. In another embodiment, n is 2. In yet another embodiment, n is 3. In yet a further embodiment, n is 4. In yet a further embodiment, n is 5. In yet a further embodiment, n is 6. In yet a further

embodiment, n is 7. In yet a further embodiment, n is 8. In yet a further embodiment, n is 9. In yet a further embodiment, n is 10.

In an embodiment, said azido linker is a compound of formula (I), wherein X is $O(CH_2CH_2O)_mCH_2CH_2$, where m is selected from 1 to 4. In an embodiment, m is selected from 1 to 3. In an embodiment, m is selected from 1 to 2. In a particular embodiment, m is 1. In another embodiment, m is 2. In yet another embodiment, m is 3. In yet a further embodiment, m is 4.

In an embodiment, said azido linker is a compound of formula (II),

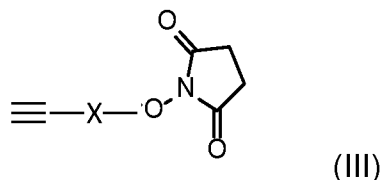


In a preferred embodiment, said azido linker is 3-azido-propylamine.

In an embodiment, 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.

In an embodiment, said agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group is an agent bearing an N-Hydroxysuccinimide (NHS) moiety and a cycloalkyne.

In an embodiment, said agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group is a compound of formula (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.

In an embodiment, said agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group is a compound of formula (III), wherein X is $CH_2O(CH_2)_nCH_2C=O$, where n is selected from 0 to 10. In an embodiment, n is selected from 0 to 5. In an embodiment, n is selected from 0 to 4. In an embodiment, n is selected from 0 to 3. In an embodiment, n is selected from 0 to 2. In a particular embodiment, n is 0. In a particular embodiment, n is 1. In another embodiment, n is 2. In yet another embodiment, n is 3. In yet a further embodiment, n is 4. In yet a further embodiment, n is 5. In yet a further embodiment, n is 6. In yet a further embodiment, n is 7. In yet a

further embodiment, n is 8. In yet a further embodiment, n is 9. In yet a further embodiment, n is 10.

In an embodiment, said agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group is a compound of formula (III), wherein X is
5 $\text{CH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_m(\text{CH}_2)_n\text{CH}_2\text{C}=\text{O}$, where n is selected from 0 to 10 and m is selected from 0 to 4. In an embodiment, n is selected from 0 to 5. In an embodiment, n is selected from 0 to 4. In an embodiment, n is selected from 0 to 3. In an embodiment, n is selected from 0 to 2. In a particular embodiment, n is 0. In a particular embodiment, n is 1. In another embodiment, n is 2. In yet another embodiment, n is 3. In yet a further
10 embodiment, n is 4. In yet a further embodiment, n is 5. In yet a further embodiment, n is 6. In yet a further embodiment, n is 7. In yet a further embodiment, n is 8. In yet a further embodiment, n is 9. In yet a further embodiment, n is 10. In an embodiment, m is selected from 0 to 3. In an embodiment, m is selected from 0 to 2. In a particular embodiment, m is 1. In a particular embodiment, m is 1. In another embodiment, m is 2. In yet another
15 embodiment, m is 3. In yet a further embodiment, m is 4.

In an embodiment, n is selected from 0 to 5 and m is selected from 0 to 3. In an embodiment, n is selected from 0 to 5 and m is selected from 0 to 2.

In an embodiment, n is selected from 0 to 4 and m is selected from 0 to 3. In an embodiment, n is selected from 0 to 4 and m is selected from 0 to 2.

20 In an embodiment, n is selected from 0 to 3 and m is selected from 0 to 3. In an embodiment, n is selected from 0 to 3 and m is selected from 0 to 2.

In an embodiment, n is selected from 0 to 2 and m is selected from 0 to 3. In an embodiment, n is selected from 0 to 2 and m is selected from 0 to 2.

In an embodiment, n is selected from 0 to 1 and m is selected from 0 to 3. In an
25 embodiment, n is selected from 0 to 1 and m is selected from 0 to 2.

In an embodiment, n is 0 and m is 0. In an embodiment, n is 1 and m is 0. In an embodiment, n is 2 and m is 0. In an embodiment, n is 3 and m is 0. In an embodiment, n is 4 and m is 0. In an embodiment, n is 5 and m is 0. In an embodiment, n is 6 and m is 0. In an embodiment, n is 7 and m is 0. In an embodiment, n is 8 and m is 0. In an
30 embodiment, n is 9 and m is 0. In an embodiment, n is 10 and m is 0.

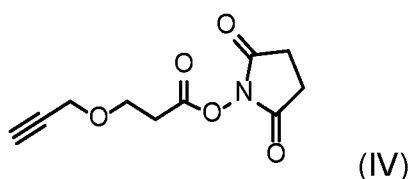
In an embodiment, n is 0 and m is 1. In an embodiment, n is 1 and m is 1. In an embodiment, n is 2 and m is 1. In an embodiment, n is 3 and m is 1. In an embodiment, n is 4 and m is 1. In an embodiment, n is 5 and m is 1. In an embodiment, n is 6 and m is 1. In an embodiment, n is 7 and m is 1. In an embodiment, n is 8 and m is 1. In an
35 embodiment, n is 9 and m is 1. In an embodiment, n is 10 and m is 1.

In an embodiment, n is 0 and m is 2. In an embodiment, n is 1 and m is 2. In an embodiment, n is 2 and m is 2. In an embodiment, n is 3 and m is 2. In an embodiment, n is 4 and m is 2. In an embodiment, n is 5 and m is 2. In an embodiment, n is 6 and m is 2. In an embodiment, n is 7 and m is 2. In an embodiment, n is 8 and m is 2. In an embodiment, n is 9 and m is 2. In an embodiment, n is 10 and m is 2.

In an embodiment, n is 0 and m is 3. In an embodiment, n is 1 and m is 3. In an embodiment, n is 2 and m is 3. In an embodiment, n is 3 and m is 3. In an embodiment, n is 4 and m is 3. In an embodiment, n is 5 and m is 3. In an embodiment, n is 6 and m is 3. In an embodiment, n is 7 and m is 3. In an embodiment, n is 8 and m is 3. In an embodiment, n is 9 and m is 3. In an embodiment, n is 10 and m is 3.

In an embodiment, n is 0 and m is 4. In an embodiment, n is 1 and m is 4. In an embodiment, n is 2 and m is 4. In an embodiment, n is 3 and m is 4. In an embodiment, n is 4 and m is 4. In an embodiment, n is 5 and m is 4. In an embodiment, n is 6 and m is 4. In an embodiment, n is 7 and m is 4. In an embodiment, n is 8 and m is 4. In an embodiment, n is 9 and m is 4. In an embodiment, n is 10 and m is 4.

In an embodiment, said agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group is a compound of formula (IV):



In an embodiment, step a) comprises reacting the saccharide with a carbonic acid derivative followed by reacting the carbonic acid derivative-activated saccharide with an azido linker in an aprotic solvent to produce an activated azido saccharide.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.01-10 molar equivalent to the amount of saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.05-10 molar equivalent to the amount of saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.1-10 molar equivalent to the amount of saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.2-10 molar equivalent to the amount of saccharide present in the reaction mixture.

5 In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.3-10 molar equivalent to the amount of saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.4-10 molar equivalent to the amount of saccharide present in the reaction mixture.

10 In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.5-10 molar equivalent to the amount of saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.8-10 molar equivalent to the amount of
15 saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 1-10 molar equivalent to the amount of saccharide present in the reaction mixture.

20 In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 2-10 molar equivalent to the amount of saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 3-10 molar equivalent to the amount of saccharide present in the reaction mixture.

25 In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 5-10 molar equivalent to the amount of saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.01-5 molar equivalent to the amount of
30 saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.05-5 molar equivalent to the amount of saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.1-5 molar equivalent to the amount of saccharide present in the reaction mixture.

5 In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.2-5 molar equivalent to the amount of saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.3-5 molar equivalent to the amount of saccharide present in the reaction mixture.

10 In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.4-5 molar equivalent to the amount of saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.5-5 molar equivalent to the amount of
15 saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.8-5 molar equivalent to the amount of saccharide present in the reaction mixture.

20 In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 1-5 molar equivalent to the amount of saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 2-5 molar equivalent to the amount of saccharide present in the reaction mixture.

25 In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 3-5 molar equivalent to the amount of saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.01-3 molar equivalent to the amount of
30 saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.05-3 molar equivalent to the amount of saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.1-3 molar equivalent to the amount of saccharide present in the reaction mixture.

5 In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.2-3 molar equivalent to the amount of saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.3-3 molar equivalent to the amount of saccharide present in the reaction mixture.

10 In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.4-3 molar equivalent to the amount of saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.5-3 molar equivalent to the amount of saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.8-3 molar equivalent to the amount of saccharide present in the reaction mixture.

20 In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 1-3 molar equivalent to the amount of saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 2-3 molar equivalent to the amount of saccharide present in the reaction mixture.

25 In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.01-2 molar equivalent to the amount of saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.05-2 molar equivalent to the amount of saccharide present in the reaction mixture.

30 In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.1-2 molar equivalent to the amount of saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.2-2 molar equivalent to the amount of saccharide present in the reaction mixture.

5 In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.3-2 molar equivalent to the amount of saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.4-2 molar equivalent to the amount of saccharide present in the reaction mixture.

10 In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.5-2 molar equivalent to the amount of saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.8-2 molar equivalent to the amount of
15 saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 1-2 molar equivalent to the amount of saccharide present in the reaction mixture.

20 In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.01-1 molar equivalent to the amount of saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.05-1 molar equivalent to the amount of saccharide present in the reaction mixture.

25 In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.1-1 molar equivalent to the amount of saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.2-1 molar equivalent to the amount of
30 saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.3-1 molar equivalent to the amount of saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.4-1 molar equivalent to the amount of saccharide present in the reaction mixture.

5 In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.5-1 molar equivalent to the amount of saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.8-1 molar equivalent to the amount of saccharide present in the reaction mixture.

10 In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.01-0.5 molar equivalent to the amount of saccharide present in the reaction mixture.

15 In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.05-0.5 molar equivalent to the amount of saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.1-0.5 molar equivalent to the amount of saccharide present in the reaction mixture.

20 In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.2-0.5 molar equivalent to the amount of saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.3-0.5 molar equivalent to the amount of saccharide present in the reaction mixture.

25 In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.4-0.5 molar equivalent to the amount of saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative

30 In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.01-0.4 molar equivalent to the amount of saccharide present in the reaction mixture.

35 In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.05-0.4 molar equivalent to the amount of saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.1-0.4 molar equivalent to the amount of saccharide present in the reaction mixture.

5 In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.2-0.4 molar equivalent to the amount of saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.3-0.4 molar equivalent to the amount of saccharide present in the reaction mixture.

10 In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.01-0.3 molar equivalent to the amount of saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.05-0.3 molar equivalent to the amount of
15 saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.1-0.3 molar equivalent to the amount of saccharide present in the reaction mixture.

20 In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative that is between 0.2-0.3 molar equivalent to the amount of saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative of about 0.01 molar equivalent to the amount of saccharide present in the reaction mixture.

25 In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative of about 0.05 molar equivalent to the amount of saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative of about 0.08 molar equivalent to the amount of saccharide
30 present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative of about 0.1 molar equivalent to the amount of saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative of about 0.2 molar equivalent to the amount of saccharide present in the reaction mixture.

5 In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative of about 0.3 molar equivalent to the amount of saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative of about 0.4 molar equivalent to the amount of saccharide present in the reaction mixture.

10 In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative of about 0.5 molar equivalent to the amount of saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative of about 1 molar equivalent to the amount of saccharide present
15 in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative of about 2 molar equivalent to the amount of saccharide present in the reaction mixture.

20 In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative of about 3 molar equivalent to the amount of saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative of about 4 molar equivalent to the amount of saccharide present in the reaction mixture.

25 In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative of about 5 molar equivalent to the amount of saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative of about 8 molar equivalent to the amount of saccharide present
30 in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative of about 10 molar equivalent to the amount of saccharide present in the reaction mixture.

In an embodiment, at step a) the isolated saccharide is reacted with a carbonic acid derivative in an aprotic solvent.

In one embodiment the isolated saccharide is reacted with a carbonic acid derivative in a solution consisting essentially of dimethylsulphoxide (DMSO) or dimethylformamide (DMF). In one embodiment the isolated saccharide is reacted with a carbonic acid derivative in a solution consisting essentially of dimethylformamide (DMF). In one embodiment the isolated saccharide is reacted with a carbonic acid derivative in a solution consisting essentially of dimethylsulphoxide (DMSO).

In an embodiment, the isolated saccharide is reacted with a carbonic acid derivative in a solution consisting essentially of dimethylacetamide. In an embodiment, the isolated saccharide is reacted with a carbonic acid derivative in a solution consisting essentially of N-methyl-2-pyrrolidone. In an embodiment, the isolated saccharide is reacted with a carbonic acid derivative in a solution consisting essentially of hexamethylphosphoramide (HMPA).

In a preferred embodiment the isolated saccharide is reacted with a carbonic acid derivative in a solution consisting essentially of dimethylsulphoxide (DMSO).

In one embodiment the isolated saccharide is reacted with a carbonic acid derivative in dimethylsulphoxide (DMSO) or dimethylformamide (DMF). In one embodiment the isolated saccharide is reacted with a carbonic acid derivative in dimethylformamide (DMF). In one embodiment the isolated saccharide is reacted with a carbonic acid derivative in dimethylsulphoxide (DMSO).

In an embodiment, the isolated saccharide is reacted with a carbonic acid derivative in dimethylacetamide. In an embodiment, the isolated saccharide is reacted with a carbonic acid derivative in N-methyl-2-pyrrolidone. In an embodiment, the isolated saccharide is reacted with a carbonic acid derivative in hexamethylphosphoramide (HMPA).

In a preferred embodiment the isolated saccharide is reacted with CDI in dimethylsulphoxide (DMSO). In an embodiment the isolated saccharide is reacted with CDI in anhydrous DMSO.

It has been surprisingly found that reacting the isolated saccharide with CDI in an environment with a moisture level of about 0.1% to 1% (v/v) allows to avoid side reactions. Therefore, in one embodiment the isolated saccharide is reacted with CDI in an aprotic solvent comprising 0.1% to 1% (v/v) water. In one embodiment the isolated saccharide is reacted with CDI in an aprotic solvent comprising 0.1% to 0.8% (v/v) water. In one

embodiment the isolated saccharide is reacted with CDI in an aprotic solvent comprising 0.1% to 0.5% (v/v) water. In one embodiment the isolated saccharide is reacted with CDI in an aprotic solvent comprising 0.1% to 0.4% (v/v) water. In one embodiment the isolated saccharide is reacted with CDI in an aprotic solvent comprising 0.1% to 0.3% (v/v) water.

5 In one embodiment the isolated saccharide is reacted with CDI in an aprotic solvent comprising 0.1% to 0.2% (v/v) water.

In one embodiment the isolated saccharide is reacted with CDI in an aprotic solvent comprising 0.2% to 1% (v/v) water. In one embodiment the isolated saccharide is reacted with CDI in an aprotic solvent comprising 0.2% to 0.8% (v/v) water. In one embodiment
10 the isolated saccharide is reacted with CDI in an aprotic solvent comprising 0.2% to 0.5% (v/v) water. In one embodiment the isolated saccharide is reacted with CDI in an aprotic solvent comprising 0.2% to 0.4% (v/v) water. In one embodiment the isolated saccharide is reacted with CDI in an aprotic solvent comprising 0.2% to 0.3% (v/v) water.

In one embodiment the isolated saccharide is reacted with CDI in an aprotic solvent
15 comprising 0.3% to 0.8% (v/v) water. In one embodiment the isolated saccharide is reacted with CDI in an aprotic solvent comprising 0.3% to 0.5% (v/v) water. In one embodiment the isolated saccharide is reacted with CDI in an aprotic solvent comprising 0.3% to 0.4% (v/v) water.

In one embodiment the isolated saccharide is reacted with CDI in an aprotic solvent
20 comprising about 0.1% (v/v) water. In one embodiment the isolated saccharide is reacted with CDI in an aprotic solvent comprising about 0.2% (v/v) water. In one embodiment the isolated saccharide is reacted with CDI in an aprotic solvent comprising about 0.3% (v/v) water. In one embodiment the isolated saccharide is reacted with CDI in an aprotic solvent comprising about 0.4% (v/v) water. In one embodiment the isolated saccharide is reacted
25 with CDI in an aprotic solvent comprising about 0.5% (v/v) water. In one embodiment the isolated saccharide is reacted with CDI in an aprotic solvent comprising about 0.6% (v/v) water. In one embodiment the isolated saccharide is reacted with CDI in an aprotic solvent comprising about 0.7% (v/v) water. In one embodiment the isolated saccharide is reacted with CDI in an aprotic solvent comprising about 0.8% (v/v) water. In one embodiment the
30 isolated saccharide is reacted with CDI in an aprotic solvent comprising about 0.9% (v/v) water.

In one embodiment the isolated saccharide is reacted with CDI in DMSO comprising 0.1% to 1% (v/v) water. In one embodiment the isolated saccharide is reacted with CDI in DMSO comprising 0.1% to 0.8% (v/v) water. In one embodiment the isolated
35 saccharide is reacted with CDI in DMSO comprising 0.1% to 0.5% (v/v) water. In one

embodiment the isolated saccharide is reacted with CDI in DMSO comprising 0.1% to 0.4% (v/v) water. In one embodiment the isolated saccharide is reacted with CDI in DMSO comprising 0.1% to 0.3% (v/v) water. In one embodiment the isolated saccharide is reacted with CDI in DMSO comprising 0.1% to 0.2% (v/v) water.

5 In one embodiment the isolated saccharide is reacted with CDI in DMSO comprising 0.2% to 1% (v/v) water. In one embodiment the isolated saccharide is reacted with CDI in DMSO comprising 0.2% to 0.8% (v/v) water. In one embodiment the isolated saccharide is reacted with CDI in DMSO comprising 0.2% to 0.5% (v/v) water. In one embodiment the isolated saccharide is reacted with CDI in DMSO comprising 0.2% to 0.4% (v/v) water. In one embodiment the isolated saccharide is reacted with CDI in DMSO comprising 0.2% to 0.3% (v/v) water.

15 In one embodiment the isolated saccharide is reacted with CDI in DMSO comprising 0.3% to 0.8% (v/v) water. In one embodiment the isolated saccharide is reacted with CDI in DMSO comprising 0.3% to 0.5% (v/v) water. In one embodiment the isolated saccharide is reacted with CDI in DMSO comprising 0.3% to 0.4% (v/v) water.

20 In one embodiment the isolated saccharide is reacted with CDI in DMSO comprising about 0.1% (v/v) water. In one embodiment the isolated saccharide is reacted with CDI in DMSO comprising about 0.2% (v/v) water. In one embodiment the isolated saccharide is reacted with CDI in DMSO comprising about 0.3% (v/v) water. In one embodiment the isolated saccharide is reacted with CDI in DMSO comprising about 0.4% (v/v) water. In one embodiment the isolated saccharide is reacted with CDI in DMSO comprising about 0.5% (v/v) water. In one embodiment the isolated saccharide is reacted with CDI in DMSO comprising about 0.6% (v/v) water. In one embodiment the isolated saccharide is reacted with CDI in DMSO comprising about 0.7% (v/v) water. In one embodiment the isolated saccharide is reacted with CDI in DMSO comprising about 0.8% (v/v) water. In one embodiment the isolated saccharide is reacted with CDI in DMSO comprising about 0.9% (v/v) water.

25 In one embodiment the free carbonic acid derivative is then quenched by the addition of water before the addition of the azido linker. Water can inactivate free CDI.

30 Therefore, in an embodiment, carbonic acid derivative activation is followed by the addition of water. In an embodiment, water is added to bring the total water content in the mixture to between about 1% to about 10% (v/v). In an embodiment, water is added to bring the total water content in the mixture to between about 1.2% to about 8% (v/v). In an embodiment, water is added to bring the total water content in the mixture to between about 1.5% to about 5% (v/v). In an embodiment, water is added to bring the total water

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content in the mixture to between about 1.5% to about 3% (v/v). In an embodiment, water is added to bring the total water content in the mixture to between about 1.5% to about 2.5% (v/v). In an embodiment, water is added to bring the total water content in the mixture to about 1 % (v/v). In an embodiment, water is added to bring the total water content in the mixture to about 1.2% (v/v). In an embodiment, water is added to bring the total water content in the mixture to about 1.4% (v/v). In an embodiment, water is added to bring the total water content in the mixture to about 1.5% (v/v). In an embodiment, water is added to bring the total water content in the mixture to about 2% (v/v). In an embodiment, water is added to bring the total water content in the mixture to about 2.5% (v/v). In an embodiment, water is added to bring the total water content in the mixture to about 3% (v/v). In an embodiment, water is added to bring the total water content in the mixture to about 5% (v/v). In an embodiment, water is added to bring the total water content in the mixture to about 7% (v/v). In an embodiment, water is added to bring the total water content in the mixture to about 10% (v/v).

Once the saccharide has been reacted with carbonic acid derivative and following an eventual quenching of carbonic acid derivative with water, the carbonic acid derivative-activated saccharide is reacted with an azido linker.

In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 0.01-10 molar equivalent to the amount of polysaccharide Repeat Unit of the activated saccharide (molar equivalent of RU).

In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 0.01-8 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 0.01-5 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 0.01-4 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 0.01-3 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 0.01-2 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

5 In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 0.01-1 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 0.01-0.5 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

10 In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 0.01-0.1 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 0.05-10 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

15 In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 0.05-8 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 0.05-5 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 0.05-4 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

25 In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 0.05-3 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 0.05-2 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

30 In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 0.05-1 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 0.05-0.5 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

5 In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 0.05-0.1 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 0.1-10 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

10 In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 0.1-8 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 0.1-5 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

15 In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 0.1-4 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 0.1-3 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

20 In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 0.1-2 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

25 In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 0.1-1 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 0.1-0.5 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

30 In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 0.5-10 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 0.5-8 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

5 In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 0.5-5 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 0.5-4 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

10 In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 0.5-3 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 0.5-2 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

15 In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 0.5-1 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

20 In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 1-10 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 1-8 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

25 In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 1-5 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

30 In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 1-4 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 1-3 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 1-2 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

5 In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 2-10 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 2-8 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

10 In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 2-5 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 2-4 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

15 In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 2-3 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

20 In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 3-10 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 3-8 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

25 In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 3-5 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

30 In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 3-4 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 4-10 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 4-8 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

5 In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 4-5 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 5-10 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

10 In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 5-8 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 8-10 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

15 In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is about 0.01 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is about 0.05 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

20 In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is about 0.1 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

25 In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is about 0.5 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is about 1 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

30 In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is about 2 molar equivalents to the amount of polysaccharide repeat unit of the activated saccharide.

In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is about 3 molar equivalents to the amount of polysaccharide repeat unit of the activated saccharide.

5 In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is about 4 molar equivalents to the amount of polysaccharide repeat unit of the activated saccharide.

In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is about 5 molar equivalents to the amount of polysaccharide repeat unit of the activated saccharide.

10 In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is about 8 molar equivalents to the amount of polysaccharide repeat unit of the activated saccharide.

In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is about 10 molar equivalents to the amount of polysaccharide repeat unit of the activated saccharide.

15 In the above embodiments, said carbonic acid derivative is preferably CDI. In another embodiment, said carbonic acid derivative is CDT.

In one embodiment the degree of activation of the activated saccharide following step a) is between 0.5 to 50%. The degree of activation of the azido saccharide being defined as the percentage of Repeating Unit linked to an azido linker.

20 In one embodiment the degree of activation of the activated saccharide following step a) is between 1 to 30%. In another embodiment the degree of activation of the activated saccharide following step a) is between 2 to 25%. In another embodiment the degree of activation of the activated saccharide following step a) is between 3 to 20%.

25 In another embodiment the degree of activation of the activated saccharide following step a) is between 3 to 15%. In another embodiment the degree of activation of the activated saccharide following step a) is between 4 to 15%. In an embodiment the degree of activation of the activated saccharide following step a) is between 1 to 6%.

In an embodiment the degree of activation of the activated saccharide following step a) is between 3 to 6%. In an embodiment the degree of activation of the activated saccharide following step a) is between 10 to 15%.

30 In an embodiment the degree of activation of the activated saccharide following step a) is about 1%. In an embodiment the degree of activation of the activated saccharide following step a) is about 2%. In an embodiment the degree of activation of the activated saccharide following step a) is about 3%. In an embodiment the degree of activation of

the activated saccharide following step a) is about 4%. In an embodiment the degree of activation of the activated saccharide following step a) is about 5%. In an embodiment the degree of activation of the activated saccharide following step a) is about 6%. In an embodiment the degree of activation of the activated saccharide following step a) is about 7%. In an embodiment the degree of activation of the activated saccharide following step a) is about 8%. In an embodiment the degree of activation of the activated saccharide following step a) is about 9%. In an embodiment the degree of activation of the activated saccharide following step a) is about 10%. In an embodiment the degree of activation of the activated saccharide following step a) is about 11%. In an embodiment the degree of activation of the activated saccharide following step a) is about 12%. In an embodiment the degree of activation of the activated saccharide following step a) is about 13%. In an embodiment the degree of activation of the activated saccharide following step a) is about 14%. In an embodiment the degree of activation of the activated saccharide following step a) is about 15%. In an embodiment the degree of activation of the activated saccharide following step a) is about 16%. In an embodiment the degree of activation of the activated saccharide following step a) is about 17%. In an embodiment the degree of activation of the activated saccharide following step a) is about 18%. In an embodiment the degree of activation of the activated saccharide following step a) is about 19%. In an embodiment the degree of activation of the activated saccharide following step a) is about 20%.

In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 0.1-10 molar equivalents to the lysines on the carrier.

In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 0.5-10 molar equivalents to the lysines on the carrier.

In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 1-10 molar equivalents to the lysines on the carrier.

In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 1.5-10 molar equivalents to the lysines on the carrier.

In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 2-10 molar equivalents to the lysines on the carrier.

In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 2.5-10 molar equivalents to the lysines on the carrier.

5 In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 3-10 molar equivalents to the lysines on the carrier.

In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 5-10 molar equivalents to the lysines on the carrier.

10 In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 7.5-10 molar equivalents to the lysines on the carrier.

In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 0.1-15 7.5 molar equivalents to the lysines on the carrier.

In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 0.5-7.5 molar equivalents to the lysines on the carrier.

20 In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 1-7.5 molar equivalents to the lysines on the carrier.

In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 1.5-7.5 molar equivalents to the lysines on the carrier.

25 In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 2-7.5 molar equivalents to the lysines on the carrier.

In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 2.5-30 7.5 molar equivalents to the lysines on the carrier.

In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 3-7.5 molar equivalents to the lysines on the carrier.

In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 5-7.5 molar equivalents to the lysines on the carrier.

5 In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 0.1-5 molar equivalents to the lysines on the carrier.

In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 0.5-5 molar equivalents to the lysines on the carrier.

10 In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 1-5 molar equivalents to the lysines on the carrier.

In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 1.5-15 5 molar equivalents to the lysines on the carrier.

In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 2-5 molar equivalents to the lysines on the carrier.

20 In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 2.5-5 molar equivalents to the lysines on the carrier.

In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 3-5 molar equivalents to the lysines on the carrier.

25 In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 0.1-3 molar equivalents to the lysines on the carrier.

In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 0.5-30 3 molar equivalents to the lysines on the carrier.

In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 1-3 molar equivalents to the lysines on the carrier.

In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 1.5-3 molar equivalents to the lysines on the carrier.

5 In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 2-3 molar equivalents to the lysines on the carrier.

In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 2.5-3 molar equivalents to the lysines on the carrier.

10 In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 0.1-2.5 molar equivalents to the lysines on the carrier.

In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 0.5-2.5 molar equivalents to the lysines on the carrier.

In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 1-2.5 molar equivalents to the lysines on the carrier.

20 In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 1.5-2.5 molar equivalents to the lysines on the carrier.

In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 2-2.5 molar equivalents to the lysines on the carrier.

25 In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 0.1-2 molar equivalents to the lysines on the carrier.

In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 0.5-2 molar equivalents to the lysines on the carrier.

30 In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 1-2 molar equivalents to the lysines on the carrier.

In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 1.5-2 molar equivalents to the lysines on the carrier.

5 In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 0.1-1.5 molar equivalents to the lysines on the carrier.

In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 0.5-1.5 molar equivalents to the lysines on the carrier.

10 In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 1-1.5 molar equivalents to the lysines on the carrier.

In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 0.1-15 1 molar equivalents to the lysines on the carrier.

In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 0.5-1 molar equivalents to the lysines on the carrier.

20 In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 0.1-0.5 molar equivalents to the lysines on the carrier.

In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is about 10 molar equivalents to the lysines on the carrier.

25 In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is about 7.5 molar equivalents to the lysines on the carrier.

In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is about 5 molar equivalents to the lysines on the carrier.

30 In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is about 3 molar equivalents to the lysines on the carrier.

In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is about 2.5 molar equivalents to the lysines on the carrier.

5 In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is about 2 molar equivalents to the lysines on the carrier.

In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is about 1.5 molar equivalents to the lysines on the carrier.

10 In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is about 1 molar equivalent to the lysines on the carrier.

In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is about 0.5 molar equivalents to the lysines on the carrier.

15 In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is about 0.1 molar equivalents to the lysines on the carrier.

In one embodiment the degree of activation of the activated carrier following step b) is between 1 and 50. The degree of activation of the activated carrier being defined as the number of lysine residues in the carrier protein that become linked to the agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group .

In an embodiment, the carrier protein is CRM₁₉₇, which contains 39 lysine residues. In said embodiment the degree of activation of the activated carrier following step b) may be between 1 to 30. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is between 5 to 20. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is between 9 to 18. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is between 8 to 11. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is between 15 to 20. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 5. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 6. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 7. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 8. In another embodiment the

degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 9. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 10. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 11. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 12. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 13. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 14. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 15. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 16. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 17. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 18. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 19. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 20. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 21. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 22. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 23. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 24. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 25.

In an embodiment, the carrier protein is SCP or a fragment thereof. In said embodiment the degree of activation of the activated carrier following step b) may be between 1 to 50. In another embodiment the degree of activation of the activated carrier (SCP) following step b) is between 5 to 50. In another embodiment the degree of activation of the activated carrier (SCP) following step b) is between 7 to 45. In another embodiment the degree of activation of the activated carrier (SCP) following step b) is between 5 to 15. In another embodiment the degree of activation of the activated carrier (SCP) following step b) is between 20 to 30. In another embodiment the degree of activation of the activated carrier (SCP) following step b) is between 30 to 50. In another embodiment the degree of activation of the activated carrier (SCP) following step b) is between 30 to 40. In another embodiment the degree of activation of the activated carrier (SCP) following step b) is between 10 to 40. In another embodiment the degree of activation of the activated carrier (SCP) following step b) is about 5. In another embodiment the degree of activation of the activated carrier (SCP) following step b) is

about 7. In another embodiment the degree of activation of the activated carrier (SCP) following step b) is about 10. In another embodiment the degree of activation of the activated carrier (SCP) following step b) is about 13. In another embodiment the degree of activation of the activated carrier (SCP) following step b) is about 15. In another embodiment the degree of activation of the activated carrier (SCP) following step b) is about 20. In another embodiment the degree of activation of the activated carrier (SCP) following step b) is about 26. In another embodiment the degree of activation of the activated carrier (SCP) following step b) is about 30. In another embodiment the degree of activation of the activated carrier (SCP) following step b) is about 35. In another embodiment the degree of activation of the activated carrier (SCP) following step b) is about 37. In another embodiment the degree of activation of the activated carrier (SCP) following step b) is about 40. In another embodiment the degree of activation of the activated carrier (SCP) following step b) is about 45. In another embodiment the degree of activation of the activated carrier (SCP) following step b) is about 50.

In an embodiment, the carrier protein is TT or a fragment thereof. In said embodiment the degree of activation of the activated carrier following step b) may be between 1 to 30. In another embodiment the degree of activation of the activated carrier (TT) following step b) is between 5 to 25. In another embodiment the degree of activation of the activated carrier (TT) following step b) is between 7 to 25. In another embodiment the degree of activation of the activated carrier (TT) following step b) is between 10 to 20. In another embodiment the degree of activation of the activated carrier (TT) following step b) is about 5. In another embodiment the degree of activation of the activated carrier (TT) following step b) is about 7. In another embodiment the degree of activation of the activated carrier (TT) following step b) is about 10. In another embodiment the degree of activation of the activated carrier (TT) following step b) is about 12. In another embodiment the degree of activation of the activated carrier (TT) following step b) is about 15. In another embodiment the degree of activation of the activated carrier (TT) following step b) is about 20. In another embodiment the degree of activation of the activated carrier (TT) following step b) is about 25. In another embodiment the degree of activation of the activated carrier (TT) following step b) is about 30.

In an embodiment, the conjugation reaction c) is carried out in aqueous buffer. In an embodiment, the conjugation reaction c) is carried out in aqueous buffer in the presence of copper (I) as catalyst. In an embodiment, the conjugation reaction c) is carried out in aqueous buffer in the presence an oxidant and of copper (I) as catalyst. In a preferred embodiment, the conjugation reaction c) is carried out in aqueous buffer in

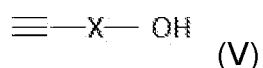
the presence of copper (I) as catalyst and ascorbate as oxidant. In an embodiment, THPTA (tris(3-hydroxypropyltriazolylmethyl)amine) and aminoguanidine may be further added to protect the protein from side reactions. Therefore, in a preferred embodiment, the conjugation reaction c) is carried out in aqueous buffer in the presence of copper (I) as catalyst and ascorbate as oxidant, wherein the reaction mixture further comprises THPTA (tris(3-hydroxypropyltriazolylmethyl)amine) and aminoguanidine.

In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at setp c) is between 0.1 and 3. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at setp c) is between 0.5 and 2. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at setp c) is between 0.6 and 1.5. In a preferred embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at setp c) is between 0.8 and 1. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at setp c) is about 0.5. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at setp c) is about 0.6. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at setp c) is about 0.7. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at setp c) is about 0.8. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at setp c) is about 0.9. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at setp c) is about 1. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at setp c) is about 1.1. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at setp c) is about 1.2. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at setp c) is about 1.3. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at setp c) is about 1.4. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at setp c) is about 1.5. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at setp c) is about 1.6. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at setp c) is about 1.7. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at setp c) is about 1.8. In an

embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at setp c) is about 1.9. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at setp c) is about 2.

5 Following the click conjugation reaction, there may remain unreacted azido groups in the conjugates, these may be capped using a suitable azido group capping agent. Therefore, in an embodiment, following step c), unreacted azido groups in the conjugates, are capped using a suitable azido group capping agent. In one embodiment this azido group capping agent is an agent bearing an alkyne group. In one embodiment this azido
10 group capping agent is an agent bearing a terminal alkyne. In one embodiment this azido group capping agent is an agent bearing a cycloalkyne.

In an embodiment, said azido group capping agent is a compound of formula (V),



wherein X is (CH₂)_n wherein n is selected from 1 to 15.

15 In one embodiment this azido group capping agent is propargyl alcohol.

Therefore, in an embodiment, following step (c) the process further comprises a step of capping the unreacted azido groups remained in the conjugates with an azido group capping agent.

In an embodiment the capping of the unreacted azido groups is performed with an
20 amount of capping agent that is between 0.05 to 20 molar equivalents to the amount of polysaccharide repeat unit of the activated saccharide.

In an embodiment the capping of the unreacted azido groups is performed with an amount of capping agent that is between 0.1 to 15 molar equivalents to the amount of polysaccharide repeat unit of the activated saccharide.

25 In an embodiment the capping of the unreacted azido groups is performed with an amount of capping agent that is between 0.5 to 10 molar equivalents to the amount of polysaccharide repeat unit of the activated saccharide.

In an embodiment the capping of the unreacted azido groups is performed with an amount of capping agent that is between 0.5 to 5 molar equivalents to the amount of
30 polysaccharide repeat unit of the activated saccharide.

In an embodiment the capping of the unreacted azido groups is performed with an amount of capping agent that is between 0.5 to 2 molar equivalents to the amount of polysaccharide repeat unit of the activated saccharide.

In an embodiment the capping of the unreacted azido groups is performed with an amount of capping agent that is between 0.5 to 1 molar equivalents to the amount of polysaccharide repeat unit of the activated saccharide.

5 In an embodiment the capping of the unreacted azido groups is performed with an amount of capping agent that is between 1 to 2 molar equivalents to the amount of polysaccharide repeat unit of the activated saccharide.

In an embodiment the capping of the unreacted azido groups is performed with an amount of capping agent that is between 0.75 to 1.5 molar equivalents to the amount of polysaccharide repeat unit of the activated saccharide.

10 In an embodiment the capping of the unreacted azido groups is performed with an amount of capping agent that is about 1 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

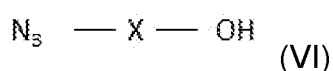
In an embodiment the capping of the unreacted azido groups is performed with an amount of capping agent that is about 1.5 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

15 In an embodiment the capping of the unreacted azido groups is performed with an amount of capping agent that is about 0.5 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

20 In an embodiment the capping of the unreacted azido groups is performed with an amount of capping agent that is about 2 molar equivalents to the amount of polysaccharide repeat unit of the activated saccharide.

Following the click conjugation reaction, unreacted alkyne groups may remain present in the conjugates, these may be capped using a suitable alkyne group capping agent. In one embodiment this alkyne group capping agent is an agent bearing an azido group.

25 In an embodiment, said alkyne group capping agent is a compound of formula (VI),



wherein X is (CH₂)_n wherein n is selected from 1 to 15.

In one embodiment this alkyne group capping agent is 3-azido-1-propanol.

30 Therefore, in an embodiment, following step (c) the process further comprises a step of capping the unreacted alkyne groups remained in the conjugates with an alkyne group capping agent.

In an embodiment the capping of the unreacted alkyne groups is performed with an amount of capping agent that is between 0.05 to 20 molar equivalents to the amount of polysaccharide repeat unit of the activated saccharide.

5 In an embodiment the capping of the unreacted alkyne groups is performed with an amount of capping agent that is between 0.1 to 15 molar equivalents to the amount of polysaccharide repeat unit of the activated saccharide.

In an embodiment the capping of the unreacted alkyne groups is performed with an amount of capping agent that is between 0.5 to 10 molar equivalents to the amount of polysaccharide repeat unit of the activated saccharide.

10 In an embodiment the capping of the unreacted alkyne groups is performed with an amount of capping agent that is between 0.5 to 5 molar equivalents to the amount of polysaccharide repeat unit of the activated saccharide.

In an embodiment the capping of the unreacted alkyne groups is performed with an amount of capping agent that is between 0.5 to 2 molar equivalents to the amount of polysaccharide repeat unit of the activated saccharide.

15 In an embodiment the capping of the unreacted alkyne groups is performed with an amount of capping agent that is between 0.5 to 1 molar equivalents to the amount of polysaccharide repeat unit of the activated saccharide.

In an embodiment the capping of the unreacted alkyne groups is performed with an amount of capping agent that is between 1 to 5 molar equivalents to the amount of polysaccharide repeat unit of the activated saccharide.

20 In an embodiment the capping of the unreacted alkyne groups is performed with an amount of capping agent that is between 1 to 2 molar equivalents to the amount of polysaccharide repeat unit of the activated saccharide.

25 In an embodiment the capping of the unreacted alkyne groups is performed with an amount of capping agent that is between 1.5 to 2.5 molar equivalents to the amount of polysaccharide repeat unit of the activated saccharide.

In an embodiment the capping of the unreacted alkyne groups is performed with an amount of capping agent that about 0.5 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

30 In an embodiment the capping of the unreacted alkyne groups is performed with an amount of capping agent that about 1 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

In an embodiment the capping of the unreacted alkyne groups is performed with an amount of capping agent that about 1.5 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

In an embodiment the capping of the unreacted alkyne groups is performed with an amount of capping agent that about 2 molar equivalentS to the amount of polysaccharide repeat unit of the activated saccharide.

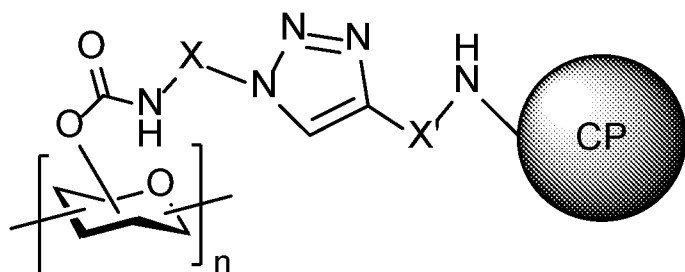
In an embodiment the capping of the unreacted alkyne groups is performed with an amount of capping agent that about 2.5 molar equivalents to the amount of polysaccharide repeat unit of the activated saccharide.

In an embodiment the capping of the unreacted alkyne groups is performed with an amount of capping agent that about 5 molar equivalents to the amount of polysaccharide repeat unit of the activated saccharide.

Following conjugation to the carrier protein, the glycoconjugate can be purified (enriched with respect to the amount of saccharide-protein conjugate) by a variety of techniques known to the skilled person. These techniques include dialysis, concentration/diafiltration operations, tangential flow filtration precipitation/elution, column chromatography (DEAE or hydrophobic interaction chromatography), and depth filtration. Therefore, in one embodiment the process for producing the glycoconjugate of the present invention comprises the step of purifying the glycoconjugate after it is produced.

In an aspect, the invention provides a glycoconjugate produced according to any of the methods disclosed herein.

In an aspect, the invention provides a capsular saccharide glycoconjugate comprising a capsular saccharide 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 $\text{CH}_2(\text{CH}_2)_{n'}$, $(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$, $\text{NHCO}(\text{CH}_2)_{n'}$, $\text{NHCO}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$, $\text{OCH}_2(\text{CH}_2)_{n'}$ and $\text{O}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$; where n' is selected from 1 to 10 and m is selected from 1 to 4,

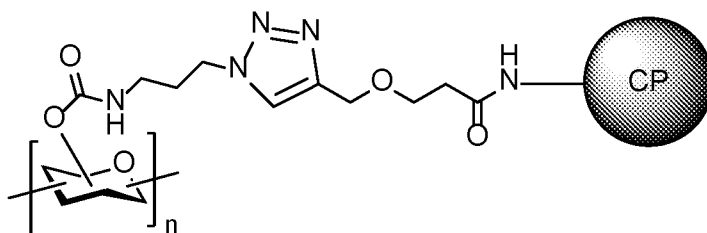
and wherein X' is selected from the group consisting of $\text{CH}_2\text{O}(\text{CH}_2)_{n''}\text{CH}_2\text{C}=\text{O}$, $\text{CH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_{m'}(\text{CH}_2)_{n''}\text{CH}_2\text{C}=\text{O}$, where n'' is selected from 0 to 10 and m' is selected from 0 to 4. Where the structure in square brackets represents a repeat unit of the capsular saccharide.

5 In an embodiment, the capsular saccharide is not a capsular saccharide from *Streptococcus pneumoniae* serotype 3.

Formula (VII) is a schematic representation of glycoconjugates of the invention. It should not be understood that a linkage is present at every repeating unit of the saccharide (the structure in square brackets). Rather, a majority of the saccharide repeating unit remains unmodified and covalent linkages between the carrier protein and the saccharide is for a minority of the saccharide repeat units. Additionally, an individual carrier protein (CP) molecule may be linked to more than one saccharide molecule and an individual saccharide molecule can be linked to more than one individual carrier protein (CP) molecule. The structure in square brackets represents a repeat unit of the capsular saccharide.

15 In a preferred embodiment, the invention provides a glycoconjugate comprising a saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (VII), wherein X is $\text{CH}_2(\text{CH}_2)_{n'}$, where n' is 2 and wherein X' is $\text{CH}_2\text{O}(\text{CH}_2)_{n''}\text{CH}_2\text{C}=\text{O}$ where n'' is 1. In an embodiment, the saccharide is not a capsular saccharide from *Streptococcus pneumoniae* serotype 3.

20 Therefore, in a preferred embodiment, the invention provides a capsular saccharide glycoconjugate comprising a capsular saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (VIII),



(VIII).

25 Where the structure in square brackets represents a repeat unit of the capsular saccharide.

Formula (VIII) is a schematic representation of the preferred glycoconjugates of the invention. It should not be understood that a linkage is present at every repeat unit of the saccharide (the structure in square brackets). Rather, a majority of the saccharide repeating unit remains unmodified and covalent linkages between the carrier protein and the saccharide is for a minority of the saccharide repeat units. Additionally, an individual

carrier protein (CP) molecule may be linked to more than one saccharide molecule and an individual saccharide molecule can be linked to more than one individual carrier protein (CP) molecule. The structure in square brackets is a schematic representation of a repeat unit of the saccharide.

- 5 In an embodiment, the saccharide is not a capsular saccharide from *Streptococcus pneumoniae* serotype 3.

In an embodiment, the invention provides a glycoconjugate comprising a saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (VII), wherein X is $\text{CH}_2(\text{CH}_2)_{n'}$, where n' is selected from 1 to 10 and
 10 wherein X' is $\text{CH}_2\text{O}(\text{CH}_2)_{n''}\text{CH}_2\text{C}=\text{O}$ where n'' is selected from 0 to 10. In an embodiment, n' is selected from 1 to 5 and n'' is selected from 0 to 10. In an embodiment, n' is selected from 1 to 5 and n'' is selected from 0 to 5. In an embodiment, n' is selected from 1 to 3 and n'' is selected from 0 to 3. In an embodiment, n' is selected from 1 to 2 and n'' is selected from 0 to 2. In a particular embodiment, n' is 1 and n'' is 0. In another
 15 embodiment, n' is 2 and n'' is 0. In yet another embodiment, n' is 3 and n'' is 0. In yet a further embodiment, n' is 4 and n'' is 0. In yet a further embodiment, n' is 5 and n'' is 0. In yet a further embodiment, n' is 6 and n'' is 0. In a particular embodiment, n' is 1 and n'' is 1. In another embodiment, n' is 2 and n'' is 1. In yet another embodiment, n' is 3 and n'' is 1. In yet a further embodiment, n' is 4 and n'' is 1. In yet a further embodiment, n' is 5
 20 and n'' is 1. In yet a further embodiment, n' is 6 and n'' is 1. In a particular embodiment, n' is 1 and n'' is 2. In another embodiment, n' is 2 and n'' is 2. In yet another embodiment, n' is 3 and n'' is 2. In yet a further embodiment, n' is 4 and n'' is 2. In yet a further embodiment, n' is 5 and n'' is 2. In yet a further embodiment, n' is 6 and n'' is 2. In a particular embodiment, n' is 1 and n'' is 3. In another embodiment, n' is 2 and n'' is 3. In
 25 yet another embodiment, n' is 3 and n'' is 3. In yet a further embodiment, n' is 4 and n'' is 3. In yet a further embodiment, n' is 5 and n'' is 3. In yet a further embodiment, n' is 6 and n'' is 3. In a particular embodiment, n' is 1 and n'' is 4. In another embodiment, n' is 2 and n'' is 4. In yet another embodiment, n' is 3 and n'' is 4. In yet a further embodiment, n' is 4 and n'' is 4. In yet a further embodiment, n' is 5 and n'' is 4. In yet a further embodiment,
 30 n' is 6 and n'' is 4. In a particular embodiment, n' is 1 and n'' is 5. In another embodiment, n' is 2 and n'' is 5. In yet another embodiment, n' is 3 and n'' is 5. In yet a further embodiment, n' is 4 and n'' is 5. In yet a further embodiment, n' is 5 and n'' is 5. In yet a further embodiment, n' is 6 and n'' is 5. In a particular embodiment, n' is 1 and n'' is 6. In another embodiment, n' is 2 and n'' is 6. In yet another embodiment, n' is 3 and n'' is 6.
 35 In yet a further embodiment, n' is 4 and n'' is 6. In yet a further embodiment, n' is 5 and

n'' is 6. In yet a further embodiment, n' is 6 and n'' is 6. Preferably, the saccharide is not a capsular saccharide from *Streptococcus pneumoniae* serotype 3.

In an embodiment, the invention provides a glycoconjugate comprising a saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (VII), wherein X is CH₂(CH₂)_{n'}, where n' is selected from 1 to 10 and wherein CH₂O(CH₂CH₂O)_{m'}(CH₂)_{n''}CH₂C=O, where n'' is selected from 0 to 10 and m' is selected from 0 to 4.

In an embodiment, n' is selected from 1 to 5, m' is selected from 0 to 4 and n'' is selected from 0 to 10. In an embodiment, n' is selected from 1 to 5, m' is selected from 0 to 4 and n'' is selected from 0 to 5. In an embodiment, n' is selected from 1 to 3, m' is selected from 0 to 2 and n'' is selected from 0 to 3. In an embodiment, n' is selected from 1 to 2, m' is selected from 0 to 2 and n'' is selected from 0 to 1.

In a particular embodiment, n' is 1, m' is 0 and n'' is 0. In another embodiment, n' is 1, m' is 1 and n'' is 0. In another embodiment, n' is 1, m' is 2 and n'' is 0. In another embodiment, n' is 1, m' is 3 and n'' is 0.

In another embodiment, n' is 2, m' is 0 and n'' is 0. In another embodiment, n' is 2, m' is 1 and n'' is 0. In another embodiment, n' is 2, m' is 2 and n'' is 0. In another embodiment, n' is 2, m' is 3 and n'' is 0.

In yet another embodiment, n' is 3, m' is 0 and n'' is 0. In yet another embodiment, n' is 3, m' is 1 and n'' is 0. In yet another embodiment, n' is 3, m' is 2 and n'' is 0. In yet another embodiment, n' is 3, m' is 3 and n'' is 0.

In yet a further embodiment, n' is 4, m' is 0 and n'' is 0. In yet a further embodiment, n' is 4, m' is 1 and n'' is 0. In yet a further embodiment, n' is 4, m' is 2 and n'' is 0. In yet a further embodiment, n' is 4, m' is 3 and n'' is 0.

In yet a further embodiment, n' is 5, m' is 0 and n'' is 0. In yet a further embodiment, n' is 5, m' is 1 and n'' is 0. In yet a further embodiment, n' is 5, m' is 2 and n'' is 0. In yet a further embodiment, n' is 5, m' is 3 and n'' is 0.

In a particular embodiment, n' is 1, m' is 0 and n'' is 1. In a particular embodiment, n' is 1, m' is 1 and n'' is 1. In a particular embodiment, n' is 1, m' is 2 and n'' is 1. In a particular embodiment, n' is 1, m' is 3 and n'' is 1.

In another embodiment, n' is 2, m' is 0 and n'' is 1. In another embodiment, n' is 2, m' is 1 and n'' is 1. In another embodiment, n' is 2, m' is 2 and n'' is 1. In another embodiment, n' is 2, m' is 3 and n'' is 1.

In yet another embodiment, n' is 3, m' is 0 and n'' is 1. In yet another embodiment, n' is 3, m' is 1 and n'' is 1. In yet another embodiment, n' is 3, m' is 2 and n'' is 1. In yet another embodiment, n' is 3, m' is 3 and n'' is 1.

In yet a further embodiment, n' is 4, m' is 0 and n'' is 1. In yet a further embodiment, n' is 4, m' is 1 and n'' is 1. In yet a further embodiment, n' is 4, m' is 2 and n'' is 1. In yet a further embodiment, n' is 4, m' is 3 and n'' is 1.

In yet a further embodiment, n' is 5, m' is 0 and n'' is 1. In yet a further embodiment, n' is 5, m' is 1 and n'' is 1. In yet a further embodiment, n' is 5, m' is 2 and n'' is 1. In yet a further embodiment, n' is 5, m' is 3 and n'' is 1.

In a particular embodiment, n' is 1, m' is 0 and n'' is 2. In a particular embodiment, n' is 1, m' is 1 and n'' is 2. In a particular embodiment, n' is 1, m' is 2 and n'' is 2. In a particular embodiment, n' is 1, m' is 3 and n'' is 2.

In another embodiment, n' is 2, m' is 0 and n'' is 2. In another embodiment, n' is 2, m' is 1 and n'' is 2. In another embodiment, n' is 2, m' is 2 and n'' is 2. In another embodiment, n' is 2, m' is 3 and n'' is 2.

In yet another embodiment, n' is 3, m' is 0 and n'' is 2. In yet another embodiment, n' is 3, m' is 1 and n'' is 2. In yet another embodiment, n' is 3, m' is 2 and n'' is 2. In yet another embodiment, n' is 3, m' is 3 and n'' is 2.

In yet a further embodiment, n' is 4, m' is 0 and n'' is 2. In yet a further embodiment, n' is 4, m' is 1 and n'' is 2. In yet a further embodiment, n' is 4, m' is 2 and n'' is 2. In yet a further embodiment, n' is 4, m' is 3 and n'' is 2.

In yet a further embodiment, n' is 5, m' is 0 and n'' is 2. In yet a further embodiment, n' is 5, m' is 1 and n'' is 2. In yet a further embodiment, n' is 5, m' is 2 and n'' is 2. In yet a further embodiment, n' is 5, m' is 3 and n'' is 2.

In a particular embodiment, n' is 1, m' is 0 and n'' is 3. In a particular embodiment, n' is 1, m' is 1 and n'' is 3. In a particular embodiment, n' is 1, m' is 2 and n'' is 3. In a particular embodiment, n' is 1, m' is 3 and n'' is 3.

In another embodiment, n' is 2, m' is 0 and n'' is 3. In another embodiment, n' is 2, m' is 1 and n'' is 3. In another embodiment, n' is 2, m' is 2 and n'' is 3. In another embodiment, n' is 2, m' is 3 and n'' is 3.

In yet another embodiment, n' is 3, m' is 0 and n'' is 3. In yet another embodiment, n' is 3, m' is 1 and n'' is 3. In yet another embodiment, n' is 3, m' is 2 and n'' is 3. In yet another embodiment, n' is 3, m' is 3 and n'' is 3.

In yet a further embodiment, n' is 4, m' is 0 and n'' is 3. In yet a further embodiment, n' is 4, m' is 1 and n'' is 3. In yet a further embodiment, n' is 4, m' is 2 and n'' is 3. In yet a further embodiment, n' is 4, m' is 3 and n'' is 3.

In yet a further embodiment, n' is 5, m' is 0 and n'' is 3. In yet a further embodiment, n' is 5, m' is 1 and n'' is 3. In yet a further embodiment, n' is 5, m' is 2 and n'' is 3. In yet a further embodiment, n' is 5, m' is 3 and n'' is 3.

In a particular embodiment, n' is 1, m' is 0 and n'' is 4. In a particular embodiment, n' is 1, m' is 1 and n'' is 4. In a particular embodiment, n' is 1, m' is 2 and n'' is 4. In a particular embodiment, n' is 1, m' is 3 and n'' is 4.

In another embodiment, n' is 2, m' is 0 and n'' is 4. In another embodiment, n' is 2, m' is 1 and n'' is 4. In another embodiment, n' is 2, m' is 2 and n'' is 4. In another embodiment, n' is 2, m' is 3 and n'' is 4.

In yet another embodiment, n' is 3, m' is 0 and n'' is 4. In yet another embodiment, n' is 3, m' is 1 and n'' is 4. In yet another embodiment, n' is 3, m' is 2 and n'' is 4. In yet another embodiment, n' is 3, m' is 3 and n'' is 4.

In yet a further embodiment, n' is 4, m' is 0 and n'' is 4. In yet a further embodiment, n' is 4, m' is 1 and n'' is 4. In yet a further embodiment, n' is 4, m' is 2 and n'' is 4. In yet a further embodiment, n' is 4, m' is 3 and n'' is 4.

In yet a further embodiment, n' is 5, m' is 0 and n'' is 4. In yet a further embodiment, n' is 5, m' is 1 and n'' is 4. In yet a further embodiment, n' is 5, m' is 2 and n'' is 4. In yet a further embodiment, n' is 5, m' is 3 and n'' is 4.

In a particular embodiment, n' is 1, m' is 0 and n'' is 5. In a particular embodiment, n' is 1, m' is 1 and n'' is 5. In a particular embodiment, n' is 1, m' is 2 and n'' is 5. In a particular embodiment, n' is 1, m' is 3 and n'' is 5.

In another embodiment, n' is 2, m' is 0 and n'' is 5. In another embodiment, n' is 2, m' is 1 and n'' is 5. In another embodiment, n' is 2, m' is 2 and n'' is 5. In another embodiment, n' is 2, m' is 3 and n'' is 5.

In yet another embodiment, n' is 3, m' is 0 and n'' is 5. In yet another embodiment, n' is 3, m' is 1 and n'' is 5. In yet another embodiment, n' is 3, m' is 2 and n'' is 5. In yet another embodiment, n' is 3, m' is 3 and n'' is 5.

In yet a further embodiment, n' is 4, m' is 0 and n'' is 5. In yet a further embodiment, n' is 4, m' is 1 and n'' is 5. In yet a further embodiment, n' is 4, m' is 2 and n'' is 5. In yet a further embodiment, n' is 4, m' is 3 and n'' is 5.

In yet a further embodiment, n' is 5, m' is 0 and n'' is 5. In yet a further embodiment, n' is 5, m' is 1 and n'' is 5. In yet a further embodiment, n' is 5, m' is 2 and n'' is 5. In yet a further embodiment, n' is 5, m' is 3 and n'' is 5.

Preferably, the saccharide is not a capsular saccharide from *Streptococcus pneumoniae* serotype 3.

In an embodiment, the invention provides a glycoconjugate comprising a saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (VII), wherein X is $(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$, where m is selected from 1 to 4 and wherein X' is $\text{CH}_2\text{O}(\text{CH}_2)_{n''}\text{CH}_2\text{C}=\text{O}$, where n'' is selected from 0 to 10. In an embodiment, m is selected from 1 to 3 and n'' is selected from 0 to 10. In an embodiment, m is selected from 1 to 3 and n'' is selected from 0 to 5. In an embodiment, m is selected from 1 to 2 and n'' is selected from 0 to 3. In an embodiment, m is selected from 1 to 2 and n'' is selected from 0 to 2. In a particular embodiment, m is 1 and n'' is 0. In another embodiment, m is 2 and n'' is 0. In yet another embodiment, m is 3 and n'' is 0. In yet a further embodiment, m is 4 and n'' is 0. In a particular embodiment, m is 1 and n'' is 1. In another embodiment, m is 2 and n'' is 1. In yet another embodiment, m is 3 and n'' is 1. In yet a further embodiment, m is 4 and n'' is 1. In a particular embodiment, m is 1 and n'' is 2. In another embodiment, m is 2 and n'' is 2. In yet another embodiment, m is 3 and n'' is 2. In yet a further embodiment, m is 4 and n'' is 2. In a particular embodiment, m is 1 and n'' is 3. In another embodiment, m is 2 and n'' is 3. In yet another embodiment, m is 3 and n'' is 3. In yet a further embodiment, m is 4 and n'' is 3. In a particular embodiment, m is 1 and n'' is 4. In another embodiment, m is 2 and n'' is 4. In yet another embodiment, m is 3 and n'' is 4. In yet a further embodiment, m is 4 and n'' is 4. In a particular embodiment, m is 1 and n'' is 5. In another embodiment, m is 2 and n'' is 5. In yet another embodiment, m is 3 and n'' is 5. In yet a further embodiment, m is 4 and n'' is 5. In a particular embodiment, m is 1 and n'' is 6. In another embodiment, m is 2 and n'' is 6. In yet another embodiment, m is 3 and n'' is 6. In yet a further embodiment, m is 4 and n'' is 6. Preferably, the saccharide is not a capsular saccharide from *Streptococcus pneumoniae* serotype 3.

In an embodiment, the invention provides a glycoconjugate comprising a saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (VII), wherein X is $(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$, where m is selected from 1 to 4 and wherein X' is $\text{CH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_{m'}(\text{CH}_2)_{n''}\text{CH}_2\text{C}=\text{O}$, where n'' is selected from 0 to 10 and m' is selected from 0 to 4.

In an embodiment, m is selected from 1 to 3, m' is selected from 0 to 4 and n'' is selected from 0 to 10. In an embodiment, m is selected from 1 to 2, m' is selected from 0 to 4 and n'' is selected from 0 to 5. In an embodiment, m is selected from 1 to 2, m' is selected from 0 to 2 and n'' is selected from 0 to 3. In an embodiment, m is selected from 1 to 2, m' is selected from 0 to 2 and n'' is selected from 0 to 1.

In a particular embodiment, m is 1, m' is 0 and n'' is 0. In another embodiment, m is 1, m' is 1 and n'' is 0. In another embodiment, m is 1, m' is 2 and n'' is 0. In another embodiment, m is 1, m' is 3 and n'' is 0.

In another embodiment, m is 2, m' is 0 and n'' is 0. In another embodiment, m is 2, m' is 1 and n'' is 0. In another embodiment, m is 2, m' is 2 and n'' is 0. In another embodiment, m is 2, m' is 3 and n'' is 0.

In yet another embodiment, m is 3, m' is 0 and n'' is 0. In yet another embodiment, m is 3, m' is 1 and n'' is 0. In yet another embodiment, m is 3, m' is 2 and n'' is 0. In yet another embodiment, m is 3, m' is 3 and n'' is 0.

In yet a further embodiment, m is 4, m' is 0 and n'' is 0. In yet a further embodiment, m is 4, m' is 1 and n'' is 0. In yet a further embodiment, m is 4, m' is 2 and n'' is 0. In yet a further embodiment, m is 4, m' is 3 and n'' is 0.

In a particular embodiment, m is 1, m' is 0 and n'' is 1. In a particular embodiment, m is 1, m' is 1 and n'' is 1. In a particular embodiment, m is 1, m' is 2 and n'' is 1. In a particular embodiment, m is 1, m' is 3 and n'' is 1.

In another embodiment, m is 2, m' is 0 and n'' is 1. In another embodiment, m is 2, m' is 1 and n'' is 1. In another embodiment, m is 2, m' is 2 and n'' is 1. In another embodiment, m is 2, m' is 3 and n'' is 1.

In yet another embodiment, m is 3, m' is 0 and n'' is 1. In yet another embodiment, m is 3, m' is 1 and n'' is 1. In yet another embodiment, m is 3, m' is 2 and n'' is 1. In yet another embodiment, m is 3, m' is 3 and n'' is 1.

In yet a further embodiment, m is 4, m' is 0 and n'' is 1. In yet a further embodiment, m is 4, m' is 1 and n'' is 1. In yet a further embodiment, m is 4, m' is 2 and n'' is 1. In yet a further embodiment, m is 4, m' is 3 and n'' is 1.

In a particular embodiment, m is 1, m' is 0 and n'' is 2. In a particular embodiment, m is 1, m' is 1 and n'' is 2. In a particular embodiment, m is 1, m' is 2 and n'' is 2. In a particular embodiment, m is 1, m' is 3 and n'' is 2.

In another embodiment, m is 2, m' is 0 and n'' is 2. In another embodiment, m is 2, m' is 1 and n'' is 2. In another embodiment, m is 2, m' is 2 and n'' is 2. In another embodiment, m is 2, m' is 3 and n'' is 2.

In yet another embodiment, m is 3, m' is 0 and n'' is 2. In yet another embodiment, m is 3, m' is 1 and n'' is 2. In yet another embodiment, m is 3, m' is 2 and n'' is 2. In yet another embodiment, m is 3, m' is 3 and n'' is 2.

In yet a further embodiment, m is 4, m' is 0 and n'' is 2. In yet a further embodiment, m is 4, m' is 1 and n'' is 2. In yet a further embodiment, m is 4, m' is 2 and n'' is 2. In yet a further embodiment, m is 4, m' is 3 and n'' is 2.

In a particular embodiment, m is 1, m' is 0 and n'' is 3. In a particular embodiment, m is 1, m' is 1 and n'' is 3. In a particular embodiment, m is 1, m' is 2 and n'' is 3. In a particular embodiment, m is 1, m' is 3 and n'' is 3.

In another embodiment, m is 2, m' is 0 and n'' is 3. In another embodiment, m is 2, m' is 1 and n'' is 3. In another embodiment, m is 2, m' is 2 and n'' is 3. In another embodiment, m is 2, m' is 3 and n'' is 3.

In yet another embodiment, m is 3, m' is 0 and n'' is 3. In yet another embodiment, m is 3, m' is 1 and n'' is 3. In yet another embodiment, m is 3, m' is 2 and n'' is 3. In yet another embodiment, m is 3, m' is 3 and n'' is 3.

In yet a further embodiment, m is 4, m' is 0 and n'' is 3. In yet a further embodiment, m is 4, m' is 1 and n'' is 3. In yet a further embodiment, m is 4, m' is 2 and n'' is 3. In yet a further embodiment, m is 4, m' is 3 and n'' is 3.

In a particular embodiment, m is 1, m' is 0 and n'' is 4. In a particular embodiment, m is 1, m' is 1 and n'' is 4. In a particular embodiment, m is 1, m' is 2 and n'' is 4. In a particular embodiment, m is 1, m' is 3 and n'' is 4.

In another embodiment, m is 2, m' is 0 and n'' is 4. In another embodiment, m is 2, m' is 1 and n'' is 4. In another embodiment, m is 2, m' is 2 and n'' is 4. In another embodiment, m is 2, m' is 3 and n'' is 4.

In yet another embodiment, m is 3, m' is 0 and n'' is 4. In yet another embodiment, m is 3, m' is 1 and n'' is 4. In yet another embodiment, m is 3, m' is 2 and n'' is 4. In yet another embodiment, m is 3, m' is 3 and n'' is 4.

In yet a further embodiment, m is 4, m' is 0 and n'' is 4. In yet a further embodiment, m is 4, m' is 1 and n'' is 4. In yet a further embodiment, m is 4, m' is 2 and n'' is 4. In yet a further embodiment, m is 4, m' is 3 and n'' is 4.

In a particular embodiment, m is 1, m' is 0 and n'' is 5. In a particular embodiment, m is 1, m' is 1 and n'' is 5. In a particular embodiment, m is 1, m' is 2 and n'' is 5. In a particular embodiment, m is 1, m' is 3 and n'' is 5.

In another embodiment, m is 2, m' is 0 and n'' is 5. In another embodiment, m is 2, m' is 1 and n'' is 5. In another embodiment, m is 2, m' is 2 and n'' is 5. In another embodiment, m is 2, m' is 3 and n'' is 5.

In yet another embodiment, m is 3, m' is 0 and n'' is 5. In yet another embodiment, m is 3, m' is 1 and n'' is 5. In yet another embodiment, m is 3, m' is 2 and n'' is 5. In yet another embodiment, m is 3, m' is 3 and n'' is 5.

In yet a further embodiment, m is 4, m' is 0 and n'' is 5. In yet a further embodiment, m is 4, m' is 1 and n'' is 5. In yet a further embodiment, m is 4, m' is 2 and n'' is 5. In yet a further embodiment, m is 4, m' is 3 and n'' is 5.

Preferably, the saccharide is not a capsular saccharide from *Streptococcus pneumoniae* serotype 3.

In an embodiment, the invention provides a glycoconjugate comprising a saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (VII), wherein X is $\text{NHCO}(\text{CH}_2)_{n'}$, where n' is selected from 1 to 10 and wherein X' is $\text{CH}_2\text{O}(\text{CH}_2)_{n''}\text{CH}_2\text{C}=\text{O}$, where n'' is selected from 0 to 10. In an embodiment, n' is selected from 1 to 5 and n'' is selected from 0 to 10. In an embodiment, n' is selected from 1 to 5 and n'' is selected from 0 to 5. In an embodiment, n' is selected from 1 to 3 and n'' is selected from 0 to 3. In an embodiment, n' is selected from 1 to 2 and n'' is selected from 0 to 2. In a particular embodiment, n' is 1 and n'' is 0. In another embodiment, n' is 2 and n'' is 0. In yet another embodiment, n' is 3 and n'' is 0. In yet a further embodiment, n' is 4 and n'' is 0. In yet a further embodiment, n' is 5 and n'' is 0. In yet a further embodiment, n' is 6 and n'' is 0. In a particular embodiment, n' is 1 and n'' is 1. In another embodiment, n' is 2 and n'' is 1. In yet another embodiment, n' is 3 and n'' is 1. In yet a further embodiment, n' is 4 and n'' is 1. In yet a further embodiment, n' is 5 and n'' is 1. In yet a further embodiment, n' is 6 and n'' is 1. In a particular embodiment, n' is 1 and n'' is 2. In another embodiment, n' is 2 and n'' is 2. In yet another embodiment, n' is 3 and n'' is 2. In yet a further embodiment, n' is 4 and n'' is 2. In yet a further embodiment, n' is 5 and n'' is 2. In yet a further embodiment, n' is 6 and n'' is 2. In a particular embodiment, n' is 1 and n'' is 3. In another embodiment, n' is 2 and n'' is 3. In yet another embodiment, n' is 3 and n'' is 3. In yet a further embodiment, n' is 4 and n'' is 3. In yet a further embodiment, n' is 5 and n'' is 3. In yet a further embodiment, n' is 6 and n'' is 3. In a particular embodiment, n' is 1 and n'' is 4. In another embodiment, n' is 2 and n'' is 4. In yet another embodiment, n' is 3 and n'' is 4. In yet a further embodiment, n' is 4 and n'' is 4. In yet a further embodiment, n' is 5 and n'' is 4. In yet a further embodiment, n' is 6 and n'' is 4. In a particular embodiment, n' is 1 and n'' is 5. In another embodiment,

n' is 2 and n'' is 5. In yet another embodiment, n' is 3 and n'' is 5. In yet a further embodiment, n' is 4 and n'' is 5. In yet a further embodiment, n' is 5 and n'' is 5. In yet a further embodiment, n' is 6 and n'' is 5. In a particular embodiment, n' is 1 and n'' is 6. In another embodiment, n' is 2 and n'' is 6. In yet another embodiment, n' is 3 and n'' is 6. In yet a further embodiment, n' is 4 and n'' is 6. In yet a further embodiment, n' is 5 and n'' is 6. In yet a further embodiment, n' is 6 and n'' is 6. Preferably, the saccharide is not a capsular saccharide from *Streptococcus pneumoniae* serotype 3.

In an embodiment, the invention provides a glycoconjugate comprising a saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (VII), wherein X is $\text{NHCO}(\text{CH}_2)_{n'}$, where n' is selected from 1 to 10 and wherein X' is $\text{CH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_{m'}(\text{CH}_2)_{n''}\text{CH}_2\text{C}=\text{O}$, where n'' is selected from 0 to 10 and m' is selected from 0 to 4.

In an embodiment, n' is selected from 1 to 5, m' is selected from 0 to 4 and n'' is selected from 0 to 10. In an embodiment, n' is selected from 1 to 5, m' is selected from 0 to 4 and n'' is selected from 0 to 5. In an embodiment, n' is selected from 1 to 3, m' is selected from 0 to 2 and n'' is selected from 0 to 3. In an embodiment, n' is selected from 1 to 2, m' is selected from 0 to 2 and n'' is selected from 0 to 1.

In a particular embodiment, n' is 1, m' is 0 and n'' is 0. In another embodiment, n' is 1, m' is 1 and n'' is 0. In another embodiment, n' is 1, m' is 2 and n'' is 0. In another embodiment, n' is 1, m' is 3 and n'' is 0.

In another embodiment, n' is 2, m' is 0 and n'' is 0. In another embodiment, n' is 2, m' is 1 and n'' is 0. In another embodiment, n' is 2, m' is 2 and n'' is 0. In another embodiment, n' is 2, m' is 3 and n'' is 0.

In yet another embodiment, n' is 3, m' is 0 and n'' is 0. In yet another embodiment, n' is 3, m' is 1 and n'' is 0. In yet another embodiment, n' is 3, m' is 2 and n'' is 0. In yet another embodiment, n' is 3, m' is 3 and n'' is 0.

In yet a further embodiment, n' is 4, m' is 0 and n'' is 0. In yet a further embodiment, n' is 4, m' is 1 and n'' is 0. In yet a further embodiment, n' is 4, m' is 2 and n'' is 0. In yet a further embodiment, n' is 4, m' is 3 and n'' is 0.

In yet a further embodiment, n' is 5, m' is 0 and n'' is 0. In yet a further embodiment, n' is 5, m' is 1 and n'' is 0. In yet a further embodiment, n' is 5, m' is 2 and n'' is 0. In yet a further embodiment, n' is 5, m' is 3 and n'' is 0.

In a particular embodiment, n' is 1, m' is 0 and n'' is 1. In a particular embodiment, n' is 1, m' is 1 and n'' is 1. In a particular embodiment, n' is 1, m' is 2 and n'' is 1. In a particular embodiment, n' is 1, m' is 3 and n'' is 1.

In another embodiment, n' is 2, m' is 0 and n'' is 1. In another embodiment, n' is 2, m' is 1 and n'' is 1. In another embodiment, n' is 2, m' is 2 and n'' is 1. In another embodiment, n' is 2, m' is 3 and n'' is 1.

In yet another embodiment, n' is 3, m' is 0 and n'' is 1. In yet another embodiment, n' is 3, m' is 1 and n'' is 1. In yet another embodiment, n' is 3, m' is 2 and n'' is 1. In yet another embodiment, n' is 3, m' is 3 and n'' is 1.

In yet a further embodiment, n' is 4, m' is 0 and n'' is 1. In yet a further embodiment, n' is 4, m' is 1 and n'' is 1. In yet a further embodiment, n' is 4, m' is 2 and n'' is 1. In yet a further embodiment, n' is 4, m' is 3 and n'' is 1.

In yet a further embodiment, n' is 5, m' is 0 and n'' is 1. In yet a further embodiment, n' is 5, m' is 1 and n'' is 1. In yet a further embodiment, n' is 5, m' is 2 and n'' is 1. In yet a further embodiment, n' is 5, m' is 3 and n'' is 1.

In a particular embodiment, n' is 1, m' is 0 and n'' is 2. In a particular embodiment, n' is 1, m' is 1 and n'' is 2. In a particular embodiment, n' is 1, m' is 2 and n'' is 2. In a particular embodiment, n' is 1, m' is 3 and n'' is 2.

In another embodiment, n' is 2, m' is 0 and n'' is 2. In another embodiment, n' is 2, m' is 1 and n'' is 2. In another embodiment, n' is 2, m' is 2 and n'' is 2. In another embodiment, n' is 2, m' is 3 and n'' is 2.

In yet another embodiment, n' is 3, m' is 0 and n'' is 2. In yet another embodiment, n' is 3, m' is 1 and n'' is 2. In yet another embodiment, n' is 3, m' is 2 and n'' is 2. In yet another embodiment, n' is 3, m' is 3 and n'' is 2.

In yet a further embodiment, n' is 4, m' is 0 and n'' is 2. In yet a further embodiment, n' is 4, m' is 1 and n'' is 2. In yet a further embodiment, n' is 4, m' is 2 and n'' is 2. In yet a further embodiment, n' is 4, m' is 3 and n'' is 2.

In yet a further embodiment, n' is 5, m' is 0 and n'' is 2. In yet a further embodiment, n' is 5, m' is 1 and n'' is 2. In yet a further embodiment, n' is 5, m' is 2 and n'' is 2. In yet a further embodiment, n' is 5, m' is 3 and n'' is 2.

In a particular embodiment, n' is 1, m' is 0 and n'' is 3. In a particular embodiment, n' is 1, m' is 1 and n'' is 3. In a particular embodiment, n' is 1, m' is 2 and n'' is 3. In a particular embodiment, n' is 1, m' is 3 and n'' is 3.

In another embodiment, n' is 2, m' is 0 and n'' is 3. In another embodiment, n' is 2, m' is 1 and n'' is 3. In another embodiment, n' is 2, m' is 2 and n'' is 3. In another embodiment, n' is 2, m' is 3 and n'' is 3.

In yet another embodiment, n' is 3, m' is 0 and n'' is 3. In yet another embodiment, n' is 3, m' is 1 and n'' is 3. In yet another embodiment, n' is 3, m' is 2 and n'' is 3. In yet another embodiment, n' is 3, m' is 3 and n'' is 3.

In yet a further embodiment, n' is 4, m' is 0 and n'' is 3. In yet a further embodiment, n' is 4, m' is 1 and n'' is 3. In yet a further embodiment, n' is 4, m' is 2 and n'' is 3. In yet a further embodiment, n' is 4, m' is 3 and n'' is 3.

In yet a further embodiment, n' is 5, m' is 0 and n'' is 3. In yet a further embodiment, n' is 5, m' is 1 and n'' is 3. In yet a further embodiment, n' is 5, m' is 2 and n'' is 3. In yet a further embodiment, n' is 5, m' is 3 and n'' is 3.

In a particular embodiment, n' is 1, m' is 0 and n'' is 4. In a particular embodiment, n' is 1, m' is 1 and n'' is 4. In a particular embodiment, n' is 1, m' is 2 and n'' is 4. In a particular embodiment, n' is 1, m' is 3 and n'' is 4.

In another embodiment, n' is 2, m' is 0 and n'' is 4. In another embodiment, n' is 2, m' is 1 and n'' is 4. In another embodiment, n' is 2, m' is 2 and n'' is 4. In another embodiment, n' is 2, m' is 3 and n'' is 4.

In yet another embodiment, n' is 3, m' is 0 and n'' is 4. In yet another embodiment, n' is 3, m' is 1 and n'' is 4. In yet another embodiment, n' is 3, m' is 2 and n'' is 4. In yet another embodiment, n' is 3, m' is 3 and n'' is 4.

In yet a further embodiment, n' is 4, m' is 0 and n'' is 4. In yet a further embodiment, n' is 4, m' is 1 and n'' is 4. In yet a further embodiment, n' is 4, m' is 2 and n'' is 4. In yet a further embodiment, n' is 4, m' is 3 and n'' is 4.

In yet a further embodiment, n' is 5, m' is 0 and n'' is 4. In yet a further embodiment, n' is 5, m' is 1 and n'' is 4. In yet a further embodiment, n' is 5, m' is 2 and n'' is 4. In yet a further embodiment, n' is 5, m' is 3 and n'' is 4.

In a particular embodiment, n' is 1, m' is 0 and n'' is 5. In a particular embodiment, n' is 1, m' is 1 and n'' is 5. In a particular embodiment, n' is 1, m' is 2 and n'' is 5. In a particular embodiment, n' is 1, m' is 3 and n'' is 5.

In another embodiment, n' is 2, m' is 0 and n'' is 5. In another embodiment, n' is 2, m' is 1 and n'' is 5. In another embodiment, n' is 2, m' is 2 and n'' is 5. In another embodiment, n' is 2, m' is 3 and n'' is 5.

In yet another embodiment, n' is 3, m' is 0 and n'' is 5. In yet another embodiment, n' is 3, m' is 1 and n'' is 5. In yet another embodiment, n' is 3, m' is 2 and n'' is 5. In yet another embodiment, n' is 3, m' is 3 and n'' is 5.

In yet a further embodiment, n' is 4, m' is 0 and n'' is 5. In yet a further embodiment, n' is 4, m' is 1 and n'' is 5. In yet a further embodiment, n' is 4, m' is 2 and n'' is 5. In yet a further embodiment, n' is 4, m' is 3 and n'' is 5.

In yet a further embodiment, n' is 5, m' is 0 and n'' is 5. In yet a further embodiment, n' is 5, m' is 1 and n'' is 5. In yet a further embodiment, n' is 5, m' is 2 and n'' is 5. In yet a further embodiment, n' is 5, m' is 3 and n'' is 5.

Preferably, the saccharide is not a capsular saccharide from *Streptococcus pneumoniae* serotype 3.

In an embodiment, the invention provides a glycoconjugate comprising a saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (VII), wherein X is $\text{NHCO}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$, where m is selected from 1 to 4 and wherein X' is $\text{CH}_2\text{O}(\text{CH}_2)_{n''}\text{CH}_2\text{C}=\text{O}$, where n'' is selected from 0 to 10. In an embodiment, m is selected from 1 to 3 and n'' is selected from 0 to 10. In an embodiment, m is selected from 1 to 3 and n'' is selected from 0 to 5. In an embodiment, m is selected from 1 to 2 and n'' is selected from 0 to 3. In an embodiment, m is selected from 1 to 2 and n'' is selected from 0 to 2. In a particular embodiment, m is 1 and n'' is 0. In another embodiment, m is 2 and n'' is 0. In yet another embodiment, m is 3 and n'' is 0. In yet a further embodiment, m is 4 and n'' is 0. In a particular embodiment, m is 1 and n'' is 1. In another embodiment, m is 2 and n'' is 1. In yet another embodiment, m is 3 and n'' is 1. In yet a further embodiment, m is 4 and n'' is 1. In a particular embodiment, m is 1 and n'' is 2. In another embodiment, m is 2 and n'' is 2. In yet another embodiment, m is 3 and n'' is 2. In yet a further embodiment, m is 4 and n'' is 2. In a particular embodiment, m is 1 and n'' is 3. In another embodiment, m is 2 and n'' is 3. In yet another embodiment, m is 3 and n'' is 3. In yet a further embodiment, m is 4 and n'' is 3. In a particular embodiment, m is 1 and n'' is 4. In another embodiment, m is 2 and n'' is 4. In yet another embodiment, m is 3 and n'' is 4. In yet a further embodiment, m is 4 and n'' is 4. In a particular embodiment, m is 1 and n'' is 5. In another embodiment, m is 2 and n'' is 5. In yet another embodiment, m is 3 and n'' is 5. In yet a further embodiment, m is 4 and n'' is 5. In a particular embodiment, m is 1 and n'' is 6. In another embodiment, m is 2 and n'' is 6. In yet another embodiment, m is 3 and n'' is 6. In yet a further embodiment, m is 4 and n'' is 6. Preferably, the saccharide is not a capsular saccharide from *Streptococcus pneumoniae* serotype 3.

In an embodiment, the invention provides a glycoconjugate comprising a saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (VII), wherein X is $\text{NHCO}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$, where m is selected

from 1 to 4 and wherein X' is $\text{CH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_{m'}(\text{CH}_2)_{n''}\text{CH}_2\text{C}=\text{O}$, where n'' is selected from 0 to 10 and m' is selected from 0 to 4.

In an embodiment, m is selected from 1 to 3, m' is selected from 0 to 4 and n'' is selected from 0 to 10. In an embodiment, m is selected from 1 to 2, m' is selected from 0 to 4 and n'' is selected from 0 to 5. In an embodiment, m is selected from 1 to 2, m' is selected from 0 to 2 and n'' is selected from 0 to 3. In an embodiment, m is selected from 1 to 2, m' is selected from 0 to 2 and n'' is selected from 0 to 1.

In a particular embodiment, m is 1, m' is 0 and n'' is 0. In another embodiment, m is 1, m' is 1 and n'' is 0. In another embodiment, m is 1, m' is 2 and n'' is 0. In another embodiment, m is 1, m' is 3 and n'' is 0.

In another embodiment, m is 2, m' is 0 and n'' is 0. In another embodiment, m is 2, m' is 1 and n'' is 0. In another embodiment, m is 2, m' is 2 and n'' is 0. In another embodiment, m is 2, m' is 3 and n'' is 0.

In yet another embodiment, m is 3, m' is 0 and n'' is 0. In yet another embodiment, m is 3, m' is 1 and n'' is 0. In yet another embodiment, m is 3, m' is 2 and n'' is 0. In yet another embodiment, m is 3, m' is 3 and n'' is 0.

In yet a further embodiment, m is 4, m' is 0 and n'' is 0. In yet a further embodiment, m is 4, m' is 1 and n'' is 0. In yet a further embodiment, m is 4, m' is 2 and n'' is 0. In yet a further embodiment, m is 4, m' is 3 and n'' is 0.

In a particular embodiment, m is 1, m' is 0 and n'' is 1. In a particular embodiment, m is 1, m' is 1 and n'' is 1. In a particular embodiment, m is 1, m' is 2 and n'' is 1. In a particular embodiment, m is 1, m' is 3 and n'' is 1.

In another embodiment, m is 2, m' is 0 and n'' is 1. In another embodiment, m is 2, m' is 1 and n'' is 1. In another embodiment, m is 2, m' is 2 and n'' is 1. In another embodiment, m is 2, m' is 3 and n'' is 1.

In yet another embodiment, m is 3, m' is 0 and n'' is 1. In yet another embodiment, m is 3, m' is 1 and n'' is 1. In yet another embodiment, m is 3, m' is 2 and n'' is 1. In yet another embodiment, m is 3, m' is 3 and n'' is 1.

In yet a further embodiment, m is 4, m' is 0 and n'' is 1. In yet a further embodiment, m is 4, m' is 1 and n'' is 1. In yet a further embodiment, m is 4, m' is 2 and n'' is 1. In yet a further embodiment, m is 4, m' is 3 and n'' is 1.

In a particular embodiment, m is 1, m' is 0 and n'' is 2. In a particular embodiment, m is 1, m' is 1 and n'' is 2. In a particular embodiment, m is 1, m' is 2 and n'' is 2. In a particular embodiment, m is 1, m' is 3 and n'' is 2.

In another embodiment, m is 2, m' is 0 and n'' is 2. In another embodiment, m is 2, m' is 1 and n'' is 2. In another embodiment, m is 2, m' is 2 and n'' is 2. In another embodiment, m is 2, m' is 3 and n'' is 2.

In yet another embodiment, m is 3, m' is 0 and n'' is 2. In yet another embodiment, m is 3, m' is 1 and n'' is 2. In yet another embodiment, m is 3, m' is 2 and n'' is 2. In yet another embodiment, m is 3, m' is 3 and n'' is 2.

In yet a further embodiment, m is 4, m' is 0 and n'' is 2. In yet a further embodiment, m is 4, m' is 1 and n'' is 2. In yet a further embodiment, m is 4, m' is 2 and n'' is 2. In yet a further embodiment, m is 4, m' is 3 and n'' is 2.

In a particular embodiment, m is 1, m' is 0 and n'' is 3. In a particular embodiment, m is 1, m' is 1 and n'' is 3. In a particular embodiment, m is 1, m' is 2 and n'' is 3. In a particular embodiment, m is 1, m' is 3 and n'' is 3.

In another embodiment, m is 2, m' is 0 and n'' is 3. In another embodiment, m is 2, m' is 1 and n'' is 3. In another embodiment, m is 2, m' is 2 and n'' is 3. In another embodiment, m is 2, m' is 3 and n'' is 3.

In yet another embodiment, m is 3, m' is 0 and n'' is 3. In yet another embodiment, m is 3, m' is 1 and n'' is 3. In yet another embodiment, m is 3, m' is 2 and n'' is 3. In yet another embodiment, m is 3, m' is 3 and n'' is 3.

In yet a further embodiment, m is 4, m' is 0 and n'' is 3. In yet a further embodiment, m is 4, m' is 1 and n'' is 3. In yet a further embodiment, m is 4, m' is 2 and n'' is 3. In yet a further embodiment, m is 4, m' is 3 and n'' is 3.

In a particular embodiment, m is 1, m' is 0 and n'' is 4. In a particular embodiment, m is 1, m' is 1 and n'' is 4. In a particular embodiment, m is 1, m' is 2 and n'' is 4. In a particular embodiment, m is 1, m' is 3 and n'' is 4.

In another embodiment, m is 2, m' is 0 and n'' is 4. In another embodiment, m is 2, m' is 1 and n'' is 4. In another embodiment, m is 2, m' is 2 and n'' is 4. In another embodiment, m is 2, m' is 3 and n'' is 4.

In yet another embodiment, m is 3, m' is 0 and n'' is 4. In yet another embodiment, m is 3, m' is 1 and n'' is 4. In yet another embodiment, m is 3, m' is 2 and n'' is 4. In yet another embodiment, m is 3, m' is 3 and n'' is 4.

In yet a further embodiment, m is 4, m' is 0 and n'' is 4. In yet a further embodiment, m is 4, m' is 1 and n'' is 4. In yet a further embodiment, m is 4, m' is 2 and n'' is 4. In yet a further embodiment, m is 4, m' is 3 and n'' is 4.

In a particular embodiment, m is 1, m' is 0 and n'' is 5. In a particular embodiment, m is 1, m' is 1 and n'' is 5. In a particular embodiment, m is 1, m' is 2 and n'' is 5. In a particular embodiment, m is 1, m' is 3 and n'' is 5.

In another embodiment, m is 2, m' is 0 and n'' is 5. In another embodiment, m is 2, m' is 1 and n'' is 5. In another embodiment, m is 2, m' is 2 and n'' is 5. In another embodiment, m is 2, m' is 3 and n'' is 5.

In yet another embodiment, m is 3, m' is 0 and n'' is 5. In yet another embodiment, m is 3, m' is 1 and n'' is 5. In yet another embodiment, m is 3, m' is 2 and n'' is 5. In yet another embodiment, m is 3, m' is 3 and n'' is 5.

In yet a further embodiment, m is 4, m' is 0 and n'' is 5. In yet a further embodiment, m is 4, m' is 1 and n'' is 5. In yet a further embodiment, m is 4, m' is 2 and n'' is 5. In yet a further embodiment, m is 4, m' is 3 and n'' is 5. Preferably, the saccharide is not a capsular saccharide from *Streptococcus pneumoniae* serotype 3.

In an embodiment, the invention provides a glycoconjugate comprising a saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (VII), wherein X is $\text{OCH}_2(\text{CH}_2)_{n'}$, where n' is selected from 1 to 10 and wherein X' is $\text{CH}_2\text{O}(\text{CH}_2)_{n''}\text{CH}_2\text{C}=\text{O}$, where n'' is selected from 0 to 10.

In an embodiment, n' is selected from 1 to 5 and n'' is selected from 0 to 10. In an embodiment, n' is selected from 1 to 5 and n'' is selected from 0 to 5. In an embodiment, n' is selected from 1 to 3 and n'' is selected from 0 to 3. In an embodiment, n' is selected from 1 to 2 and n'' is selected from 0 to 2. In a particular embodiment, n' is 1 and n'' is 0. In another embodiment, n' is 2 and n'' is 0. In yet another embodiment, n' is 3 and n'' is 0. In yet a further embodiment, n' is 4 and n'' is 0. In yet a further embodiment, n' is 5 and n'' is 0. In yet a further embodiment, n' is 6 and n'' is 0. In a particular embodiment, n' is 1 and n'' is 1. In another embodiment, n' is 2 and n'' is 1. In yet another embodiment, n' is 3 and n'' is 1. In yet a further embodiment, n' is 4 and n'' is 1. In yet a further embodiment, n' is 5 and n'' is 1. In yet a further embodiment, n' is 6 and n'' is 1. In a particular embodiment, n' is 1 and n'' is 2. In another embodiment, n' is 2 and n'' is 2. In yet another embodiment, n' is 3 and n'' is 2. In yet a further embodiment, n' is 4 and n'' is 2. In yet a further embodiment, n' is 5 and n'' is 2. In yet a further embodiment, n' is 6 and n'' is 2. In a particular embodiment, n' is 1 and n'' is 3. In another embodiment, n' is 2 and n'' is 3. In yet another embodiment, n' is 3 and n'' is 3. In yet a further embodiment, n' is 4 and n'' is 3. In yet a further embodiment, n' is 5 and n'' is 3. In yet a further embodiment, n' is 6 and n'' is 3. In a particular embodiment, n' is 1 and n'' is 4. In another embodiment, n' is 2 and n'' is 4. In yet another embodiment, n' is 3 and n'' is 4. In yet a further

embodiment, n' is 4 and n'' is 4. In yet a further embodiment, n' is 5 and n'' is 4. In yet a further embodiment, n' is 6 and n'' is 4. In a particular embodiment, n' is 1 and n'' is 5. In another embodiment, n' is 2 and n'' is 5. In yet another embodiment, n' is 3 and n'' is 5. In yet a further embodiment, n' is 4 and n'' is 5. In yet a further embodiment, n' is 5 and n'' is 5. In yet a further embodiment, n' is 6 and n'' is 5. In a particular embodiment, n' is 1 and n'' is 6. In another embodiment, n' is 2 and n'' is 6. In yet another embodiment, n' is 3 and n'' is 6. In yet a further embodiment, n' is 4 and n'' is 6. In yet a further embodiment, n' is 5 and n'' is 6. In yet a further embodiment, n' is 6 and n'' is 6. Preferably, the saccharide is not a capsular saccharide from *Streptococcus pneumoniae* serotype 3.

In an embodiment, the invention provides a glycoconjugate comprising a saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (VII), wherein X is $\text{OCH}_2(\text{CH}_2)_{n'}$, where n' is selected from 1 to 10 and wherein X' is $\text{CH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_{m'}(\text{CH}_2)_{n''}\text{CH}_2\text{C}=\text{O}$, where n'' is selected from 0 to 10 and m' is selected from 0 to 4.

In an embodiment, n' is selected from 1 to 5, m' is selected from 0 to 4 and n'' is selected from 0 to 10. In an embodiment, n' is selected from 1 to 5, m' is selected from 0 to 4 and n'' is selected from 0 to 5. In an embodiment, n' is selected from 1 to 3, m' is selected from 0 to 2 and n'' is selected from 0 to 3. In an embodiment, n' is selected from 1 to 2, m' is selected from 0 to 2 and n'' is selected from 0 to 1.

In a particular embodiment, n' is 1, m' is 0 and n'' is 0. In another embodiment, n' is 1, m' is 1 and n'' is 0. In another embodiment, n' is 1, m' is 2 and n'' is 0. In another embodiment, n' is 1, m' is 3 and n'' is 0.

In another embodiment, n' is 2, m' is 0 and n'' is 0. In another embodiment, n' is 2, m' is 1 and n'' is 0. In another embodiment, n' is 2, m' is 2 and n'' is 0. In another embodiment, n' is 2, m' is 3 and n'' is 0.

In yet another embodiment, n' is 3, m' is 0 and n'' is 0. In yet another embodiment, n' is 3, m' is 1 and n'' is 0. In yet another embodiment, n' is 3, m' is 2 and n'' is 0. In yet another embodiment, n' is 3, m' is 3 and n'' is 0.

In yet a further embodiment, n' is 4, m' is 0 and n'' is 0. In yet a further embodiment, n' is 4, m' is 1 and n'' is 0. In yet a further embodiment, n' is 4, m' is 2 and n'' is 0. In yet a further embodiment, n' is 4, m' is 3 and n'' is 0.

In yet a further embodiment, n' is 5, m' is 0 and n'' is 0. In yet a further embodiment, n' is 5, m' is 1 and n'' is 0. In yet a further embodiment, n' is 5, m' is 2 and n'' is 0. In yet a further embodiment, n' is 5, m' is 3 and n'' is 0.

In a particular embodiment, n' is 1, m' is 0 and n'' is 1. In a particular embodiment, n' is 1, m' is 1 and n'' is 1. In a particular embodiment, n' is 1, m' is 2 and n'' is 1. In a particular embodiment, n' is 1, m' is 3 and n'' is 1.

In another embodiment, n' is 2, m' is 0 and n'' is 1. In another embodiment, n' is 2, m' is 1 and n'' is 1. In another embodiment, n' is 2, m' is 2 and n'' is 1. In another embodiment, n' is 2, m' is 3 and n'' is 1.

In yet another embodiment, n' is 3, m' is 0 and n'' is 1. In yet another embodiment, n' is 3, m' is 1 and n'' is 1. In yet another embodiment, n' is 3, m' is 2 and n'' is 1. In yet another embodiment, n' is 3, m' is 3 and n'' is 1.

In yet a further embodiment, n' is 4, m' is 0 and n'' is 1. In yet a further embodiment, n' is 4, m' is 1 and n'' is 1. In yet a further embodiment, n' is 4, m' is 2 and n'' is 1. In yet a further embodiment, n' is 4, m' is 3 and n'' is 1.

In yet a further embodiment, n' is 5, m' is 0 and n'' is 1. In yet a further embodiment, n' is 5, m' is 1 and n'' is 1. In yet a further embodiment, n' is 5, m' is 2 and n'' is 1. In yet a further embodiment, n' is 5, m' is 3 and n'' is 1.

In a particular embodiment, n' is 1, m' is 0 and n'' is 2. In a particular embodiment, n' is 1, m' is 1 and n'' is 2. In a particular embodiment, n' is 1, m' is 2 and n'' is 2. In a particular embodiment, n' is 1, m' is 3 and n'' is 2.

In another embodiment, n' is 2, m' is 0 and n'' is 2. In another embodiment, n' is 2, m' is 1 and n'' is 2. In another embodiment, n' is 2, m' is 2 and n'' is 2. In another embodiment, n' is 2, m' is 3 and n'' is 2.

In yet another embodiment, n' is 3, m' is 0 and n'' is 2. In yet another embodiment, n' is 3, m' is 1 and n'' is 2. In yet another embodiment, n' is 3, m' is 2 and n'' is 2. In yet another embodiment, n' is 3, m' is 3 and n'' is 2.

In yet a further embodiment, n' is 4, m' is 0 and n'' is 2. In yet a further embodiment, n' is 4, m' is 1 and n'' is 2. In yet a further embodiment, n' is 4, m' is 2 and n'' is 2. In yet a further embodiment, n' is 4, m' is 3 and n'' is 2.

In yet a further embodiment, n' is 5, m' is 0 and n'' is 2. In yet a further embodiment, n' is 5, m' is 1 and n'' is 2. In yet a further embodiment, n' is 5, m' is 2 and n'' is 2. In yet a further embodiment, n' is 5, m' is 3 and n'' is 2.

In a particular embodiment, n' is 1, m' is 0 and n'' is 3. In a particular embodiment, n' is 1, m' is 1 and n'' is 3. In a particular embodiment, n' is 1, m' is 2 and n'' is 3. In a particular embodiment, n' is 1, m' is 3 and n'' is 3.

In another embodiment, n' is 2, m' is 0 and n'' is 3. In another embodiment, n' is 2, m' is 1 and n'' is 3. In another embodiment, n' is 2, m' is 2 and n'' is 3. In another embodiment, n' is 2, m' is 3 and n'' is 3.

In yet another embodiment, n' is 3, m' is 0 and n'' is 3. In yet another embodiment, n' is 3, m' is 1 and n'' is 3. In yet another embodiment, n' is 3, m' is 2 and n'' is 3. In yet another embodiment, n' is 3, m' is 3 and n'' is 3.

In yet a further embodiment, n' is 4, m' is 0 and n'' is 3. In yet a further embodiment, n' is 4, m' is 1 and n'' is 3. In yet a further embodiment, n' is 4, m' is 2 and n'' is 3. In yet a further embodiment, n' is 4, m' is 3 and n'' is 3.

In yet a further embodiment, n' is 5, m' is 0 and n'' is 3. In yet a further embodiment, n' is 5, m' is 1 and n'' is 3. In yet a further embodiment, n' is 5, m' is 2 and n'' is 3. In yet a further embodiment, n' is 5, m' is 3 and n'' is 3.

In a particular embodiment, n' is 1, m' is 0 and n'' is 4. In a particular embodiment, n' is 1, m' is 1 and n'' is 4. In a particular embodiment, n' is 1, m' is 2 and n'' is 4. In a particular embodiment, n' is 1, m' is 3 and n'' is 4.

In another embodiment, n' is 2, m' is 0 and n'' is 4. In another embodiment, n' is 2, m' is 1 and n'' is 4. In another embodiment, n' is 2, m' is 2 and n'' is 4. In another embodiment, n' is 2, m' is 3 and n'' is 4.

In yet another embodiment, n' is 3, m' is 0 and n'' is 4. In yet another embodiment, n' is 3, m' is 1 and n'' is 4. In yet another embodiment, n' is 3, m' is 2 and n'' is 4. In yet another embodiment, n' is 3, m' is 3 and n'' is 4.

In yet a further embodiment, n' is 4, m' is 0 and n'' is 4. In yet a further embodiment, n' is 4, m' is 1 and n'' is 4. In yet a further embodiment, n' is 4, m' is 2 and n'' is 4. In yet a further embodiment, n' is 4, m' is 3 and n'' is 4.

In yet a further embodiment, n' is 5, m' is 0 and n'' is 4. In yet a further embodiment, n' is 5, m' is 1 and n'' is 4. In yet a further embodiment, n' is 5, m' is 2 and n'' is 4. In yet a further embodiment, n' is 5, m' is 3 and n'' is 4.

In a particular embodiment, n' is 1, m' is 0 and n'' is 5. In a particular embodiment, n' is 1, m' is 1 and n'' is 5. In a particular embodiment, n' is 1, m' is 2 and n'' is 5. In a particular embodiment, n' is 1, m' is 3 and n'' is 5.

In another embodiment, n' is 2, m' is 0 and n'' is 5. In another embodiment, n' is 2, m' is 1 and n'' is 5. In another embodiment, n' is 2, m' is 2 and n'' is 5. In another embodiment, n' is 2, m' is 3 and n'' is 5.

In yet another embodiment, n' is 3, m' is 0 and n'' is 5. In yet another embodiment, n' is 3, m' is 1 and n'' is 5. In yet another embodiment, n' is 3, m' is 2 and n'' is 5. In yet another embodiment, n' is 3, m' is 3 and n'' is 5.

In yet a further embodiment, n' is 4, m' is 0 and n'' is 5. In yet a further embodiment, n' is 4, m' is 1 and n'' is 5. In yet a further embodiment, n' is 4, m' is 2 and n'' is 5. In yet a further embodiment, n' is 4, m' is 3 and n'' is 5.

In yet a further embodiment, n' is 5, m' is 0 and n'' is 5. In yet a further embodiment, n' is 5, m' is 1 and n'' is 5. In yet a further embodiment, n' is 5, m' is 2 and n'' is 5. In yet a further embodiment, n' is 5, m' is 3 and n'' is 5.

Preferably, the saccharide is not a capsular saccharide from *Streptococcus pneumoniae* serotype 3.

In an embodiment, the invention provides a glycoconjugate comprising a saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (VII), wherein X is $O(CH_2CH_2O)_mCH_2CH_2$, where m is selected from 1 to 4 and wherein X' is $CH_2O(CH_2)_{n''}CH_2C=O$, where n'' is selected from 0 to 10.

In an embodiment, m is selected from 1 to 3 and n'' is selected from 0 to 10. In an embodiment, m is selected from 1 to 3 and n'' is selected from 0 to 5. In an embodiment, m is selected from 1 to 2 and n'' is selected from 0 to 3. In an embodiment, m is selected from 1 to 2 and n'' is selected from 0 to 2. In a particular embodiment, m is 1 and n'' is 0.

In another embodiment, m is 2 and n'' is 0. In yet another embodiment, m is 3 and n'' is 0. In yet a further embodiment, m is 4 and n'' is 0. In a particular embodiment, m is 1 and n'' is 1. In another embodiment, m is 2 and n'' is 1. In yet another embodiment, m is 3 and n'' is 1. In yet a further embodiment, m is 4 and n'' is 1. In a particular embodiment, m is 1 and n'' is 2. In another embodiment, m is 2 and n'' is 2. In yet another embodiment, m is 3 and n'' is 2. In yet a further embodiment, m is 4 and n'' is 2. In a particular embodiment, m is 1 and n'' is 3. In another embodiment, m is 2 and n'' is 3. In yet another embodiment, m is 3 and n'' is 3. In yet a further embodiment, m is 4 and n'' is 3. In a particular embodiment, m is 1 and n'' is 4. In another embodiment, m is 2 and n'' is 4. In yet another embodiment, m is 3 and n'' is 4. In yet a further embodiment, m is 4 and n'' is 4. In a particular embodiment, m is 1 and n'' is 5. In another embodiment, m is 2 and n'' is 5. In yet another embodiment, m is 3 and n'' is 5. In yet a further embodiment, m is 4 and n'' is 5. In a particular embodiment, m is 1 and n'' is 6. In another embodiment, m is 2 and n'' is 6. In yet another embodiment, m is 3 and n'' is 6. In yet a further embodiment, m is 4 and n'' is 6. Preferably, the saccharide is not a capsular saccharide from *Streptococcus*

pneumoniae serotype 3.

In an embodiment, the invention provides a glycoconjugate comprising a saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (VII), wherein X is $O(CH_2CH_2O)_mCH_2CH_2$, where m is selected from 1 to 4 and wherein X' is $CH_2O(CH_2CH_2O)_{m'}(CH_2)_{n''}CH_2C=O$, where n'' is selected from 0 to 10 and m' is selected from 0 to 4.

In an embodiment, m is selected from 1 to 3, m' is selected from 0 to 4 and n'' is selected from 0 to 10. In an embodiment, m is selected from 1 to 2, m' is selected from 0 to 4 and n'' is selected from 0 to 5. In an embodiment, m is selected from 1 to 2, m' is selected from 0 to 2 and n'' is selected from 0 to 3. In an embodiment, m is selected from 1 to 2, m' is selected from 0 to 2 and n'' is selected from 0 to 1.

In a particular embodiment, m is 1, m' is 0 and n'' is 0. In another embodiment, m is 1, m' is 1 and n'' is 0. In another embodiment, m is 1, m' is 2 and n'' is 0. In another embodiment, m is 1, m' is 3 and n'' is 0.

In another embodiment, m is 2, m' is 0 and n'' is 0. In another embodiment, m is 2, m' is 1 and n'' is 0. In another embodiment, m is 2, m' is 2 and n'' is 0. In another embodiment, m is 2, m' is 3 and n'' is 0.

In yet another embodiment, m is 3, m' is 0 and n'' is 0. In yet another embodiment, m is 3, m' is 1 and n'' is 0. In yet another embodiment, m is 3, m' is 2 and n'' is 0. In yet another embodiment, m is 3, m' is 3 and n'' is 0.

In yet a further embodiment, m is 4, m' is 0 and n'' is 0. In yet a further embodiment, m is 4, m' is 1 and n'' is 0. In yet a further embodiment, m is 4, m' is 2 and n'' is 0. In yet a further embodiment, m is 4, m' is 3 and n'' is 0.

In a particular embodiment, m is 1, m' is 0 and n'' is 1. In a particular embodiment, m is 1, m' is 1 and n'' is 1. In a particular embodiment, m is 1, m' is 2 and n'' is 1. In a particular embodiment, m is 1, m' is 3 and n'' is 1.

In another embodiment, m is 2, m' is 0 and n'' is 1. In another embodiment, m is 2, m' is 1 and n'' is 1. In another embodiment, m is 2, m' is 2 and n'' is 1. In another embodiment, m is 2, m' is 3 and n'' is 1.

In yet another embodiment, m is 3, m' is 0 and n'' is 1. In yet another embodiment, m is 3, m' is 1 and n'' is 1. In yet another embodiment, m is 3, m' is 2 and n'' is 1. In yet another embodiment, m is 3, m' is 3 and n'' is 1.

In yet a further embodiment, m is 4, m' is 0 and n'' is 1. In yet a further embodiment, m is 4, m' is 1 and n'' is 1. In yet a further embodiment, m is 4, m' is 2 and n'' is 1. In yet a further embodiment, m is 4, m' is 3 and n'' is 1.

In a particular embodiment, m is 1, m' is 0 and n'' is 2. In a particular embodiment, m is 1, m' is 1 and n'' is 2. In a particular embodiment, m is 1, m' is 2 and n'' is 2. In a particular embodiment, m is 1, m' is 3 and n'' is 2.

In another embodiment, m is 2, m' is 0 and n'' is 2. In another embodiment, m is 2, m' is 1 and n'' is 2. In another embodiment, m is 2, m' is 2 and n'' is 2. In another embodiment, m is 2, m' is 3 and n'' is 2.

In yet another embodiment, m is 3, m' is 0 and n'' is 2. In yet another embodiment, m is 3, m' is 1 and n'' is 2. In yet another embodiment, m is 3, m' is 2 and n'' is 2. In yet another embodiment, m is 3, m' is 3 and n'' is 2.

In yet a further embodiment, m is 4, m' is 0 and n'' is 2. In yet a further embodiment, m is 4, m' is 1 and n'' is 2. In yet a further embodiment, m is 4, m' is 2 and n'' is 2. In yet a further embodiment, m is 4, m' is 3 and n'' is 2.

In a particular embodiment, m is 1, m' is 0 and n'' is 3. In a particular embodiment, m is 1, m' is 1 and n'' is 3. In a particular embodiment, m is 1, m' is 2 and n'' is 3. In a particular embodiment, m is 1, m' is 3 and n'' is 3.

In another embodiment, m is 2, m' is 0 and n'' is 3. In another embodiment, m is 2, m' is 1 and n'' is 3. In another embodiment, m is 2, m' is 2 and n'' is 3. In another embodiment, m is 2, m' is 3 and n'' is 3.

In yet another embodiment, m is 3, m' is 0 and n'' is 3. In yet another embodiment, m is 3, m' is 1 and n'' is 3. In yet another embodiment, m is 3, m' is 2 and n'' is 3. In yet another embodiment, m is 3, m' is 3 and n'' is 3.

In yet a further embodiment, m is 4, m' is 0 and n'' is 3. In yet a further embodiment, m is 4, m' is 1 and n'' is 3. In yet a further embodiment, m is 4, m' is 2 and n'' is 3. In yet a further embodiment, m is 4, m' is 3 and n'' is 3.

In a particular embodiment, m is 1, m' is 0 and n'' is 4. In a particular embodiment, m is 1, m' is 1 and n'' is 4. In a particular embodiment, m is 1, m' is 2 and n'' is 4. In a particular embodiment, m is 1, m' is 3 and n'' is 4.

In another embodiment, m is 2, m' is 0 and n'' is 4. In another embodiment, m is 2, m' is 1 and n'' is 4. In another embodiment, m is 2, m' is 2 and n'' is 4. In another embodiment, m is 2, m' is 3 and n'' is 4.

In yet another embodiment, m is 3, m' is 0 and n'' is 4. In yet another embodiment, m is 3, m' is 1 and n'' is 4. In yet another embodiment, m is 3, m' is 2 and n'' is 4. In yet another embodiment, m is 3, m' is 3 and n'' is 4.

In yet a further embodiment, m is 4, m' is 0 and n'' is 4. In yet a further embodiment, m is 4, m' is 1 and n'' is 4. In yet a further embodiment, m is 4, m' is 2 and n'' is 4. In yet a further embodiment, m is 4, m' is 3 and n'' is 4.

In a particular embodiment, m is 1, m' is 0 and n'' is 5. In a particular embodiment, m is 1, m' is 1 and n'' is 5. In a particular embodiment, m is 1, m' is 2 and n'' is 5. In a particular embodiment, m is 1, m' is 3 and n'' is 5.

In another embodiment, m is 2, m' is 0 and n'' is 5. In another embodiment, m is 2, m' is 1 and n'' is 5. In another embodiment, m is 2, m' is 2 and n'' is 5. In another embodiment, m is 2, m' is 3 and n'' is 5.

In yet another embodiment, m is 3, m' is 0 and n'' is 5. In yet another embodiment, m is 3, m' is 1 and n'' is 5. In yet another embodiment, m is 3, m' is 2 and n'' is 5. In yet another embodiment, m is 3, m' is 3 and n'' is 5.

In yet a further embodiment, m is 4, m' is 0 and n'' is 5. In yet a further embodiment, m is 4, m' is 1 and n'' is 5. In yet a further embodiment, m is 4, m' is 2 and n'' is 5. In yet a further embodiment, m is 4, m' is 3 and n'' is 5.

Preferably, the saccharide is not a capsular saccharide from *Streptococcus pneumoniae* serotype 3.

Preferably, the saccharide of the glycoconjugate of the present invention is not a capsular saccharide from *Streptococcus pneumoniae* serotype 3.

1.5 Carrier protein of the glycoconjugates of the invention

A component of the glycoconjugate is a carrier protein to which the saccharide is conjugated. The terms "protein carrier" or "carrier protein" or "carrier" may be used interchangeably herein. Carrier proteins should be amenable to standard conjugation procedures.

In a preferred embodiment, the carrier protein of the glycoconjugate of the invention is selected in the group consisting of: DT (Diphtheria toxoid), TT (tetanus toxoid) or fragment C of TT, CRM₁₉₇ (a nontoxic but antigenically identical variant of diphtheria toxin), other DT mutants (such as CRM₁₇₆, CRM₂₂₈, CRM₄₅ (Uchida et al. (1973) J. Biol. Chem. 218:3838-3844), CRM₉, CRM₁₀₂, CRM₁₀₃ or CRM₁₀₇; and other mutations described by Nicholls and Youle in Genetically Engineered Toxins, Ed: Frankel, Marcel Dekker Inc. (1992); deletion or mutation of Glu-148 to Asp, Gln or Ser and/or Ala 158 to Gly and other mutations disclosed in U.S. Patent Nos. 4,709,017 and 4,950,740; mutation of at least one or more residues Lys 516, Lys 526, Phe 530 and/or Lys 534 and other mutations disclosed in U.S. Patent Nos. 5,917,017 and 6,455,673; or fragment disclosed

in U.S. Patent No. 5,843,711, pneumococcal pneumolysin (ply) (Kuo et al. (1995) Infect Immun 63:2706-2713) including ply detoxified in some fashion, for example dPLY-GMBS (WO 2004/081515, WO 2006/032499) or dPLY-formol, PhtX, including PhtA, PhtB, PhtD, PhtE (sequences of PhtA, PhtB, PhtD or PhtE are disclosed in WO 00/37105 and WO 00/39299) and fusions of Pht proteins, for example PhtDE fusions, PhtBE fusions, Pht A-E (WO 01/98334, WO 03/054007, WO 2009/000826), OMPC (meningococcal outer membrane protein), which is usually extracted from *Neisseria meningitidis* serogroup B (EP0372501), PorB (from *N. meningitidis*), PD (*Haemophilus influenzae* protein D; see, e.g., EP0594610 B), or immunologically functional equivalents thereof, synthetic peptides (EP0378881, EP0427347), heat shock proteins (WO 93/17712, WO 94/03208), pertussis proteins (WO 98/58668, EP0471177), cytokines, lymphokines, growth factors or hormones (WO 91/01146), artificial proteins comprising multiple human CD4+ T cell epitopes from various pathogen derived antigens (Falugi et al. (2001) Eur J Immunol 31:3816-3824) such as N19 protein (Baraldoi et al. (2004) Infect Immun 72:4884-4887) pneumococcal surface protein PspA (WO 02/091998), iron uptake proteins (WO 01/72337), toxin A or B of *Clostridium difficile* (WO 00/61761), transferrin binding proteins, pneumococcal adhesion protein (PsaA), recombinant *Pseudomonas aeruginosa* exotoxin A (in particular non-toxic mutants thereof (such as exotoxin A bearing a substitution at glutamic acid 553 (Douglas et al. (1987) J. Bacteriol. 169(11):4967-4971)). Other proteins, such as ovalbumin, keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA) or purified protein derivative of tuberculin (PPD) also can be used as carrier proteins. Other suitable carrier proteins include inactivated bacterial toxins such as cholera toxoid (e.g., as described in WO 2004/083251), *Escherichia coli* LT, *E. coli* ST, and exotoxin A from *P. aeruginosa*. Another suitable carrier protein is a C5a peptidase from *Streptococcus* (SCP).

In a preferred embodiment, the carrier protein of the glycoconjugate of the invention is TT, DT, DT mutants (such as CRM₁₉₇) or a C5a peptidase from *Streptococcus* (SCP).

In an embodiment, the carrier protein of the glycoconjugate of the invention is DT (Diphtheria toxoid). In another embodiment, the carrier protein of the glycoconjugate of the invention is TT (tetanus toxoid).

In another embodiment, the carrier protein of the glycoconjugate of the invention is PD (*H. influenzae* protein D; see, e.g., EP0594610 B).

In a preferred embodiment, the carrier protein of the glycoconjugate of the invention is CRM₁₉₇ or a C5a peptidase from *Streptococcus* (SCP).

In a preferred embodiment, the carrier protein of the glycoconjugate of the invention is CRM₁₉₇. The CRM₁₉₇ protein is a nontoxic form of diphtheria toxin but is immunologically indistinguishable from the diphtheria toxin. CRM₁₉₇ is produced by *Corynebacterium diphtheriae* infected by the nontoxigenic phage β 197^{tox-} created by nitrosoguanidine mutagenesis of the toxigenic corynephage beta (Uchida et al. (1971) Nature New Biology 233:8-11). The CRM₁₉₇ protein has the same molecular weight as the diphtheria toxin but differs therefrom by a single base change (guanine to adenine) in the structural gene. This single base change causes an amino acid substitution (glutamic acid for glycine) in the mature protein and eliminates the toxic properties of diphtheria toxin. The CRM₁₉₇ protein is a safe and effective T-cell dependent carrier for saccharides. Further details about CRM₁₉₇ and production thereof can be found, e.g., in U.S. Patent No. 5,614,382.

In an embodiment, the carrier protein of the glycoconjugate of the invention is the A chain of CRM₁₉₇ (see CN103495161). In an embodiment, the carrier protein of the glycoconjugate of the invention is the A chain of CRM₁₉₇ obtained via expression by genetically recombinant *E. coli* (see CN103495161).

In other preferred embodiments, the carrier protein of the glycoconjugate of the invention is SCP (Streptococcal C5a Peptidase). Two important species of β -hemolytic streptococci, *Streptococcus pyogenes* (group A *Streptococcus*, GAS) and *Streptococcus agalactiae* (group B *Streptococcus*, GBS), which cause a variety of serious human infections that range from mild cases of pharyngitis and impetigo to serious invasive diseases such as necrotizing fasciitis (GAS) and neonatal sepsis (GBS) have developed a way to defeat this immune response. All human isolates of β -hemolytic streptococci, including GAS and GBS, produce a highly conserved cell-wall protein SCP (Streptococcal C5a Peptidase) that specifically inactivates C5a. The *scp* genes from GAS and GBS encode a polypeptide containing between 1,134 and 1,181 amino acids (Brown et al., PNAS, 2005, vol. 102, no. 51 pages 18391–18396). The first 31 residues are the export signal presequence and are removed upon passing through the cytoplasmic membrane. The next 68 residues serve as a pro-sequence and must be removed to produce active SCP. The next 10 residues can be removed without loss of protease activity. At the other end, starting with Lys-1034, are four consecutive 17-residue motifs followed by a cell sorting and cell-wall attachment signal. This combined signal is composed of a 20-residue hydrophilic sequence containing an LPTTND sequence, a 17-residue hydrophobic sequence, and a short basic carboxyl terminus.

SCP can be divided in domains (see figure 1B of Brown *et al.*, PNAS, 2005, vol. 102, no. 51 pages 18391–18396). These domains are the Pre/Pro domain (which comprises the export signal presequence (commonly the first 31 residues) and the pro-sequence (commonly the next 68 residues)), the protease domain (which is splitted in two part (protease part 1 commonly residues 89–333/334 and protease domain part 2 and commonly residues 467/468–583/584), the protease-associated domain (PA domain) (commonly residues 333/334–467/468), three fibronectin type III (Fn) domains (Fn1, commonly residues 583/584–712/713; Fn2, commonly residues 712/713–928/929/930; commonly Fn3, residues 929/930–1029/1030/1031) and a cell wall anchor domain (commonly residues 1029/1030/1031 to the C-terminus).

In an embodiment, the carrier protein of the glycoconjugate of the invention is an SCP from GBS (SCPB). An exemple of SCPB is provided at SEQ ID.NO: 3 of WO97/26008. See also SEQ ID NO: 3 of WO00/34487.

In another preferred embodiments, the carrier protein of the glycoconjugate of the invention is an SCP from GAS (SCPA). Examples of SCPA can be found at SEQ ID.No.1 and SEQ ID.No.2 of WO97/26008. See also SEQ ID NO: 1, 2 and 23 of WO00/34487.

In a preferred embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive SCP.

In other preferred embodiments, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive SCP from GBS (SCPB).

In another preferred embodiments, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive SCP from GAS (SCPA).

In an embodiment, the carrier protein of the glycoconjugate of the invention is a fragment of an SCP. In an embodiment, the carrier protein of the glycoconjugate of the invention is a fragment of an SCPA. Preferably, the carrier protein of the glycoconjugate of the invention is a fragment of an SCPB.

In an embodiment, the carrier protein of the glycoconjugate of the invention is a fragment of an SCP which comprises the protease domain, the protease-associated domain (PA domain) and the three fibronectin type III (Fn) domains but does not comprise the export signal presequence, the pro-sequence and the cell wall anchor domain.

In an embodiment, the carrier protein of the glycoconjugate of the invention is a fragment of an SCP which comprises the protease domain, the protease-associated domain (PA domain) and the three fibronectin type III (Fn) domains but does not comprise the export signal presequence, the pro-sequence and the cell wall anchor domain.

In an embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive fragment of an SCP which comprises the protease domain, the protease-associated domain (PA domain) and two of the three fibronectin type III (Fn) domains but does not comprise the export signal presequence, the pro-sequence and the cell wall anchor domain.

In an embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive fragment of an SCP. In an embodiment, said enzymatically inactive fragment of SCP comprises the protease domain, the protease-associated domain (PA domain) and the three fibronectin type III (Fn) domains but does not comprise the export signal presequence, the pro-sequence and the cell wall anchor domain.

In an embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive fragment of an SCPA. In an embodiment, said enzymatically inactive fragment of an SCPA comprises the protease domain, the protease-associated domain (PA domain) and the three fibronectin type III (Fn) domains but does not comprise the export signal presequence, the pro-sequence and the cell wall anchor domain.

In a preferred embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive fragment of SCPB. Preferably, said enzymatically inactive fragment of SCPB comprises the protease domain, the protease-associated domain (PA domain) and the three fibronectin type III (Fn) domains but does not comprise the export signal presequence, the pro-sequence and the cell wall anchor domain.

In an embodiment, the enzymatic activity of SCP is inactivated by replacing at least one amino acid of the wild type sequence. In an embodiment, said replacement is selected from the group consisting of D130A, H193A, N295A and S512A. The numbers indicate the amino acid residue position in the peptidase according to the numbering of SEQ ID NO: 1 of WO00/34487.

Therefore, in an embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive SCP where said inactivation is accomplished by replacing at least one amino acid of the wild type sequence. Preferably, said replacement of at least one amino acid is in the protease domain. In an embodiment, said replacement of at least one amino acid is in part 1 of the protease domain. In an embodiment, said replacement of at least one amino acid is in part 2 of the protease domain. In an embodiment, said replacement is selected from the group consisting of D130A, H193A, N295A and S512A. In an embodiment, said replacement is D130A. In another embodiment, said replacement is H193A. In another embodiment, said replacement is N295A. In yet another embodiment, said replacement is S512A.

In an embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive SCPA where said inactivation is accomplished by replacing at least one amino acid of the wild type sequence. Preferably, said replacement of at least one amino acid is in the protease domain. In an embodiment, said replacement of at least one amino acid is in part 1 of the protease domain. In an embodiment, said replacement of at least one amino acid is in part 2 of the protease domain. In an embodiment, said replacement is selected from the group consisting of D130A, H193A, N295A and S512A. In an embodiment, said replacement is D130A. In another embodiment, said replacement is H193A. In another embodiment, said replacement is N295A. In yet another embodiment, said replacement is S512A.

In an embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive SCPB where said inactivation is accomplished by replacing at least one amino acid of the wild type sequence. Preferably, said replacement of at least one amino acid is in the protease domain. In an embodiment, said replacement of at least one amino acid is in part 1 of the protease domain. In an embodiment, said replacement of at least one amino acid is in part 2 of the protease domain. In an embodiment, said replacement is selected from the group consisting of D130A, H193A, N295A and S512A. In an embodiment, said replacement is D130A. In another embodiment, said replacement is H193A. In another embodiment, said replacement is N295A. In yet another embodiment, said replacement is S512A.

In an embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive fragment of an SCP where said inactivation is accomplished by replacing at least one amino acid of the wild type sequence. Preferably, said replacement of at least one amino acid is in the protease domain. In an embodiment, said replacement of at least one amino acid is in part 1 of the protease domain. In an embodiment, said replacement of at least one amino acid is in part 2 of the protease domain. In an embodiment, said replacement is selected from the group consisting of D130A, H193A, N295A and S512A. In an embodiment, said replacement is D130A. In another embodiment, said replacement is H193A. In another embodiment, said replacement is N295A. In yet another embodiment, said replacement is S512A.

In an embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive fragment of SCP which comprises the protease domain, the protease-associated domain (PA domain) and the three fibronectin type III (Fn) domains but does not comprise the export signal presequence, the pro-sequence and the cell wall anchor domain, where said inactivation is accomplished by replacing at least one amino

acid of the wild type sequence. Preferably, said replacement of at least one amino acid is in the protease domain. In an embodiment, said replacement of at least one amino acid is in part 1 of the protease domain. In an embodiment, said replacement of at least one amino acid is in part 2 of the protease domain. In an embodiment, said replacement is selected from the group consisting of D130A, H193A, N295A and S512A. In an embodiment, said replacement is D130A. In another embodiment, said replacement is H193A. In another embodiment, said replacement is N295A. In yet another embodiment, said replacement is S512A.

In an embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive fragment of SCPA which comprises the protease domain, the protease-associated domain (PA domain) and the three fibronectin type III (Fn) domains but does not comprise the export signal presequence, the pro-sequence and the cell wall anchor domain, where said inactivation is accomplished by replacing at least one amino acid of the wild type sequence. Preferably, said replacement of at least one amino acid is in the protease domain. In an embodiment, said replacement of at least one amino acid is in part 1 of the protease domain. In an embodiment, said replacement of at least one amino acid is in part 2 of the protease domain. In an embodiment, said replacement is selected from the group consisting of D130A, H193A, N295A and S512A. In an embodiment, said replacement is D130A. In another embodiment, said replacement is H193A. In another embodiment, said replacement is N295A. In yet another embodiment, said replacement is S512A.

In an embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive fragment of SCPB which comprises the protease domain, the protease-associated domain (PA domain) and the three fibronectin type III (Fn) domains but does not comprise the export signal presequence, the pro-sequence and the cell wall anchor domain, where said inactivation is accomplished by replacing at least one amino acid of the wild type sequence. Preferably, said replacement of at least one amino acid is in the protease domain. In an embodiment, said replacement of at least one amino acid is in part 1 of the protease domain. In an embodiment, said replacement of at least one amino acid is in part 2 of the protease domain. In an embodiment, said replacement is selected from the group consisting of D130A, H193A, N295A and S512A. In an embodiment, said replacement is D130A. In another embodiment, said replacement is H193A. In another embodiment, said replacement is N295A. In yet another embodiment, said replacement is S512A.

In an embodiment, the enzymatic activity of SCP is inactivated by replacing at least two amino acids of the wild type sequence. In an embodiment, said at least two amino acids replacements are selected from the group consisting of D130A, H193A, N295A and S512A. In an embodiment, said at least two amino acids replacements are D130A and H193A. In an embodiment, said at least two amino acids replacements are D130A and N295A. In an embodiment, said at least two amino acids replacements are D130A and S512A. In an embodiment, said at least two amino acids replacements are H193A and N295A. In an embodiment, said at least two amino acids replacements are H193A and S512A. In an embodiment, said at least two amino acids replacements are N295A and S512A.

Therefore, in an embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive SCP where said inactivation is accomplished by replacing at least two amino acids of the wild type sequence. Preferably, said replacement of at least two amino acids is in the protease domain. In an embodiment, said replacement of at least two amino acid is in part 1 of the protease domain. In an embodiment, said replacement of at least two amino acid is in part 2 of the protease domain. In an embodiment, said at least two amino acids replacements are selected from the group consisting of D130A, H193A, N295A and S512A. In an embodiment, said at least two amino acids replacements are D130A and H193A. In an embodiment, said at least two amino acids replacements are D130A and N295A. Preferably, said at least two amino acids replacements are D130A and S512A. In an embodiment, said at least two amino acids replacements are H193A and N295A. In an embodiment, said at least two amino acids replacements are H193A and S512A. In an embodiment, said at least two amino acids replacements are N295A and S512A.

In an embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive SCPA where said inactivation is accomplished by replacing at least two amino acids of the wild type sequence. Preferably, said replacement of at least two amino acids is in the protease domain. In an embodiment, said replacement of at least two amino acids is in part 1 of the protease domain. In an embodiment, said replacement of at least two amino acid is in part 2 of the protease domain. In an embodiment, said at least two amino acids replacements are selected from the group consisting of D130A, H193A, N295A and S512A. In an embodiment, said at least two amino acids replacements are D130A and H193A. In an embodiment, said at least two amino acids replacements are D130A and N295A. Preferably, said at least two amino acids replacements are D130A and S512A. In an embodiment, said at least two amino acids

replacements are H193A and N295A. In an embodiment, said at least two amino acids replacements are H193A and S512A. In an embodiment, said at least two amino acids replacements are N295A and S512A.

5 In an embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive SCPB where said inactivation is accomplished by replacing at least two amino acids of the wild type sequence. Preferably, said replacement of at least two amino acids is in the protease domain. In an embodiment, said replacement of at least two amino acids is in part 1 of the protease domain. In an embodiment, said replacement of at least two amino acid is in part 2 of the protease domain. In an embodiment, said at
10 least two amino acids replacements are selected from the group consisting of D130A, H193A, N295A and S512A. In an embodiment, said at least two amino acids replacements are D130A and H193A. In an embodiment, said at least two amino acids replacements are D130A and N295A. Preferably, said at least two amino acids replacements are D130A and S512A. In an embodiment, said at least two amino acids
15 replacements are H193A and N295A. In an embodiment, said at least two amino acids replacements are H193A and S512A. In an embodiment, said at least two amino acids replacements are N295A and S512A.

In an embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive fragment of an SCP where said inactivation is accomplished by
20 replacing at least two amino acids of the wild type sequence. Preferably, said replacement of at least two amino acids is in the protease domain. In an embodiment, said replacement of at least two amino acids is in part 1 of the protease domain. In an embodiment, said replacement of at least two amino acid is in part 2 of the protease domain. In an embodiment, said at least two amino acids replacements are selected from the group
25 consisting of D130A, H193A, N295A and S512A. In an embodiment, said at least two amino acids replacements are D130A and H193A. In an embodiment, said at least two amino acids replacements are D130A and N295A. Preferably, said at least two amino acids replacements are D130A and S512A. In an embodiment, said at least two amino acids replacements are H193A and N295A. In an embodiment, said at least two amino
30 acids replacements are H193A and S512A. In an embodiment, said at least two amino acids replacements are N295A and S512A.

In an embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive fragment of SCP which comprises the protease domain, the protease-associated domain (PA domain) and the three fibronectin type III (Fn) domains
35 but does not comprise the export signal presequence, the pro-sequence and the cell wall

anchor domain, where said inactivation is accomplished by replacing at least two amino acids of the wild type sequence. Preferably, said replacement of at least two amino acids is in the protease domain. In an embodiment, said replacement of at least two amino acids is in part 1 of the protease domain. In an embodiment, said replacement of at least two amino acid is in part 2 of the protease domain. In an embodiment, said at least two amino acids replacements are selected from the group consisting of D130A, H193A, N295A and S512A. In an embodiment, said at least two amino acids replacements are D130A and H193A. In an embodiment, said at least two amino acids replacements are D130A and N295A. Preferably, said at least two amino acids replacements are D130A and S512A. In an embodiment, said at least two amino acids replacements are H193A and N295A. In an embodiment, said at least two amino acids replacements are H193A and S512A. In an embodiment, said at least two amino acids replacements are N295A and S512A.

In an embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive fragment of SCPA which comprises the protease domain, the protease-associated domain (PA domain) and the three fibronectin type III (Fn) domains but does not comprise the export signal presequence, the pro-sequence and the cell wall anchor domain, where said inactivation is accomplished by replacing at least two amino acids of the wild type sequence. Preferably, said replacement of at least two amino acids is in the protease domain. In an embodiment, said replacement of at least two amino acids is in part 1 of the protease domain. In an embodiment, said replacement of at least one amino acids is in part 2 of the protease domain. In an embodiment, said at least two amino acids replacements are selected from the group consisting of D130A, H193A, N295A and S512A. In an embodiment, said at least two amino acids replacements are D130A and H193A. In an embodiment, said at least two amino acids replacements are D130A and N295A. Preferably, said at least two amino acids replacements are D130A and S512A. In an embodiment, said at least two amino acids replacements are H193A and N295A. In an embodiment, said at least two amino acids replacements are H193A and S512A. In an embodiment, said at least two amino acids replacements are N295A and S512A.

In an embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive fragment of SCPB which comprises the protease domain, the protease-associated domain (PA domain) and the three fibronectin type III (Fn) domains but does not comprise the export signal presequence, the pro-sequence and the cell wall anchor domain, where said inactivation is accomplished by replacing at least two amino

acids of the wild type sequence. Preferably, said replacement of at least two amino acids is in the protease domain. In an embodiment, said replacement of at least two amino acids is in part 1 of the protease domain. In an embodiment, said replacement of at least two amino acids is in part 2 of the protease domain. In an embodiment, said at least two amino acids replacements are selected from the group consisting of D130A, H193A, N295A and S512A. In an embodiment, said at least two amino acids replacements are D130A and H193A. In an embodiment, said at least two amino acids replacements are D130A and N295A. Preferably, said at least two amino acids replacements are D130A and S512A. In an embodiment, said at least two amino acids replacements are H193A and N295A. In an embodiment, said at least two amino acids replacements are H193A and S512A. In an embodiment, said at least two amino acids replacements are N295A and S512A.

In an embodiment, the enzymatic activity of SCP is inactivated by replacing at least three amino acids of the wild type sequence. In an embodiment, said at least three amino acids replacements are selected from the group consisting of D130A, H193A, N295A and S512A. In an embodiment, said at least three amino acids replacements are D130A, H193A and N295A. In an embodiment, said at least three amino acids replacements are D130A, H193A and S512A. In an embodiment, said at least three amino acids replacements are D130A, N295A and S512A. In an embodiment, said at least three amino acids replacements are H193A, N295A and S512A.

Therefore, in an embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive SCP where said inactivation is accomplished by replacing at least three amino acids of the wild type sequence. Preferably, said replacement of at least three amino acids is in the protease domain. In an embodiment, said replacement of at least three amino acid is in part 1 of the protease domain. In an embodiment, said replacement of at least three amino acid is in part 2 of the protease domain. In an embodiment, said at least three amino acids replacements are selected from the group consisting of D130A, H193A, N295A and S512A. In an embodiment, said at least three amino acids replacements are D130A, H193A and N295A. In an embodiment, said at least three amino acids replacements are D130A, H193A and S512A. In an embodiment, said at least three amino acids replacements are D130A, N295A and S512A. In an embodiment, said at least three amino acids replacements are H193A, N295A and S512A.

In an embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive SCPA where said inactivation is accomplished by replacing at least

three amino acids of the wild type sequence. Preferably, said replacement of at least three amino acids is in the protease domain. In an embodiment, said replacement of at least three amino acids is in part 1 of the protease domain. In an embodiment, said replacement of at least three amino acid is in part 2 of the protease domain. In an embodiment, said at least three amino acids replacements are selected from the group consisting of D130A, H193A, N295A and S512A. In an embodiment, said at least three amino acids replacements are D130A, H193A and N295A. In an embodiment, said at least three amino acids replacements are D130A, H193A and S512A. In an embodiment, said at least three amino acids replacements are D130A, N295A and S512A. In an embodiment, said at least three amino acids replacements are H193A, N295A and S512A.

In an embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive SCPB where said inactivation is accomplished by replacing at least three amino acids of the wild type sequence. Preferably, said replacement of at least three amino acids is in the protease domain. In an embodiment, said replacement of at least three amino acids is in part 1 of the protease domain. In an embodiment, said replacement of at least three amino acid is in part 2 of the protease domain. In an embodiment, said at least three amino acids replacements are selected from the group consisting of D130A, H193A, N295A and S512A. In an embodiment, said at least three amino acids replacements are D130A, H193A and N295A. In an embodiment, said at least three amino acids replacements are D130A, H193A and S512A. In an embodiment, said at least three amino acids replacements are D130A, N295A and S512A. In an embodiment, said at least three amino acids replacements are H193A, N295A and S512A.

In an embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive fragment of an SCP where said inactivation is accomplished by replacing at least three amino acids of the wild type sequence. Preferably, said replacement of at least three amino acids is in the protease domain. In an embodiment, said replacement of at least three amino acids is in part 1 of the protease domain. In an embodiment, said replacement of at least three amino acid is in part 2 of the protease domain. In an embodiment, said at least three amino acids replacements are selected from the group consisting of D130A, H193A, N295A and S512A. In an embodiment, said at least three amino acids replacements are D130A, H193A and N295A. In an embodiment, said at least three amino acids replacements are D130A, H193A and S512A. In an embodiment, said at least three amino acids replacements are D130A,

N295A and S512A. In an embodiment, said at least three amino acids replacements are H193A, N295A and S512A.

In an embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive fragment of SCP which comprises the protease domain, the protease-associated domain (PA domain) and the three fibronectin type III (Fn) domains but does not comprise the export signal presequence, the pro-sequence and the cell wall anchor domain, where said inactivation is accomplished by replacing at least three amino acids of the wild type sequence. Preferably, said replacement of at least three amino acids is in the protease domain. In an embodiment, said replacement of at least three amino acids is in part 1 of the protease domain. In an embodiment, said replacement of at least three amino acid is in part 2 of the protease domain. In an embodiment, said at least three amino acids replacements are selected from the group consisting of D130A, H193A, N295A and S512A. In an embodiment, said at least three amino acids replacements are D130A, H193A and N295A. In an embodiment, said at least three amino acids replacements are D130A, H193A and S512A. In an embodiment, said at least three amino acids replacements are D130A, N295A and S512A. In an embodiment, said at least three amino acids replacements are H193A, N295A and S512A.

In an embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive fragment of SCPA which comprises the protease domain, the protease-associated domain (PA domain) and the three fibronectin type III (Fn) domains but does not comprise the export signal presequence, the pro-sequence and the cell wall anchor domain, where said inactivation is accomplished by replacing at least three amino acids of the wild type sequence. Preferably, said replacement of at least three amino acids is in the protease domain. In an embodiment, said replacement of at least three amino acids is in part 1 of the protease domain. In an embodiment, said replacement of at least three amino acids is in part 2 of the protease domain. In an embodiment, said at least three amino acids replacements are selected from the group consisting of D130A, H193A, N295A and S512A. In an embodiment, said at least three amino acids replacements are D130A, H193A and N295A. In an embodiment, said at least three amino acids replacements are D130A, H193A and S512A. In an embodiment, said at least three amino acids replacements are D130A, N295A and S512A. In an embodiment, said at least three amino acids replacements are H193A, N295A and S512A.

In an embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive fragment of SCPB which comprises the protease domain, the protease-associated domain (PA domain) and the three fibronectin type III (Fn) domains

but does not comprise the export signal presequence, the pro-sequence and the cell wall anchor domain, where said inactivation is accomplished by replacing at least three amino acids of the wild type sequence. Preferably, said replacement of at least three amino acids is in the protease domain. In an embodiment, said replacement of at least three amino acids is in part 1 of the protease domain. In an embodiment, said replacement of at least three amino acids is in part 2 of the protease domain. In an embodiment, said at least three amino acids replacements are selected from the group consisting of D130A, H193A, N295A and S512A. In an embodiment, said at least three amino acids replacements are D130A, H193A and N295A. In an embodiment, said at least three amino acids replacements are D130A, H193A and S512A. In an embodiment, said at least three amino acids replacements are D130A, N295A and S512A. In an embodiment, said at least three amino acids replacements are H193A, N295A and S512A.

In an embodiment, the enzymatic activity of SCP is inactivated by replacing at least four amino acids of the wild type sequence. In an embodiment, said at least four amino acids replacements are D130A, H193A, N295A and S512A.

Therefore, in an embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive SCP where said inactivation is accomplished by replacing at least four amino acids of the wild type sequence. Preferably, said replacement of at least four amino acids is in the protease domain. In an embodiment, said replacement of at least four amino acid is in part 1 of the protease domain. In an embodiment, said replacement of at least four amino acid is in part 2 of the protease domain. In an embodiment, said at least four amino acids replacements are D130A, H193A, N295A and S512A

In an embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive SCPA where said inactivation is accomplished by replacing at least four amino acids of the wild type sequence. Preferably, said replacement of at least four amino acids is in the protease domain. In an embodiment, said replacement of at least four amino acids is in part 1 of the protease domain. In an embodiment, said replacement of at least four amino acid is in part 2 of the protease domain. In an embodiment, said at least four amino acids replacements are D130A, H193A, N295A and S512A

In an embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive SCPB where said inactivation is accomplished by replacing at least four amino acids of the wild type sequence. Preferably, said replacement of at least four amino acids is in the protease domain. In an embodiment, said replacement of at least four amino acids is in part 1 of the protease domain. In an embodiment, said replacement

of at least four amino acid is in part 2 of the protease domain. In an embodiment, said at least four amino acids replacements are D130A, H193A, N295A and S512A

In an embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive fragment of an SCP where said inactivation is accomplished by replacing at least four amino acids of the wild type sequence. Preferably, said replacement of at least four amino acids is in the protease domain. In an embodiment, said replacement of at least four amino acids is in part 1 of the protease domain. In an embodiment, said replacement of at least four amino acid is in part 2 of the protease domain. In an embodiment, said at least four amino acids replacements are D130A, H193A, N295A and S512A

In an embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive fragment of SCP which comprises the protease domain, the protease-associated domain (PA domain) and the three fibronectin type III (Fn) domains but does not comprise the export signal presequence, the pro-sequence and the cell wall anchor domain, where said inactivation is accomplished by replacing at least four amino acids of the wild type sequence. Preferably, said replacement of at least four amino acids is in the protease domain. In an embodiment, said replacement of at least four amino acids is in part 1 of the protease domain. In an embodiment, said replacement of at least four amino acid is in part 2 of the protease domain. In an embodiment, said at least four amino acids replacements are D130A, H193A, N295A and S512A

In an embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive fragment of SCPA which comprises the protease domain, the protease-associated domain (PA domain) and the three fibronectin type III (Fn) domains but does not comprise the export signal presequence, the pro-sequence and the cell wall anchor domain, where said inactivation is accomplished by replacing at least four amino acids of the wild type sequence. Preferably, said replacement of at least four amino acids is in the protease domain. In an embodiment, said replacement of at least four amino acids is in part 1 of the protease domain. In an embodiment, said replacement of at least one amino acids is in part 2 of the protease domain. In an embodiment, said at least four amino acids replacements are D130A, H193A, N295A and S512A

In an embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive fragment of SCPB which comprises the protease domain, the protease-associated domain (PA domain) and the three fibronectin type III (Fn) domains but does not comprise the export signal presequence, the pro-sequence and the cell wall anchor domain, where said inactivation is accomplished by replacing at least four amino

acids of the wild type sequence. Preferably, said replacement of at least four amino acids is in the protease domain. In an embodiment, said replacement of at least four amino acids is in part 1 of the protease domain. In an embodiment, said replacement of at least four amino acids is in part 2 of the protease domain. In an embodiment, said at least four amino acids replacements are D130A, H193A, N295A and S512A

In a particular embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive fragment of SCP which consists of SEQ ID NO: 41.

In a particular embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive fragment of SCP which consists of SEQ ID NO: 42.

SEQ ID NO: 41 :

MAKTADTPATSKATIRDLNDPSQVKTLQEKAGKGAGTVVAVIAAGFDKNH
 EAWRLTDKAKARYQSKEDLEKAKKEHGITYGEWWNDKVAYYHDYSKDGKT
 AVDQEHGTHVSGILSGNAPSETKEPYRLEGAMPEAQLLLMRVEIVNGLAD
 YARNYAQAIRDAINLGAKVINMSFGNAALAYANLPDETKKAFDYAKSKGV
 SIVTSAGNDSSFSGGKTRLPLADHPDYGVVGTPAAADSTLTVASYSPPDKQL
 TETVTVKTDQDQKEMPVLSTNRFEPNKAYDYAYANRGTKEDDFKDVKGK
 IALIERGDIDFKDKIAKAKKAGAVGVLIYDNQDKGFPIELPNVDQMPAAF
 ISRKDGLLLKDNPPQKTITFNATPKVLPTASGTKLSRFSSWGLTADGNIKP
 DIAAPGQDILSSVANNKYAKLSGTAMSAPLVAGIMGLLQEYETQYPDMT
 PSERLDLAKKVLMSATALYDEDEKAYFSPRQQGAGAVDAKKASAATMYV
 TDKDNTSSKVHLNNVSDKFEVTVTVHNKSDKPQELYYQATVQTDKVDGKH
 FALAPKALYETSWQKITIPANSSKQVTPIDASRFSDLLAQMKNGYFLE
 GFVRFKQDPKKEELMSIPYIGFRGDFGNLSALEKPIYDSKDGSSYYHEAN
 SDAKDQLDGDGLQFYALKNNFTALTTESNPWTIIKAVKEGVENIEDIESS
 EITETIFAGTFAKQDDDDSHYYIHRHANGKPYAAISPNGDGNRDYVQFQGT
 FLRNAKNLVAEVLDEKGNVWWTSEVTEQVVKNYNNDLASTLGSTRFEKTR
 WDGKDKDGKVVANGTYTYRVRYTPISSGAKEQHTDFDVVDNTTPEVATS
 ATFSTEDRRLTLASKPKTSQPVYRERIAITYMDEDLPTTEYISPNEGDGTF
 TLPEEAETMEGATVPLKMSDFTYVVEDMAGNITYTPVTKLLEGHSNKPEQ

SEQ ID NO: 41 is 950 amino acids long.

SEQ ID NO: 42 :

AKTADTPATSKATIRDLNDPSQVKTLQEKAGKGAGTVVAVIAAGFDKNH

EAWRLTDKAKARYQSKEDLEKAKKEHGITYGEWWNDKVAYYHDYSKDGKT
 AVDQEHGTHVSGILSGNAPSETKEPYRLEGAMPEAQLLLMRVEIVNGLAD
 YARNYAQAIRDAINLGAKVINMSFGNAALAYANLPDETKKAFDYAKSKGV
 SIVTSAGNDSSFSGGKTRLPLADHPDYGVVGTPAAADSTLTVASYSPDKQL
 5 TETVTVKTADQQDKEMPVLSTNRFEPNKAYDYAYANRGTKEDDFKDVKGK
 IALIERGDIDFKDKIAKAKKAGAVGVLIYDNQDKGFPIELPNVDQMPAAF
 ISRKDGLLLKDNPQKTITFNATPKVLPTASGTKLSRFSSWGLTADGNIKP
 DIAAPGQDILSSVANNKYAKLSGTAMSAPLVAGIMGLLQEYETQYPDMT
 PSERLDLAKKVLMSATALYDEDEKAYFSPRQQGAGAVDAKKASAATMYV
 10 TDKDNTSSKVHLNNVSDKFEVTVTVHNKSDKPQELYYQATVQTDKVDGKH
 FALAPKALYETSWQKITIPANSSKQVTPIDASRFSDLLAQMKNGYFLE
 GFVRFKQDPKKEELMSIPYIGFRGDFGNLSALEKPIYDSKDGSSYYHEAN
 SDAKDQLDGDGLQFYALKNNFTALTTESNPWTIIKAVKEGVENIEDIESS
 EITETIFAGTFAKQDDDDSHYYIHRHANGKPYAAISPNGDGNRDYVQFQGT
 15 FLRNAKNLVAEVLDEKGNVWVTSEVTEQVVKNYNNDLASTLGSTRFEKTR
 WDGKDKDGKVVANGTYTYRVRYTPISSGAKEQHTDFDVVDNTTPEVATS
 ATFSTEDRRLTLASKPKTSQPVYRERIAITYMDEDLPTTEYISPNEGTF
 TLPEEAETMEGATVPLKMSDFTYVVEDMAGNITYTPVTKLLEGHSNKPEQ

20 SEQ ID NO: 42 is 949 amino acids long.

In a particular embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive fragment of SCP consisting of a polypeptide having at least 90% identity with SEQ ID NO: 41.

25 In a particular embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive fragment of SCP consisting of a polypeptide having at least 95% identity with SEQ ID NO: 41.

In a particular embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive fragment of SCP consisting of a polypeptide having
 30 at least 99% identity with SEQ ID NO: 41.

In a particular embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive fragment of SCP consisting of a polypeptide having at least 99.5% identity with SEQ ID NO: 41.

In a particular embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive fragment of SCP consisting of a polypeptide having at least 99.8% identity with SEQ ID NO: 41.

In a particular embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive fragment of SCP consisting of a polypeptide having at least 99.85% identity with SEQ ID NO: 41.

In a particular embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive fragment of SCP consisting of a polypeptide having at least 90% identity with SEQ ID NO: 42.

In a particular embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive fragment of SCP consisting of a polypeptide having at least 95% identity with SEQ ID NO: 42.

In a particular embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive fragment of SCP consisting of a polypeptide having at least 99% identity with SEQ ID NO: 42.

In a particular embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive fragment of SCP consisting of a polypeptide having at least 99.5% identity with SEQ ID NO: 42.

In a particular embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive fragment of SCP consisting of a polypeptide having at least 99.8% identity with SEQ ID NO: 42.

In a particular embodiment, the carrier protein of the glycoconjugate of the invention is an enzymatically inactive fragment of SCP consisting of a polypeptide having at least 99.85% identity with SEQ ID NO: 42.

2 Immunogenic compositions

2.1 Combinations of glycoconjugates of the invention

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention (as disclosed at section 1 above).

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising from 1 to 25 different glycoconjugates.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising from 1 to 25 glycoconjugates from different serotypes of *S. pneumoniae* (1 to 25 pneumococcal conjugates). In one

embodiment the invention relates to an immunogenic composition comprising glycoconjugates from 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 or 25 different serotypes of *S. pneumoniae*. In one embodiment the immunogenic composition comprises glycoconjugates from 16 or 20 different serotypes of *S. pneumoniae*. In an embodiment the immunogenic composition is a 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20-valent pneumococcal conjugate compositions. In an embodiment the immunogenic composition is a 14, 15, 16, 17, 18 or 19-valent pneumococcal conjugate compositions. In an embodiment the immunogenic composition is a 16-valent pneumococcal conjugate composition. In an embodiment the immunogenic composition is a 19-valent pneumococcal conjugate composition. In an embodiment the immunogenic composition is a 20-valent pneumococcal conjugate composition.

In an embodiment the immunogenic composition is a 21, 22, 23, 24 or 25-valent pneumococcal conjugate compositions. In an embodiment the immunogenic composition is a 21-valent pneumococcal conjugate composition. In an embodiment the immunogenic composition is a 22-valent pneumococcal conjugate composition. In an embodiment the immunogenic composition is a 23-valent pneumococcal conjugate composition. In an embodiment the immunogenic composition is a 24-valent pneumococcal conjugate composition. In an embodiment the immunogenic composition is a 25-valent pneumococcal conjugate composition.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F. In an embodiment the immunogenic composition is an 11-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F. In an embodiment the immunogenic composition is a 13-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and further comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F. In an embodiment the immunogenic composition is a 15-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F

and 33F. In an embodiment the immunogenic composition is a 20-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and further comprising glycoconjugates from *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F and 33F. In an embodiment the immunogenic composition is a 21-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 9N, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F and 33F. In an embodiment the immunogenic composition is a 21-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 2, 4, 5, 6A, 6B, 7F, 8, 9V, 9N, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F and 33F. In an embodiment the immunogenic composition is a 22-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 2, 4, 5, 6A, 6B, 7F, 8, 9V, 9N, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 22F, 23F and 33F. In an embodiment the immunogenic composition is a 23-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising glycoconjugates from *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 9N, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F. In an embodiment the immunogenic composition is a 24-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15A, 15B, 18C, 19A, 19F, 22F, 23F and 33F. In an embodiment the immunogenic composition is a 21-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23A,

23F and 33F. In an embodiment the immunogenic composition is a 21-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23B, 23F and 33F. In an embodiment the immunogenic composition is a 21-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, 24F and 33F. In an embodiment the immunogenic composition is a 21-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, 33F and 35B. In an embodiment the immunogenic composition is a 21-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15A, 15B, 18C, 19A, 19F, 22F, 23A, 23F and 33F. In an embodiment the immunogenic composition is a 22-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and further comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15A, 15B, 18C, 19A, 19F, 22F, 23B, 23F and 33F. In an embodiment the immunogenic composition is a 22-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15A, 15B, 18C, 19A, 19F, 22F, 23F, 24F and 33F. In an embodiment the immunogenic composition is a 22-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15A, 15B, 18C, 19A, 19F, 22F,

23F, 33F and 35B. In an embodiment the immunogenic composition is a 22-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23A, 23B, 23F and 33F. In an embodiment the immunogenic composition is a 22-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23A, 23F, 24F and 33F. In an embodiment the immunogenic composition is a 22-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23A, 23F, 33F and 35B. In an embodiment the immunogenic composition is a 22-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23B, 23F, 24F and 33F. In an embodiment the immunogenic composition is a 22-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23B, 23F, 33F and 35B. In an embodiment the immunogenic composition is a 22-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, 24F, 33F and 35B. In an embodiment the immunogenic composition is a 22-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15A, 15B, 18C, 19A, 19F, 22F,

23A, 23B, 23F and 33F. In an embodiment the immunogenic composition is a 23-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15A, 15B, 18C, 19A, 19F, 22F, 23A, 23F, 24F and 33F. In an embodiment the immunogenic composition is a 23-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15A, 15B, 18C, 19A, 19F, 22F, 23A, 23F, 33F and 35B. In an embodiment the immunogenic composition is a 23-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15A, 15B, 18C, 19A, 19F, 22F, 23B, 23F, 24F and 33F. In an embodiment the immunogenic composition is a 23-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15A, 15B, 18C, 19A, 19F, 22F, 23B, 23F, 33F and 35B. In an embodiment the immunogenic composition is a 23-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15A, 15B, 18C, 19A, 19F, 22F, 23F, 24F, 33F and 35B. In an embodiment the immunogenic composition is a 23-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23A, 23B, 23F, 33F and 35B. In an embodiment the immunogenic composition is a 23-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23A,

23B, 23F, 24F and 33F. In an embodiment the immunogenic composition is a 23-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23A, 23F, 24F, 33F and 35B. In an embodiment the immunogenic composition is a 23-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23B, 23F, 24F, 33F and 35B. In an embodiment the immunogenic composition is a 23-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15A, 15B, 18C, 19A, 19F, 22F, 23A, 23B, 23F, 24F and 33F. In an embodiment the immunogenic composition is a 24-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15A, 15B, 18C, 19A, 19F, 22F, 23A, 23B, 23F, 33F and 35B. In an embodiment the immunogenic composition is a 24-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15A, 15B, 18C, 19A, 19F, 22F, 23A, 23F, 24F, 33F and 35B. In an embodiment the immunogenic composition is a 24-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15A, 15B, 18C, 19A, 19F, 22F, 23B, 23F, 24F, 33F and 35B. In an embodiment the immunogenic composition is a 24-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23A,

23B, 23F, 24F, 33F and 35B. In an embodiment the immunogenic composition is a 24-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15A, 15B, 18C, 19A, 19F, 22F, 23A, 23B, 23F, 24F, 33F and 35B. In an embodiment the immunogenic composition is a 25-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention selected from the group consisting of glycoconjugates from *S. pneumoniae* serotypes 2, 7C, 9N, 10B, 15A, 16F, 17F, 20, 21, 22A, 23A, 23B, 24B, 24F, 27, 29, 31, 33B, 34, 35B, 35F and 38.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention selected from the group consisting of glycoconjugates from *S. pneumoniae* serotypes 2, 7C, 9N, 10B, 15A, 16F, 17F, 20, 21, 22A, 23A, 23B, 24B, 24F, 27, 29, 31, 33B, 34, 35B, 35F and 38. In an embodiment the immunogenic composition is a 21-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising twenty one glycoconjugates selected from the group consisting of glycoconjugates from *S. pneumoniae* serotypes 2, 7C, 9N, 10B, 15A, 16F, 17F, 20, 21, 22A, 23A, 23B, 24B, 24F, 27, 29, 31, 33B, 34, 35B, 35F and 38. In an embodiment the immunogenic composition is a 22-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising at least one glycoconjugate selected from the group consisting of glycoconjugates from *S. pneumoniae* serotypes 2, 7C, 9N, 10B, 15A, 16F, 17F, 19A, 19F, 20, 21, 22A, 23A, 23B, 24B, 24F, 27, 29, 31, 33B, 34, 35B, 35F and 38.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising twenty two glycoconjugates selected from the group consisting of glycoconjugates from *S. pneumoniae* serotypes 2, 7C, 9N, 10B, 15A, 16F, 17F, 19A, 19F, 20, 21, 22A, 23A, 23B, 24B, 24F, 27, 29, 31, 33B, 34, 35B, 35F and 38. In an embodiment the immunogenic composition is a 23-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising twenty three glycoconjugates selected

from the group consisting of glycoconjugates from *S. pneumoniae* serotypes 2, 7C, 9N, 10B, 15A, 16F, 17F, 19A, 19F, 20, 21, 22A, 23A, 23B, 24B, 24F, 27, 29, 31, 33B, 34, 35B, 35F and 38. In an embodiment the immunogenic composition is a 24-valent pneumococcal conjugate compositions.

5 In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising glycoconjugates from *S. pneumoniae* serotypes 2, 9N, 15A, 17F, 20, 23A, 23B, 24F and 35B. In an embodiment the immunogenic composition is a 10-valent pneumococcal conjugate compositions.

10 In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising glycoconjugates from *S. pneumoniae* serotypes 2, 9N, 15A, 17F, 19A, 19F, 20, 23A, 23B, 24F and 35B. In an embodiment the immunogenic composition is a 12-valent pneumococcal conjugate compositions.

15 In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising glycoconjugates from *S. pneumoniae* serotypes 2, 7C, 9N, 10B, 15A, 16F, 17F, 20, 21, 22A, 23A, 23B, 24B, 24F, 27, 29, 31, 33B, 34, 35B, 35F and 38. In an embodiment the immunogenic composition is a 23-valent pneumococcal conjugate compositions.

20 In an embodiment the invention relates to an immunogenic composition comprising a glycoconjugate of the invention and comprising glycoconjugates from *S. pneumoniae* serotypes 2, 7C, 9N, 10B, 15A, 16F, 17F, 19A, 19F, 20, 21, 22A, 23A, 23B, 24B, 24F, 27, 29, 31, 33B, 34, 35B, 35F and 38. In an embodiment the immunogenic composition is a 25-valent pneumococcal conjugate compositions.

25 In a preferred embodiment, the saccharides are each individually conjugated to different molecules of the protein carrier (each molecule of protein carrier only having one type of saccharide conjugated to it). In said embodiment, the capsular saccharides are said to be individually conjugated to the carrier protein. Preferably, all the glycoconjugates of the above immunogenic compositions are individually conjugated to the carrier protein.

30 In an embodiment of any of the above immunogenic compositions, the glycoconjugate of the invention is conjugated to CRM₁₉₇. In an embodiment of any of the above immunogenic compositions, the glycoconjugate of the invention is conjugated to SCP.

35 In an embodiment of any of the above immunogenic compositions, the glycoconjugate from *S. pneumoniae* serotype 22F is conjugated to CRM₁₉₇. In an embodiment of any of the above immunogenic compositions, the glycoconjugate from *S. pneumoniae* serotype 33F is conjugated to CRM₁₉₇. In an embodiment of any of the above

immunogenic compositions, the glycoconjugate from *S. pneumoniae* serotype 15B is conjugated to CRM₁₉₇. In an embodiment of any of the above immunogenic compositions, the glycoconjugate from *S. pneumoniae* serotype 12F is conjugated to CRM₁₉₇. In an embodiment of any of the above immunogenic compositions, the glycoconjugate from *S. pneumoniae* serotype 10A is conjugated to CRM₁₉₇. In an embodiment of any of the above immunogenic compositions, the glycoconjugate from *S. pneumoniae* serotype 11A is conjugated to CRM₁₉₇. In an embodiment of any of the above immunogenic compositions, the glycoconjugate from *S. pneumoniae* serotype 8 is conjugated to CRM₁₉₇. In an embodiment of any of the above immunogenic compositions, the glycoconjugates from *S. pneumoniae* serotypes 4, 6B, 9V, 14, 18C, 19F and 23F are conjugated to CRM₁₉₇. In an embodiment of any of the above immunogenic compositions, the glycoconjugates from *S. pneumoniae* serotypes 1, 5 and 7F are conjugated to CRM₁₉₇. In an embodiment of any of the above immunogenic compositions, the glycoconjugates from *S. pneumoniae* serotypes 6A and 19A are conjugated to CRM₁₉₇.

In an embodiment of any of the above immunogenic compositions, the glycoconjugates of any of the above immunogenic compositions are all individually conjugated to CRM₁₉₇.

In an embodiment of any of the above immunogenic compositions, the glycoconjugate of the invention is conjugated to SCP and the other glycoconjugate(s) is/are all individually conjugated to CRM₁₉₇.

In an embodiment, the glycoconjugates from *S. pneumoniae* serotypes 1, 4, 5, 6B, 7F, 9V, 14 and/or 23F of any of the above immunogenic compositions are individually conjugated to PD.

In an embodiment, the glycoconjugate from *S. pneumoniae* serotype 18C of any of the above immunogenic compositions is conjugated to TT.

In an embodiment, the glycoconjugate from *S. pneumoniae* serotype 19F of any of the above immunogenic compositions is conjugated to DT.

In an embodiment, the glycoconjugates from *S. pneumoniae* serotypes 1, 4, 5, 6B, 7F, 9V, 14 and/or 23F of any of the above immunogenic compositions are individually conjugated to PD, the glycoconjugate from *S. pneumoniae* serotype 18C is conjugated to TT and the glycoconjugate from *S. pneumoniae* serotype 19F is conjugated to DT.

In an embodiment the above immunogenic compositions comprise from 8 to 25 different serotypes of *S. pneumoniae*.

Compositions of the invention may include a small amount of free carrier. When a given carrier protein is present in both free and conjugated form in a composition of the

invention, the unconjugated form is preferably no more than 5% of the total amount of the carrier protein in the composition as a whole, and more preferably present at less than 2% by weight.

2.2 Dosage of the immunogenic compositions of the invention

The amount of glycoconjugate(s) in each dose is selected as an amount which induces an immunoprotective response without significant, adverse side effects in typical vaccinees. Such amount will vary depending upon which specific immunogen is employed and how it is presented.

The amount of a particular glycoconjugate in an immunogenic composition can be calculated based on total saccharide for that conjugate (conjugated and non-conjugated). For example, a glycoconjugate with 20% free saccharide will have about 80 µg of conjugated saccharide and about 20 µg of nonconjugated saccharide in a 100 µg saccharide dose. The amount of glycoconjugate can vary depending upon the bacteria and bacteria serotype. The saccharide concentration can be determined by the uronic acid assay.

The "immunogenic amount" of the different saccharide components in the immunogenic composition, may diverge and each may comprise about 0.5 µg, about 0.75 µg, about 1 µg, about 2 µg, about 3 µg, about 4 µg, about 5 µg, about 6 µg, about 7 µg, about 8 µg, about 9 µg, about 10 µg, about 15 µg, about 20 µg, about 30 µg, about 40 µg, about 50 µg, about 60 µg, about 70 µg, about 80 µg, about 90 µg, or about 100 µg of any particular saccharide antigen.

Generally, each dose will comprise 0.1 µg to 100 µg of saccharide. In an embodiment each dose will comprise 0.1 µg to 100 µg of saccharide. In a preferred embodiment each dose will comprise 0.5 µg to 20 µg. In a preferred embodiment each dose will comprise 1.0 µg to 10 µg. In an even preferred embodiment, each dose will comprise 2.0 µg to 5.0 µg of serotype 3 polysaccharide. Any whole number integer within any of the above ranges is contemplated as an embodiment of the disclosure.

In an embodiment, each dose will comprise about 0.5 µg of saccharide. In an embodiment, each dose will comprise about 0.55 µg of saccharide. In an embodiment, each dose will comprise about 0.75 µg of saccharide. In an embodiment, each dose will comprise about 1.0 µg of saccharide. In an embodiment, each dose will comprise about 1.1 µg of saccharide. In an embodiment, each dose will comprise about 1.5 µg of saccharide. In an embodiment, each dose will comprise about 2.0 µg of saccharide. In an embodiment, each dose will comprise about 2.2 µg of saccharide. In an embodiment,

each dose will comprise about 2.5 µg of saccharide. In an embodiment, each dose will comprise about 3.0 µg of saccharide. In an embodiment, each dose will comprise about 3.5 µg of saccharide. In an embodiment, each dose will comprise about 4.0 µg of saccharide. In an embodiment, each dose will comprise about 4.4 µg of saccharide. In an embodiment, each dose will comprise about 5.0 µg of saccharide. In an embodiment, each dose will comprise about 5.5 µg of saccharide. In an embodiment, each dose will comprise about 6.0 µg of saccharide.

Generally, each dose will comprise 0.1 µg to 100 µg of saccharide for a given bacteria or serotype. In an embodiment each dose will comprise 0.1 µg to 100 µg of saccharide for a given bacteria or serotype. In a preferred embodiment each dose will comprise 0.5 µg to 20 µg. In a preferred embodiment each dose will comprise 1.0 µg to 10 µg. In an even preferred embodiment, each dose will comprise 2.0 µg to 5.0 µg of saccharide for a given bacteria or serotype. Any whole number integer within any of the above ranges is contemplated as an embodiment of the disclosure.

In an embodiment, each dose will comprise about 0.5 µg of saccharide for each particular glycoconjugate. In an embodiment, each dose will comprise about 0.55 µg of saccharide for each particular glycoconjugate. In an embodiment, each dose will comprise about 0.75 µg of saccharide for each particular glycoconjugate. In an embodiment, each dose will comprise about 1.0 µg of saccharide for each particular glycoconjugate. In an embodiment, each dose will comprise about 1.1 µg of saccharide for each particular glycoconjugate. In an embodiment, each dose will comprise about 1.5 µg of saccharide for each particular glycoconjugate. In an embodiment, each dose will comprise about 2.0 µg of saccharide for each particular glycoconjugate. In an embodiment, each dose will comprise about 2.2 µg of saccharide for each particular glycoconjugate. In an embodiment, each dose will comprise about 2.5 µg of saccharide for each particular glycoconjugate. In an embodiment, each dose will comprise about 3.0 µg of saccharide for each particular glycoconjugate. In an embodiment, each dose will comprise about 3.5 µg of saccharide for each particular glycoconjugate. In an embodiment, each dose will comprise about 4.0 µg of saccharide for each particular glycoconjugate. In an embodiment, each dose will comprise about 4.4 µg of saccharide for each particular glycoconjugate. In an embodiment, each dose will comprise about 5.0 µg of saccharide for each particular glycoconjugate. In an embodiment, each dose will comprise about 5.5 µg of saccharide for each particular glycoconjugate. In an embodiment, each dose will comprise about 6.0 µg of saccharide for each particular glycoconjugate.

2.3 Carrier amount

Generally, each dose will comprise 10 µg to 150 µg of carrier protein, particularly 15 µg to 100 µg of carrier protein, more particularly 25 µg to 75 µg of carrier protein, and even more particularly 40 µg to 60 µg of carrier protein. In an embodiment, said carrier protein is CRM₁₉₇. In an embodiment, said carrier protein is SCP.

In an embodiment, each dose will comprise about 10 µg, about 15 µg, about 20 µg, about 25 µg, about 26 µg, about 27 µg, about 28 µg, about 29 µg, about 30 µg, about 31 µg, about 32 µg, about 33 µg, about 34 µg, about 35 µg, about 36 µg, about 37 µg, about 38 µg, about 39 µg, about 40 µg, about 41 µg, about 42 µg, about 43 µg, about 44 µg, about 45 µg, about 46 µg, about 47 µg, about 48 µg, about 49 µg, about 50 µg, about 51 µg, about 52 µg, about 53 µg, about 54 µg, about 55 µg, about 56 µg, about 57 µg, about 58 µg, about 59 µg, about 60 µg, about 61 µg, about 62 µg, about 63 µg, about 64 µg, about 65 µg, about 66 µg, about 67 µg, about 68 µg, about 69 µg, about 70 µg, about 71 µg, about 72 µg, about 73 µg, about 74 µg or about 75 µg of carrier protein.

In an embodiment, each dose will comprise about 25 µg, about 26 µg, about 27 µg, about 28 µg, about 29 µg, about 30 µg, about 31 µg, about 32 µg, about 33 µg, about 34 µg, about 35 µg, about 36 µg, about 37 µg, about 38 µg, about 39 µg, about 40 µg, about 41 µg, about 42 µg, about 43 µg, about 44 µg, about 45 µg, about 46 µg, about 47 µg, about 48 µg, about 49 µg, about 50 µg, about 51 µg, about 52 µg, about 53 µg, about 54 µg, about 55 µg, about 56 µg, about 57 µg, about 58 µg, about 59 µg, about 60 µg, about 61 µg, about 62 µg, about 63 µg, about 64 µg, about 65 µg, about 66 µg, about 67 µg, about 68 µg, about 69 µg, about 70 µg, about 71 µg, about 72 µg, about 73 µg, about 74 µg or about 75 µg of carrier protein.

In an embodiment, each dose will comprise about 30 µg of carrier protein. In an embodiment, each dose will comprise about 31 µg of carrier protein. In an embodiment, each dose will comprise about 32 µg of carrier protein. In an embodiment, each dose will comprise about 33 µg of carrier protein. In an embodiment, each dose will comprise about 34 µg of carrier protein. In an embodiment, each dose will comprise about 45 µg of carrier protein.

In an embodiment, each dose will comprise about 40 µg of carrier protein. In an embodiment, each dose will comprise about 41 µg of carrier protein. In an embodiment, each dose will comprise about 42 µg of carrier protein. In an embodiment, each dose will comprise about 43 µg of carrier protein. In an embodiment, each dose will comprise about 44 µg of carrier protein. In an embodiment, each dose will comprise about 45 µg of carrier protein.

In an embodiment, each dose will comprise about 48 µg of carrier protein. In an embodiment, each dose will comprise about 49 µg of carrier protein. In an embodiment, each dose will comprise about 50 µg of carrier protein. In an embodiment, each dose will comprise about 51 µg of carrier protein. In an embodiment, each dose will comprise about 52 µg of carrier protein. In an embodiment, each dose will comprise about 53 µg of carrier protein.

In an embodiment, said carrier protein is CRM₁₉₇.

In an embodiment, said carrier protein is SCP.

2.4 Further antigens

Immunogenic compositions of the invention comprise conjugated saccharide antigen(s) (glycoconjugate(s)). They may also further include antigen(s) from other pathogen(s), particularly from bacteria and/or viruses. Preferred further antigens are selected from: a diphtheria toxoid (D), a tetanus toxoid (T), a pertussis antigen (P), which is typically acellular (Pa), a hepatitis B virus (HBV) surface antigen (HBsAg), a hepatitis A virus (HAV) antigen, a conjugated *Haemophilus influenzae* type b capsular saccharide (Hib), inactivated poliovirus vaccine (IPV).

In an embodiment, the immunogenic compositions of the invention comprise D-T-Pa. In an embodiment, the immunogenic compositions of the invention comprise D-T-Pa-Hib, D-T-Pa-IPV or D-T-Pa-HBsAg. In an embodiment, the immunogenic compositions of the invention comprise D-T-Pa-HBsAg-IPV or D-T-Pa-HBsAg-Hib. In an embodiment, the immunogenic compositions of the invention comprise D-T-Pa-HBsAg-IPV-Hib.

Pertussis antigens: *Bordetella pertussis* causes whooping cough. Pertussis antigens in vaccines are either cellular (whole cell, in the form of inactivated *B. pertussis* cells) or acellular. Preparation of cellular pertussis antigens is well documented (e.g., it may be obtained by heat inactivation of phase I culture of *B. pertussis*). Preferably, however, the invention uses acellular antigens. Where acellular antigens are used, it is preferred to use one, two or (preferably) three of the following antigens: (1) detoxified pertussis toxin (pertussis toxoid, or PT); (2) filamentous hemagglutinin (FHA); (3) pertactin (also known as the 69 kilodalton outer membrane protein). FHA and pertactin may be treated with formaldehyde prior to use according to the invention. PT is preferably detoxified by treatment with formaldehyde and/or glutaraldehyde. Acellular pertussis antigens are preferably adsorbed onto one or more aluminum salt adjuvants. As an alternative, they may be added in an unadsorbed state. Where pertactin is added then it is preferably already adsorbed onto an aluminum hydroxide adjuvant. PT and FHA may

be adsorbed onto an aluminum hydroxide adjuvant or an aluminum phosphate. Adsorption of all of PT, FHA and pertactin to aluminum hydroxide is most preferred.

Inactivated poliovirus vaccine: Poliovirus causes poliomyelitis. Rather than use oral poliovirus vaccine, preferred embodiments of the invention use IPV. Prior to administration to patients, polioviruses must be inactivated, and this can be achieved by treatment with formaldehyde. Poliomyelitis can be caused by one of three types of poliovirus. The three types are similar and cause identical symptoms, but they are antigenically different and infection by one type does not protect against infection by others. It is therefore preferred to use three poliovirus antigens in the invention: poliovirus Type 1 (e.g., Mahoney strain), poliovirus Type 2 (e.g., MEF-1 strain), and poliovirus Type 3 (e.g., Saukett strain). The viruses are preferably grown, purified and inactivated individually, and are then combined to give a bulk trivalent mixture for use with the invention.

Diphtheria toxoid: *Corynebacterium diphtheriae* causes diphtheria. Diphtheria toxin can be treated (e.g., using formalin or formaldehyde) to remove toxicity while retaining the ability to induce specific anti-toxin antibodies after injection. These diphtheria toxoids are used in diphtheria vaccines. Preferred diphtheria toxoids are those prepared by formaldehyde treatment. The diphtheria toxoid can be obtained by growing *C. diphtheriae* in growth medium, followed by formaldehyde treatment, ultrafiltration and precipitation. The toxoided material may then be treated by a process comprising sterile filtration and/or dialysis. The diphtheria toxoid is preferably adsorbed onto an aluminum hydroxide adjuvant.

Tetanus toxoid: *Clostridium tetani* causes tetanus. Tetanus toxin can be treated to give a protective toxoid. The toxoids are used in tetanus vaccines. Preferred tetanus toxoids are those prepared by formaldehyde treatment. The tetanus toxoid can be obtained by growing *C. tetani* in growth medium, followed by formaldehyde treatment, ultrafiltration and precipitation. The material may then be treated by a process comprising sterile filtration and/or dialysis.

Hepatitis A virus antigens: Hepatitis A virus (HAV) is one of the known agents which causes viral hepatitis. A preferred HAV component is based on inactivated virus, and inactivation can be achieved by formalin treatment.

Hepatitis B virus (HBV) is one of the known agents which causes viral hepatitis. The major component of the capsid is a protein known as HBV surface antigen or, more commonly, HBsAg, which is typically a 226-amino acid polypeptide with a molecular weight of ~24 kDa. All existing hepatitis B vaccines contain HBsAg, and when this antigen is

administered to a normal vaccinee it stimulates the production of anti-HBsAg antibodies which protect against HBV infection.

For vaccine manufacture, HBsAg has been made in two ways: purification of the antigen in particulate form from the plasma of chronic hepatitis B carriers or expression of the protein by recombinant DNA methods (e.g., recombinant expression in yeast cells). Unlike native HBsAg (i.e., as in the plasma-purified product), yeast-expressed HBsAg is generally non-glycosylated, and this is the most preferred form of HBsAg for use with the invention.

Conjugated *Haemophilus influenzae* type b antigens: *Haemophilus influenzae* type b (Hib) causes bacterial meningitis. Hib vaccines are typically based on the capsular saccharide antigen, the preparation of which is well documented. The Hib saccharide can be conjugated to a carrier protein in order to enhance its immunogenicity, especially in children. Typical carrier proteins are tetanus toxoid, diphtheria toxoid, CRM₁₉₇, *H.influenzae* protein D, and an outer membrane protein complex from serogroup B meningococcus. The saccharide moiety of the conjugate may comprise full-length polyribosylribitol phosphate (PRP) as prepared from Hib bacteria, and/or fragments of full-length PRP. Hib conjugates may or may not be adsorbed to an aluminum salt adjuvant.

2.5 Adjuvant(s)

In some embodiments, the immunogenic compositions disclosed herein may further comprise at least one, two or three adjuvants. In some embodiments, the immunogenic compositions disclosed herein may further comprise at least one adjuvant. In some embodiments, the immunogenic compositions disclosed herein may further comprise one adjuvant. In some embodiments, the immunogenic compositions disclosed herein may further comprise two adjuvants. The term "adjuvant" refers to a compound or mixture that enhances the immune response to an antigen. Antigens may act primarily as a delivery system, primarily as an immune modulator or have strong features of both.

Suitable adjuvants include those suitable for use in mammals, including humans. Examples of known suitable delivery-system type adjuvants that can be used in humans include, but are not limited to, alum (e.g., aluminum phosphate, aluminum sulfate or aluminum hydroxide), calcium phosphate, liposomes, oil-in-water emulsions such as MF59 (4.3% w/v squalene, 0.5% w/v polysorbate 80 (Tween 80), 0.5% w/v sorbitan trioleate (Span 85)), water-in-oil emulsions such as Montanide, and poly(D,L-lactide-co-glycolide) (PLG) microparticles or nanoparticles.

In an embodiment, the immunogenic compositions disclosed herein comprise aluminum salts (alum) as adjuvant (e.g., aluminum phosphate, aluminum sulfate or aluminum hydroxide). In a preferred embodiment, the immunogenic compositions disclosed herein comprise aluminum phosphate or aluminum hydroxide as adjuvant. In a preferred embodiment, the immunogenic compositions disclosed herein comprise aluminum phosphate as adjuvant.

Further exemplary adjuvants to enhance effectiveness of the immunogenic compositions as disclosed herein include, but are not limited to: (1) oil-in-water emulsion formulations (with or without other specific immunostimulating agents such as muramyl peptides (see below) or bacterial cell wall components), such as for example (a) SAF, containing 10% Squalene, 0.4% Tween 80, 5% pluronic-blocked polymer L121, and th-MDP either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion, and (b) RIBI™ adjuvant system (RAS), (Ribi Immunochem, Hamilton, MT) containing 2% Squalene, 0.2% Tween 80, and one or more bacterial cell wall components such as monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (DETOX™); (2) saponin adjuvants, such as QS21, STIMULON™ (Cambridge Bioscience, Worcester, MA), ABISCO® (Isconova, Sweden), or ISCOMATRIX® (Commonwealth Serum Laboratories, Australia), may be used or particles generated therefrom such as ISCOMs (immunostimulating complexes), which ISCOMs may be devoid of additional detergent (e.g., WO 00/07621); (3) Complete Freund's Adjuvant (CFA) and Incomplete Freund's Adjuvant (IFA); (4) cytokines, such as interleukins (e.g., IL-1, IL-2, IL-4, IL-5, IL-6, IL-7, IL-12 (e.g., WO 99/44636)), interferons (e.g., gamma interferon), macrophage colony stimulating factor (M-CSF), tumor necrosis factor (TNF), etc.; (5) monophosphoryl lipid A (MPL) or 3-O-deacylated MPL (3dMPL) (see, e.g., GB-2220221, EP0689454), optionally in the substantial absence of alum when used with pneumococcal saccharides (see, e.g., WO 00/56358); (6) combinations of 3dMPL with, for example, QS21 and/or oil-in-water emulsions (see, e.g., EP0835318, EP0735898, EP0761231); (7) a polyoxyethylene ether or a polyoxyethylene ester (see, e.g., WO 99/52549); (8) a polyoxyethylene sorbitan ester surfactant in combination with an octoxynol (e.g., WO 01/21207) or a polyoxyethylene alkyl ether or ester surfactant in combination with at least one additional non-ionic surfactant such as an octoxynol (e.g., WO 01/21152); (9) a saponin and an immunostimulatory oligonucleotide (e.g., a CpG oligonucleotide) (e.g., WO 00/62800); (10) an immunostimulant and a particle of metal salt (see, e.g., WO 00/23105); (11) a saponin and an oil-in-water emulsion (e.g., WO 99/11241); (12) a saponin (e.g., QS21) + 3dMPL + IM2 (optionally + a sterol) (e.g., WO

98/57659); (13) other substances that act as immunostimulating agents to enhance the efficacy of the composition. Muramyl peptides include N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-25 acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutarninyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine MTP-PE), etc.

In an embodiment of the present invention, the immunogenic compositions as disclosed herein comprise a CpG Oligonucleotide as adjuvant. A CpG oligonucleotide as used herein refers to an immunostimulatory CpG oligodeoxynucleotide (CpG ODN), and accordingly these terms are used interchangeably unless otherwise indicated.

Immunostimulatory CpG oligodeoxynucleotides contain one or more immunostimulatory CpG motifs that are unmethylated cytosine-guanine dinucleotides, optionally within certain preferred base contexts. The methylation status of the CpG immunostimulatory motif generally refers to the cytosine residue in the dinucleotide. An immunostimulatory oligonucleotide containing at least one unmethylated CpG dinucleotide is an oligonucleotide which contains a 5' unmethylated cytosine linked by a phosphate bond to a 3' guanine, and which activates the immune system through binding to Toll-like receptor 9 (TLR-9). In another embodiment the immunostimulatory oligonucleotide may contain one or more methylated CpG dinucleotides, which will activate the immune system through TLR9 but not as strongly as if the CpG motif(s) was/were unmethylated. CpG immunostimulatory oligonucleotides may comprise one or more palindromes that in turn may encompass the CpG dinucleotide. CpG oligonucleotides have been described in a number of issued patents, published patent applications, and other publications, including U.S. Patent Nos. 6,194,388; 6,207,646; 6,214,806; 6,218,371; 6,239,116; and 6,339,068. In an embodiment of the present invention, the immunogenic compositions as disclosed herein comprise any of the CpG Oligonucleotide described at page 3, line 22, to page 12, line 36, of WO 2010/125480.

Different classes of CpG immunostimulatory oligonucleotides have been identified. These are referred to as A, B, C and P class, and are described in greater detail at page 3, line 22, to page 12, line 36, of WO 2010/125480. Methods of the invention embrace the use of these different classes of CpG immunostimulatory oligonucleotides.

In an embodiment of the present invention, the immunogenic compositions as disclosed herein comprise an A class CpG oligonucleotide. Preferably, the "A class" CpG oligonucleotide of the invention has the following nucleic acid sequence: 5' GGGGACGACGTCGTGGGGGGG 3' (SEQ ID NO: 1). Some non-limiting examples of A-

Class oligonucleotides include: 5'

G*G*G_G_A_C_G_A_C_G_T_C_G_T_G_G*G*G*G*G 3' (SEQ ID NO: 2); wherein "*" refers to a phosphorothioate bond and "_" refers to a phosphodiester bond.

In an embodiment of the present invention, the immunogenic compositions as disclosed herein comprise a B class CpG Oligonucleotide. In one embodiment, the CpG oligonucleotide for use in the present invention is a B class CpG oligonucleotide represented by at least the formula: 5' X₁X₂CGX₃X₄ 3', wherein X₁, X₂, X₃, and X₄ are nucleotides. In one embodiment, X₂ is adenine, guanine, or thymine. In another embodiment, X₃ is cytosine, adenine, or thymine.

The B class CpG oligonucleotide sequences of the invention are those broadly described above as well as disclosed in WO 96/02555, WO 98/18810 and U.S. Patent Nos. 6,194,388; 6,207,646; 6,214,806; 6,218,371; 6,239,116 and 6,339,068. Exemplary sequences include but are not limited to those disclosed in these latter applications and patents.

In an embodiment, the "B class" CpG oligonucleotide of the invention has the following nucleic acid sequence:

5' TCGTCGTTTTTCGGTGCTTTT 3' (SEQ ID NO: 3), or

5' TCGTCGTTTTTCGGTCGTTTT 3' (SEQ ID NO: 4), or

5' TCGTCGTTTTGTCGTTTTGTCGTT 3' (SEQ ID NO: 5), or

5' TCGTCGTTTCGTCGTTTTGTCGTT 3' (SEQ ID NO: 6), or

5' TCGTCGTTTTGTCGTTTTTTTCGA 3' (SEQ ID NO: 7).

In any of these sequences, all of the linkages may be all phosphorothioate bonds. In another embodiment, in any of these sequences, one or more of the linkages may be phosphodiester, preferably between the "C" and the "G" of the CpG motif making a semi-soft CpG oligonucleotide. In any of these sequences, an ethyl-uridine or a halogen may substitute for the 5' T; examples of halogen substitutions include but are not limited to bromo-uridine or iodo-uridine substitutions.

Some non-limiting examples of B-Class oligonucleotides include:

5' T*C*G*T*C*G*T*T*T*T*T*C*G*G*T*G*C*T*T*T*T 3' (SEQ ID NO: 8), or

5' T*C*G*T*C*G*T*T*T*T*T*T*C*G*G*T*C*G*T*T*T*T 3' (SEQ ID NO: 9), or

5' T*C*G*T*C*G*T*T*T*T*G*T*C*G*T*T*T*T*G*T*C*G*T*T 3' (SEQ ID NO: 10), or

5' T*C*G*T*C*G*T*T*T*T*C*G*T*C*G*T*T*T*T*G*T*C*G*T*T 3' (SEQ ID NO: 11), or

5' T*C*G*T*C*G*T*T*T*T*G*T*C*G*T*T*T*T*T*T*T*C*G*A 3' (SEQ ID NO: 12).

wherein "*" refers to a phosphorothioate bond.

In an embodiment of the present invention, the immunogenic compositions as disclosed herein comprise a C class CpG Oligonucleotide. In an embodiment, the "C class" CpG oligonucleotides of the invention have the following nucleic acid sequence:

- 5' TCGCGTCGTTTCGGCGCGCGCCG 3' (SEQ ID NO: 13), or
 5 5' TCGTCGACGTTTCGGCGCGCGCCG 3' (SEQ ID NO: 14), or
 5' TCGGACGTTTCGGCGCGCGCCG 3' (SEQ ID NO: 15), or
 5' TCGGACGTTTCGGCGCGCGCCG 3' (SEQ ID NO: 16), or
 5' TCGCGTCGTTTCGGCGCGCGCCG 3' (SEQ ID NO: 17), or
 5' TCGACGTTTCGGCGCGCGCCG 3' (SEQ ID NO: 18), or
 10 5' TCGACGTTTCGGCGCGCGCCG 3' (SEQ ID NO: 19), or
 5' TCGCGTCGTTTCGGCGCGCCG 3' (SEQ ID NO: 20), or
 5' TCGCGACGTTTCGGCGCGCGCCG 3' (SEQ ID NO: 21), or
 5' TCGTCGTTTTTCGGCGCGCGCCG 3' (SEQ ID NO: 22), or
 5' TCGTCGTTTTTCGGCGCGCGCCG 3' (SEQ ID NO: 23), or
 15 5' TCGTCGTTTTACGGCGCCGTGCCG 3' (SEQ ID NO: 24), or
 5' TCGTCGTTTTTCGGCGCGCGCCGT 3' (SEQ ID NO: 25).

In any of these sequences, all of the linkages may be all phosphorothioate bonds. In another embodiment, in any of these sequences, one or more of the linkages may be phosphodiester, preferably between the "C" and the "G" of the CpG motif making a semi-
 20 soft CpG oligonucleotide.

Some non-limiting examples of C-Class oligonucleotides include:

- 5' T*C_G*C_G*T*T*C_G*G*C*G*C_G*C*G*C*C*G 3' (SEQ ID NO: 26), or
 5' T*C_G*T*C_G*A*C_G*T*T*C_G*G*C*G*C_G*C*G*C*C*G 3' (SEQ ID NO: 27), or
 5' T*C_G*G*A*C_G*T*T*C_G*G*C*G*C_G*C*G*C*C*G 3' (SEQ ID NO: 28), or
 25 5' T*C_G*G*A*C_G*T*T*C_G*G*C*G*C*G*C*C*G 3' (SEQ ID NO: 29), or
 5' T*C_G*C_G*T*T*C_G*T*T*C_G*G*C*G*C*G*C*C*G 3' (SEQ ID NO: 30), or
 5' T*C_G*A*C_G*T*T*C_G*G*C*G*C_G*C*G*C*C*G 3' (SEQ ID NO: 31), or
 5' T*C_G*A*C_G*T*T*C_G*G*C*G*C*G*C*C*G 3' (SEQ ID NO: 32), or
 5' T*C_G*C_G*T*T*C_G*T*T*C_G*G*C*G*C*C*G 3' (SEQ ID NO: 33), or
 30 5' T*C_G*C_G*A*C_G*T*T*C_G*G*C*G*C_G*C*G*C*C*G 3' (SEQ ID NO: 34), or
 5' T*C*G*T*C*G*T*T*T*T*C*G*G*C*G*C*G*C*G*C*C*G 3' (SEQ ID NO: 35), or
 5' T*C*G*T*C*G*T*T*T*T*C*G*G*C*G*G*C*G*C*G*C*C*G 3' (SEQ ID NO: 36), or
 5' T*C*G*T*C_G*T*T*T*T*A*C_G*G*C*G*C*C_G*T*G*C*C*G 3' (SEQ ID NO: 37), or
 5' T*C_G*T*C*G*T*T*T*T*C*G*G*C*G*C*G*C*G*C*G*T 3' (SEQ ID NO: 38)
 35 wherein "*" refers to a phosphorothioate bond and "_" refers to a phosphodiester bond.

In any of these sequences, an ethyl-uridine or a halogen may substitute for the 5' T; examples of halogen substitutions include but are not limited to bromo-uridine or iodo-uridine substitutions.

In an embodiment of the present invention, the immunogenic compositions as disclosed herein comprise a P class CpG Oligonucleotide. In an embodiment, the CpG oligonucleotide for use in the present invention is a P class CpG oligonucleotide containing a 5' TLR activation domain and at least two palindromic regions, one palindromic region being a 5' palindromic region of at least 6 nucleotides in length and connected to a 3' palindromic region of at least 8 nucleotides in length either directly or through a spacer, wherein the oligonucleotide includes at least one YpR dinucleotide. In an embodiment, said oligonucleotide is not T*C_G*T*C_G*A*C_G*T*T*C_G*G*C*G*C_G*C*G*C*C*G (SEQ ID NO: 27). In one embodiment the P class CpG oligonucleotide includes at least one unmethylated CpG dinucleotide. In another embodiment the TLR activation domain is TCG, TTCG, TTTCG, TYpR, TTYpR, TTTYpR, UCG, UUCG, UUUCG, TTT, or TTTT. In yet another embodiment the TLR activation domain is within the 5' palindromic region. In another embodiment the TLR activation domain is immediately 5' to the 5' palindromic region. In an embodiment, the "P class" CpG oligonucleotides of the invention have the following nucleic acid sequence: 5' TCGTCGACGATCGGCGCGCGCCG 3' (SEQ ID NO: 39).

In said sequences, all of the linkages may be all phosphorothioate bonds. In another embodiment, one or more of the linkages may be phosphodiester, preferably between the "C" and the "G" of the CpG motif making a semi-soft CpG oligonucleotide. In any of these sequences, an ethyl-uridine or a halogen may substitute for the 5' T; examples of halogen substitutions include but are not limited to bromo-uridine or iodo-uridine substitutions.

A non-limiting example of P-Class oligonucleotides include:

5' T*C_G*T*C_G*A*C_G*A*T*C_G*G*C*G*C_G*C*G*C*C*G 3' (SEQ ID NO: 40)

wherein "*" refers to a phosphorothioate bond and "_" refers to a phosphodiester bond.

In one embodiment the oligonucleotide includes at least one phosphorothioate linkage. In another embodiment all internucleotide linkages of the oligonucleotide are phosphorothioate linkages. In another embodiment the oligonucleotide includes at least one phosphodiester-like linkage. In another embodiment the phosphodiester-like linkage is a phosphodiester linkage. In another embodiment a lipophilic group is conjugated to the oligonucleotide. In one embodiment the lipophilic group is cholesterol.

In an embodiment, all the internucleotide linkages of the CpG oligonucleotides disclosed herein are phosphodiester bonds ("soft" oligonucleotides, as described in WO 2007/026190). In another embodiment, CpG oligonucleotides of the invention are rendered resistant to degradation (e.g., are stabilized). A "stabilized oligonucleotide" refers to an oligonucleotide that is relatively resistant to *in vivo* degradation (e.g., via an 5
exo- or endo-nuclease). Nucleic acid stabilization can be accomplished via backbone modifications. Oligonucleotides having phosphorothioate linkages provide maximal activity and protect the oligonucleotide from degradation by intracellular exo- and endo-nucleases.

10 The immunostimulatory oligonucleotides may have a chimeric backbone, which have combinations of phosphodiester and phosphorothioate linkages. For purposes of the instant invention, a chimeric backbone refers to a partially stabilized backbone, wherein at least one internucleotide linkage is phosphodiester or phosphodiester-like, and wherein at least one other internucleotide linkage is a stabilized internucleotide linkage, 15
wherein the at least one phosphodiester or phosphodiester-like linkage and the at least one stabilized linkage are different. When the phosphodiester linkage is preferentially located within the CpG motif such molecules are called "semi-soft" as described in WO 2007/026190.

Other modified oligonucleotides include combinations of phosphodiester, 20
phosphorothioate, methylphosphonate, methylphosphorothioate, phosphorodithioate, and/or p-ethoxy linkages.

Mixed backbone modified ODN may be synthesized as described in WO 2007/026190.

25 The size of the CpG oligonucleotide (i.e., the number of nucleotide residues along the length of the oligonucleotide) also may contribute to the stimulatory activity of the oligonucleotide. For facilitating uptake into cells, CpG oligonucleotide of the invention preferably have a minimum length of 6 nucleotide residues. Oligonucleotides of any size greater than 6 nucleotides (even many kb long) are capable of inducing an immune response if sufficient immunostimulatory motifs are present, because larger 30
oligonucleotides are degraded inside cells. In certain embodiments, the CpG oligonucleotides are 6 to 100 nucleotides long, preferentially 8 to 30 nucleotides long. In important embodiments, nucleic acids and oligonucleotides of the invention are not plasmids or expression vectors.

In an embodiment, the CpG oligonucleotide disclosed herein comprise substitutions or modifications, such as in the bases and/or sugars as described at paragraphs 134 to 147 of WO 2007/026190.

In an embodiment, the CpG oligonucleotide of the present invention is chemically modified. Examples of chemical modifications are known to the skilled person and are described, for example in Uhlmann et al. (1990) Chem. Rev. 90:543; S. Agrawal, Ed., Humana Press, Totowa, USA 1993; Crooke et al. (1996) Annu. Rev. Pharmacol. Toxicol. 36:107-129; and Hunziker et al. (1995) Mod. Synth. Methods 7:331-417. An oligonucleotide according to the invention may have one or more modifications, wherein each modification is located at a particular phosphodiester internucleoside bridge and/or at a particular β -D-ribose unit and/or at a particular natural nucleoside base position in comparison to an oligonucleotide of the same sequence which is composed of natural DNA or RNA.

In some embodiments of the invention, CpG-containing nucleic acids might be simply mixed with immunogenic carriers according to methods known to those skilled in the art (see, e.g., WO 03/024480).

In a particular embodiment of the present invention, any of the immunogenic compositions disclosed herein comprise from 2 μ g to 100 mg of CpG oligonucleotide. In a particular embodiment of the present invention, the immunogenic composition of the invention comprises 0.1 mg to 50 mg of CpG oligonucleotide, preferably from 0.2 mg to 10 mg CpG oligonucleotide, more preferably from 0.3 mg to 5 mg CpG oligonucleotide. In a particular embodiment of the present invention, the immunogenic composition of the invention comprises from 0.3 mg to 5 mg CpG oligonucleotide. Even preferably, the immunogenic composition of the invention may comprise from 0.5 to 2 mg CpG oligonucleotide. Most preferably, the immunogenic composition of the invention may comprise from 0.75 to 1.5 mg CpG oligonucleotide. In a preferred embodiment, any of the immunogenic composition disclosed herein may comprise about 1 mg CpG oligonucleotide.

3 Formulation

The immunogenic compositions of the invention may be formulated in liquid form (i.e., solutions or suspensions) or in a lyophilized form. In an embodiment, the immunogenic composition of the invention is formulated in a liquid form. In an embodiment, the immunogenic composition of the invention is formulated in a lyophilized form. Liquid formulations may advantageously be administered directly from their

packaged form and are thus ideal for injection without the need for reconstitution in aqueous medium as otherwise required for lyophilized compositions of the invention.

Formulation of the immunogenic composition of the present disclosure can be accomplished using art-recognized methods. For instance, the individual polysaccharides and/or conjugates can be formulated with a physiologically acceptable vehicle to prepare the composition. Examples of such vehicles include, but are not limited to, water, buffered saline, polyols (e.g., glycerol, propylene glycol, liquid polyethylene glycol) and dextrose solutions.

The present disclosure provides an immunogenic composition comprising any of combination of glycoconjugates disclosed herein and a pharmaceutically acceptable excipient, carrier, or diluent.

In an embodiment, the immunogenic composition of the disclosure is in liquid form, preferably in aqueous liquid form.

Immunogenic compositions of the disclosure may comprise one or more of a buffer, a salt, a divalent cation, a non-ionic detergent, a cryoprotectant such as a sugar, and an anti-oxidant such as a free radical scavenger or chelating agent, or any multiple combinations thereof.

In an embodiment, the immunogenic compositions of the disclosure comprise a buffer. In an embodiment, said buffer has a pKa of about 3.5 to about 7.5. In some embodiments, the buffer is phosphate, succinate, histidine or citrate. In some embodiments, the buffer is succinate. In some embodiments, the buffer is histidine. In certain embodiments, the buffer is succinate at a final concentration of 1 mM to 10 mM. In one particular embodiment, the final concentration of the succinate buffer is about 5 mM.

In an embodiment, the immunogenic compositions of the disclosure comprise a salt. In some embodiments, the salt is selected from the groups consisting of magnesium chloride, potassium chloride, sodium chloride and a combination thereof. In one particular embodiment, the salt is sodium chloride. In one particular embodiment, the immunogenic compositions of the invention comprise sodium chloride at 150 mM.

In an embodiment, the immunogenic compositions of the disclosure comprise a surfactant. In an embodiment, the surfactant is selected from the group consisting of polysorbate 20 (TWEENTM20), polysorbate 40 (TWEENTM40), polysorbate 60 (TWEENTM60), polysorbate 65 (TWEENTM65), polysorbate 80 (TWEENTM80), polysorbate 85 (TWEENTM85), TRITONTM N-101, TRITONTM X-100, octoxynol 40, nonoxynol-9, triethanolamine, triethanolamine polypeptide oleate, polyoxyethylene-660

hydroxystearate (PEG-15, Solutol H 15), polyoxyethylene-35-ricinoleate (CREMOPHOR® EL), soy lecithin and a poloxamer.

In one particular embodiment, the surfactant is polysorbate 80. In some said embodiment, the final concentration of polysorbate 80 in the formulation is at least 0.0001% to 10% polysorbate 80 weight to weight (w/w). In some said embodiments, the final concentration of polysorbate 80 in the formulation is at least 0.001% to 1% polysorbate 80 weight to weight (w/w). In some said embodiments, the final concentration of polysorbate 80 in the formulation is at least 0.01% to 1% polysorbate 80 weight to weight (w/w). In other embodiments, the final concentration of polysorbate 80 in the formulation is 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09% or 0.1% polysorbate 80 (w/w). In another embodiment, the final concentration of the polysorbate 80 in the formulation is 0.02% polysorbate 80 (w/w). In another embodiment, the final concentration of the polysorbate 80 in the formulation is 0.01% polysorbate 80 (w/w). In another embodiment, the final concentration of the polysorbate 80 in the formulation is 0.03% polysorbate 80 (w/w). In another embodiment, the final concentration of the polysorbate 80 in the formulation is 0.04% polysorbate 80 (w/w). In another embodiment, the final concentration of the polysorbate 80 in the formulation is 0.05% polysorbate 80 (w/w). In another embodiment, the final concentration of the polysorbate 80 in the formulation is 1% polysorbate 80 (w/w).

In one particular embodiment, the surfactant is polysorbate 20. In some said embodiment, the final concentration of polysorbate 20 in the formulation is at least 0.0001% to 10% polysorbate 20 weight to weight (w/w). In some said embodiments, the final concentration of polysorbate 20 in the formulation is at least 0.001% to 1% polysorbate 20 weight to weight (w/w). In some said embodiments, the final concentration of polysorbate 20 in the formulation is at least 0.01% to 1% polysorbate 20 weight to weight (w/w). In other embodiments, the final concentration of polysorbate 20 in the formulation is 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09% or 0.1% polysorbate 20 (w/w). In another embodiment, the final concentration of the polysorbate 20 in the formulation is 0.02% polysorbate 20 (w/w). In another embodiment, the final concentration of the polysorbate 20 in the formulation is 0.01% polysorbate 20 (w/w). In another embodiment, the final concentration of the polysorbate 20 in the formulation is 0.03% polysorbate 20 (w/w). In another embodiment, the final concentration of the polysorbate 20 in the formulation is 0.04% polysorbate 80 (w/w). In another embodiment, the final concentration of the polysorbate 20 in the formulation is

0.05% polysorbate 20 (w/w). In another embodiment, the final concentration of the polysorbate 20 in the formulation is 1% polysorbate 20 (w/w).

In one particular embodiment, the surfactant is polysorbate 40. In some said embodiment, the final concentration of polysorbate 40 in the formulation is at least 0.0001% to 10% polysorbate 40 weight to weight (w/w). In some said embodiments, the final concentration of polysorbate 40 in the formulation is at least 0.001% to 1% polysorbate 40 weight to weight (w/w). In some said embodiments, the final concentration of polysorbate 40 in the formulation is at least 0.01% to 1% polysorbate 40 weight to weight (w/w). In other embodiments, the final concentration of polysorbate 40 in the formulation is 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09% or 0.1% polysorbate 40 (w/w). In another embodiment, the final concentration of the polysorbate 40 in the formulation is 1% polysorbate 40 (w/w).

In one particular embodiment, the surfactant is polysorbate 60. In some said embodiment, the final concentration of polysorbate 60 in the formulation is at least 0.0001% to 10% polysorbate 60 weight to weight (w/w). In some said embodiments, the final concentration of polysorbate 60 in the formulation is at least 0.001% to 1% polysorbate 60 weight to weight (w/w). In some said embodiments, the final concentration of polysorbate 60 in the formulation is at least 0.01% to 1% polysorbate 60 weight to weight (w/w). In other embodiments, the final concentration of polysorbate 60 in the formulation is 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09% or 0.1% polysorbate 60 (w/w). In another embodiment, the final concentration of the polysorbate 60 in the formulation is 1% polysorbate 60 (w/w).

In one particular embodiment, the surfactant is polysorbate 65. In some said embodiment, the final concentration of polysorbate 65 in the formulation is at least 0.0001% to 10% polysorbate 65 weight to weight (w/w). In some said embodiments, the final concentration of polysorbate 65 in the formulation is at least 0.001% to 1% polysorbate 65 weight to weight (w/w). In some said embodiments, the final concentration of polysorbate 65 in the formulation is at least 0.01% to 1% polysorbate 65 weight to weight (w/w). In other embodiments, the final concentration of polysorbate 65 in the formulation is 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09% or 0.1% polysorbate 65 (w/w). In another embodiment, the final concentration of the polysorbate 65 in the formulation is 1% polysorbate 65 (w/w).

In one particular embodiment, the surfactant is polysorbate 85. In some said embodiment, the final concentration of polysorbate 85 in the formulation is at least 0.0001% to 10% polysorbate 85 weight to weight (w/w). In some said embodiments, the

final concentration of polysorbate 85 in the formulation is at least 0.001% to 1% polysorbate 85 weight to weight (w/w). In some said embodiments, the final concentration of polysorbate 85 in the formulation is at least 0.01% to 1% polysorbate 85 weight to weight (w/w). In other embodiments, the final concentration of polysorbate 85 in the formulation is 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09% or 0.1% polysorbate 85 (w/w). In another embodiment, the final concentration of the polysorbate 85 in the formulation is 1% polysorbate 85 (w/w).

In certain embodiments, the immunogenic composition of the disclosure has a pH of 5.5 to 7.5, more preferably a pH of 5.6 to 7.0, even more preferably a pH of 5.8 to 6.0.

In one embodiment, the present disclosure provides a container filled with any of the immunogenic compositions disclosed herein. In one embodiment, the container is selected from the group consisting of a vial, a syringe, a flask, a fermentor, a bioreactor, a bag, a jar, an ampoule, a cartridge and a disposable pen. In certain embodiments, the container is siliconized.

In an embodiment, the container of the present disclosure is made of glass, metals (e.g., steel, stainless steel, aluminum, etc.) and/or polymers (e.g., thermoplastics, elastomers, thermoplastic-elastomers). In an embodiment, the container of the present disclosure is made of glass.

In one embodiment, the present disclosure provides a syringe filled with any of the immunogenic compositions disclosed herein. In certain embodiments, the syringe is siliconized and/or is made of glass.

A typical dose of the immunogenic composition of the invention for injection has a volume of 0.1 mL to 2 mL. In an embodiment, the immunogenic composition of the invention for injection has a volume of 0.2 mL to 1 mL, even more preferably a volume of about 0.5 mL.

4 Uses of the glycoconjugate and immunogenic compositions of the invention

The glycoconjugates disclosed herein may be use as antigens. For example, they may be part of a vaccine.

Therefore, in an embodiment, the immunogenic compositions of the invention are for use as a medicament.

In an embodiment, the immunogenic compositions of the invention are for use as a vaccine.

Therefore, in an embodiment, the immunogenic compositions described herein are for use in generating an immune response in a subject. In one aspect, the subject is a

mammal, such as a human, non-human primate, cat, sheep, pig, horse, bovine or dog. In one aspect, the subject is a human.

The immunogenic compositions described herein may be used in therapeutic or prophylactic methods for preventing, treating or ameliorating a bacterial infection, disease or condition in a subject. Thus, in one aspect, the disclosure provides a method of preventing, treating or ameliorating an infection, disease or condition associated with a bacterial infection in a subject, comprising administering to the subject an immunologically effective amount of an immunogenic composition of the disclosure.

The immunogenic composition of the present disclosure can be used to protect or treat a human susceptible to a bacterial infection, by means of administering the immunogenic composition via a systemic or mucosal route. In an embodiment, the immunogenic composition of the invention is administered by intramuscular, intraperitoneal, intradermal or subcutaneous routes. In an embodiment, the immunogenic composition of the invention is administered by intramuscular, intraperitoneal, intradermal or subcutaneous injection. In an embodiment, the immunogenic composition of the invention is administered by intramuscular or subcutaneous injection. In an embodiment, the immunogenic composition of the invention is administered by intramuscular injection. In an embodiment, the immunogenic composition of the invention is administered by subcutaneous injection.

5 Subject to be treated with the immunogenic compositions of the invention

As disclosed herein, the immunogenic compositions described herein may be used in various therapeutic or prophylactic methods for preventing, treating or ameliorating a bacterial infection, disease or condition in a subject.

In a preferred embodiment, said subject is a human. In a most preferred embodiment, said subject is a newborn (i.e., under three months of age), an infant (i.e., from 3 months to one year of age) or a toddler (i.e., from one year to four years of age). In an embodiment, the immunogenic compositions disclosed herein are for use as a vaccine.

In such embodiment, the subject to be vaccinated may be less than 1 year of age. For example, the subject to be vaccinated can be about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11 or about 12 months of age. In an embodiment, the subject to be vaccinated is about 2, about 4 or about 6 months of age. In another embodiment, the subject to be vaccinated is less than 2 years of age. For example, the subject to be vaccinated can be about 12 to about 15 months of age. In

some cases, as little as one dose of the immunogenic composition according to the invention is needed, but under some circumstances, a second, third or fourth dose may be given (see section 8 below).

In an embodiment of the present invention, the subject to be vaccinated is a human adult 50 years of age or older, more preferably a human adult 55 years of age or older. In an embodiment, the subject to be vaccinated is a human adult 65 years of age or older, 70 years of age or older, 75 years of age or older or 80 years of age or older.

In an embodiment the subject to be vaccinated is an immunocompromised individual, in particular a human. An immunocompromised individual is generally defined as a person who exhibits an attenuated or reduced ability to mount a normal humoral or cellular defense to challenge by infectious agents.

In an embodiment of the present invention, the immunocompromised subject to be vaccinated suffers from a disease or condition that impairs the immune system and results in an antibody response that is insufficient to protect against or treat pneumococcal disease.

In an embodiment, said disease is a primary immunodeficiency disorder. Preferably, said primary immunodeficiency disorder is selected from the group consisting of: combined T- and B-cell immunodeficiencies, antibody deficiencies, well-defined syndromes, immune dysregulation diseases, phagocyte disorders, innate immunity deficiencies, autoinflammatory disorders, and complement deficiencies. In an embodiment, said primary immunodeficiency disorder is selected from the one disclosed on page 24, line 11, to page 25, line 19, of WO 2010/125480.

In a particular embodiment of the present invention, the immunocompromised subject to be vaccinated suffers from a disease selected from the groups consisting of: HIV-infection, acquired immunodeficiency syndrome (AIDS), cancer, chronic heart or lung disorders, congestive heart failure, diabetes mellitus, chronic liver disease, alcoholism, cirrhosis, spinal fluid leaks, cardiomyopathy, chronic bronchitis, emphysema, chronic obstructive pulmonary disease (COPD), spleen dysfunction (such as sickle cell disease), lack of spleen function (asplenia), blood malignancy, leukemia, multiple myeloma, Hodgkin's disease, lymphoma, kidney failure, nephrotic syndrome and asthma.

In an embodiment of the present invention, the immunocompromised subject to be vaccinated suffers from malnutrition.

In a particular embodiment of the present invention, the immunocompromised subject to be vaccinated is taking a drug or treatment that lowers the body's resistance to

infection. In an embodiment, said drug is selected from the one disclosed on page 26, line 33, to page 26, line 4, of WO 2010/125480.

In a particular embodiment of the present invention, the immunocompromised subject to be vaccinated is a smoker.

In a particular embodiment of the present invention, the immunocompromised subject to be vaccinated has a white blood cell count (leukocyte count) below 5×10^9 cells per liter, or below 4×10^9 cells per liter, or below 3×10^9 cells per liter, or below 2×10^9 cells per liter, or below 1×10^9 cells per liter, or below 0.5×10^9 cells per liter, or below 0.3×10^9 cells per liter, or below 0.1×10^9 cells per liter.

White blood cell count (leukocyte count): The number of white blood cells (WBC) in the blood. The WBC is usually measured as part of the CBC (complete blood count). White blood cells are the infection-fighting cells in the blood and are distinct from the red (oxygen-carrying) blood cells known as erythrocytes. There are different types of white blood cells, including neutrophils (polymorphonuclear leukocytes; PMN), band cells (slightly immature neutrophils), T-type lymphocytes (T-cells), B-type lymphocytes (B-cells), monocytes, eosinophils, and basophils. All the types of white blood cells are reflected in the white blood cell count. The normal range for the white blood cell count is usually between 4,300 and 10,800 cells per cubic millimeter of blood. This can also be referred to as the leukocyte count and can be expressed in international units as $4.3 - 10.8 \times 10^9$ cells per liter.

In a particular embodiment of the present invention, the immunocompromised subject to be vaccinated suffers from neutropenia. In a particular embodiment of the present invention, the immunocompromised subject to be vaccinated has a neutrophil count below 2×10^9 cells per liter, or below 1×10^9 cells per liter, or below 0.5×10^9 cells per liter, or below 0.1×10^9 cells per liter, or below 0.05×10^9 cells per liter.

A low white blood cell count or "neutropenia" is a condition characterized by abnormally low levels of neutrophils in the circulating blood. Neutrophils are a specific kind of white blood cell that help to prevent and fight infections. The most common reason that cancer patients experience neutropenia is as a side effect of chemotherapy. Chemotherapy-induced neutropenia increases a patient's risk of infection and disrupts cancer treatment.

In a particular embodiment of the present invention, the immunocompromised subject to be vaccinated has a CD4+ cell count below $500/\text{mm}^3$, or CD4+ cell count below $300/\text{mm}^3$, or CD4+ cell count below $200/\text{mm}^3$, CD4+ cell count below $100/\text{mm}^3$, CD4+ cell count below $75/\text{mm}^3$, or CD4+ cell count below $50/\text{mm}^3$.

CD4 cell tests are normally reported as the number of cells in mm³. Normal CD4 counts are between 500 and 1,600, and CD8 counts are between 375 and 1,100. CD4 counts drop dramatically in people with HIV.

In an embodiment of the invention, any of the immunocompromised subjects disclosed herein is a human male. In an embodiment of the invention, any of the immunocompromised subjects disclosed herein is a human female.

6 Regimen

In some cases, as little as one dose of the immunogenic composition according to the invention is needed, but under some circumstances, such as conditions of greater immune deficiency, a second, third or fourth dose may be given. Following an initial vaccination, subjects can receive one or several booster immunizations adequately spaced.

In an embodiment, the schedule of vaccination of the immunogenic composition according to the invention is a single dose. In a particular embodiment, said single dose schedule is for healthy persons being at least 2 years of age.

In an embodiment, the schedule of vaccination of the immunogenic composition according to the invention is a multiple dose schedule. In a particular embodiment, said multiple dose schedule consists of a series of 2 doses separated by an interval of about 1 month to about 2 months. In a particular embodiment, said multiple dose schedule consists of a series of 2 doses separated by an interval of about 1 month, or a series of 2 doses separated by an interval of about 2 months.

In another embodiment, said multiple dose schedule consists of a series of 3 doses separated by an interval of about 1 month to about 2 months. In another embodiment, said multiple dose schedule consists of a series of 3 doses separated by an interval of about 1 month, or a series of 3 doses separated by an interval of about 2 months.

In another embodiment, said multiple dose schedule consists of a series of 3 doses separated by an interval of about 1 month to about 2 months followed by a fourth dose about 10 months to about 13 months after the first dose. In another embodiment, said multiple dose schedule consists of a series of 3 doses separated by an interval of about 1 month followed by a fourth dose about 10 months to about 13 months after the first dose, or a series of 3 doses separated by an interval of about 2 months followed by a fourth dose about 10 months to about 13 months after the first dose.

In an embodiment, the multiple dose schedule consists of at least one dose (e.g., 1, 2 or 3 doses) in the first year of age followed by at least one toddler dose.

In an embodiment, the multiple dose schedule consists of a series of 2 or 3 doses separated by an interval of about 1 month to about 2 months (for example 28-56 days between doses), starting at 2 months of age, and followed by a toddler dose at 12-18 months of age. In an embodiment, said multiple dose schedule consists of a series of 3 doses separated by an interval of about 1 month to about 2 months (for example 28-56 days between doses), starting at 2 months of age, and followed by a toddler dose at 12-15 months of age. In another embodiment, said multiple dose schedule consists of a series of 2 doses separated by an interval of about 2 months, starting at 2 months of age, and followed by a toddler dose at 12-18 months of age.

In an embodiment, the multiple dose schedule consists of a 4-dose series of vaccine at 2, 4, 6, and 12-15 months of age.

In an embodiment, a prime dose is given at day 0 and one or more boosts are given at intervals that range from about 2 to about 24 weeks, preferably with a dosing interval of 4-8 weeks.

In an embodiment, a prime dose is given at day 0 and a boost is given about 3 months later.

7. The invention also provides the following embodiments as defined in the following numbered paragraphs 1 to 96

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

(a) reacting an isolated capsular saccharide with a carbonic acid derivative and an azido linker in an aprotic solvent to produce an activated azido saccharide,

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

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

2. The method of paragraph 1 wherein, the isolated saccharide is sized before the activation step (a).

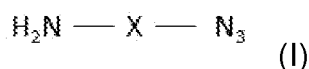
3. The method of paragraph 2 wherein, the isolated capsular saccharide is sized to a weight average molecular weight between 200 kDa and 800 kDa.

4. The method of any one of paragraphs 1-3 wherein, said carbonic acid derivative is selected from the group consisting of 1,1'-carbonyldiimidazole (CDI), 1,1'-carbonyl-di-(1,2,4-triazole) (CDT), disuccinimidyl carbonate (DSC) and N-hydroxysuccinimidyl chloroformate.

5. The method of any one of paragraphs 1-3 wherein, said carbonic acid derivative is 1,1'-carbonyldiimidazole (CDI).

6. The method of any one of paragraphs 1-3 wherein, said carbonic acid derivative is 1,1'-Carbonyl-di-(1,2,4-triazole) (CDT).

7. The method of any one of paragraphs 1-6 wherein said azido linker is a compound of formula (I),



wherein X is selected from the group consisting of $\text{CH}_2(\text{CH}_2)_n$, $(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$, $\text{NHCO}(\text{CH}_2)_n$, $\text{NHCO}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$, $\text{OCH}_2(\text{CH}_2)_n$ and $\text{O}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$; where n is selected from 1 to 10 and m is selected from 1 to 4.

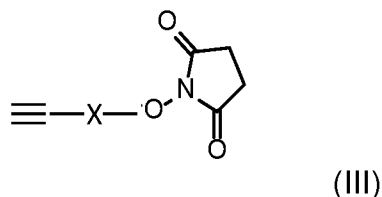
8. The method of any one of paragraphs 1-6 wherein said azido linker is a compound of formula (II),



9. The method of any one of paragraphs 1-8 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.

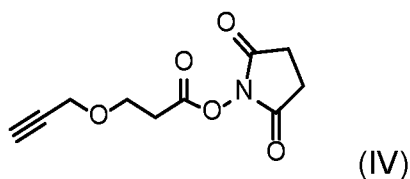
10. The method of any one of paragraphs 1-8 wherein, said agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group is an agent bearing an N-Hydroxysuccinimide (NHS) moiety and a cycloalkyne.

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



where X is selected from the group consisting of $\text{CH}_2\text{O}(\text{CH}_2)_n\text{CH}_2\text{C}=\text{O}$ and $\text{CH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_m(\text{CH}_2)_n\text{CH}_2\text{C}=\text{O}$, where n is selected from 0 to 10 and m is selected from 0 to 4.

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



13. The method of any one of paragraphs 1-12 wherein, step a) comprises reacting the capsular saccharide with a carbonic acid derivative followed by reacting the carbonic acid derivative-activated capsular saccharide with an azido linker in an aprotic solvent to produce an activated azido capsular saccharide.

14. The method of any one of paragraphs 1-13 wherein, at step a) the isolated capsular saccharide is reacted with said carbonic acid derivative in an aprotic solvent.

15. The method of any one of paragraphs 1-13 wherein, at step a) the isolated capsular saccharide is reacted with a carbonic acid derivative in a solution consisting essentially of dimethylsulphoxide (DMSO).

16. The method of any one of paragraphs 1-14 wherein, at step a) the isolated capsular saccharide is reacted with CDI in an aprotic solvent comprising 0.1% to 1% (v/v) water.

17. The method of any one of paragraphs 1-14 wherein, at step a) the isolated capsular saccharide is reacted with CDI in DMSO comprising 0.1% to 1% (v/v) water.

18. The method of any one of paragraphs 1-17 wherein at step a) carbonic acid derivative activation is followed by the addition of water.

19. The method of paragraph 18 wherein water is added to bring the total water content in the mixture to between about 1% to about 10% (v/v).

20. The method of any one of paragraphs 1-19 wherein step a) further comprises reacting the carbonic acid derivative-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 activated saccharide.

21. The method of any on of paragraphs 1-20 wherien the degree of activation of the activated saccharide following step a) is between 0.5 to 50%.

22. The method of any one of paragraphs 1-21 wherein step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group that is 0.1-10 molar equivalents to the lysines on the carrier.

23. The method of any one of paragraphs 1-22 wherein the degree of activation of the activated carrier following step b) is between 1 and 50.

24. The method of any one of paragraphs 1-23 wherein the conjugation reaction c) is carried out in aqueous buffer in the presence of copper (I) as catalyst.

5 25. The method of any one of paragraphs 1-23 wherein the conjugation reaction c) is carried out in aqueous buffer in the presence of an oxidant and of copper (I) as catalyst.

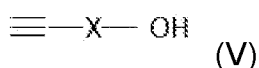
26. The method of any one of paragraphs 1-23 wherein the conjugation reaction c) is carried out in aqueous buffer in the presence of copper (I) as catalyst and ascorbate as oxidant, wherein the reaction mixture further comprises THPTA (tris(3-

10 hydroxypropyltriazolylmethyl)amine) and aminoguanidine.

27. The method of any one of paragraphs 1-26 wherein the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at setp c) is between 0.1 and 3.

15 28. The method of any one of paragraphs 1-27 wherein following step c), the method further comprises a step of capping the unreacted azido groups remained in the conjugate with an azido group capping agent.

29. The method of paragraph 28 wherein, said azido group capping agent is a compound of formula (V),



20 wherein X is (CH₂)_n wherein n is selected from 1 to 15.

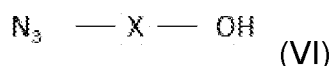
30. The method of paragraph 28 wherein, said azido group capping agent is propargyl alcohol.

25 31. The method of any one of paragraphs 28-30 wherein the capping of the unreacted azido groups is performed with an amount of capping agent that is between 0.05 to 20 molar equivalents to the amount of saccharide repeat unit of the activated saccharide.

32. The method of any one of paragraphs 1-31 wherein following step c), the method further comprises a step of capping the unreacted alkyne groups remained in the conjugate with an alkyne group capping agent.

30 33. The method of paragraph 32 wherein said alkyne group capping agent is an agent bearing an azido group.

34. The method of paragraph 33 wherein said alkyne group capping agent is a compound of formula (VI),



wherein X is $(\text{CH}_2)_n$ wherein n is selected from 1 to 15.

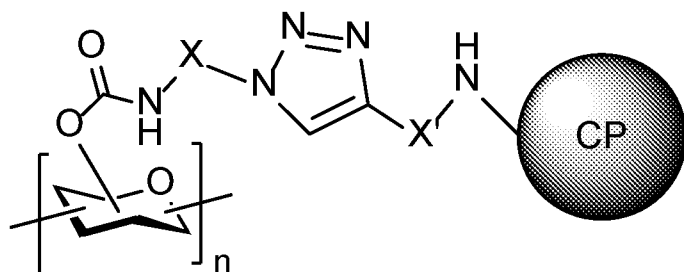
35. The method of paragraph 32 wherein said alkyne group capping agent is 3-azido-1-propanol.

36. The method of any one of paragraphs 32-35 wherein the capping of the unreacted alkyne groups is performed with an amount of capping agent that is between 0.05 to 20 molar equivalents to the amount of saccharide repeat unit of the activated saccharide.

37. The method of any one of paragraphs 1-36 wherein the method further comprises the step of purifying the glycoconjugate after it is produced.

38. A capsular saccharide glycoconjugate produced according to any one of the methods of paragraphs 1 to 37.

39. A capsular saccharide glycoconjugate comprising a capsular saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (VII):



(VII),

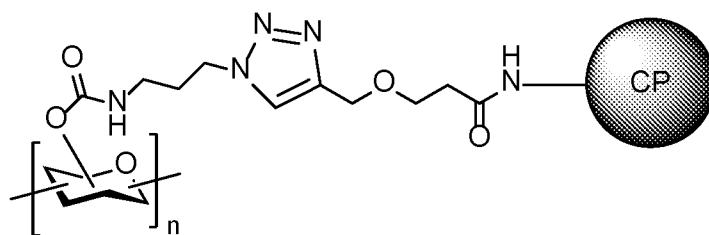
wherein X is selected from the group consisting of $\text{CH}_2(\text{CH}_2)_{n'}$, $(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$, $\text{NHCO}(\text{CH}_2)_{n'}$, $\text{NHCO}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$, $\text{OCH}_2(\text{CH}_2)_{n'}$ and $\text{O}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$;

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 $\text{CH}_2\text{O}(\text{CH}_2)_{n''}\text{CH}_2\text{C}=\text{O}$, $\text{CH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_{m'}(\text{CH}_2)_{n''}\text{CH}_2\text{C}=\text{O}$, where n'' is selected from 0 to 10 and m' is selected from 0 to 4.

40. A capsular saccharide glycoconjugate comprising a capsular saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (VII), wherein X is $\text{CH}_2(\text{CH}_2)_{n'}$, where n' is 2 and wherein X' is $\text{CH}_2\text{O}(\text{CH}_2)_{n''}\text{CH}_2\text{C}=\text{O}$ where n'' is 1.

41. A capsular saccharide glycoconjugate comprising a capsular saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (VIII),



(VIII).

- 5 42. The capsular saccharide glycoconjugate of any one of paragraphs 38 to 41 comprising a capsular saccharide wherein the weight average molecular weight (Mw) of said capsular saccharide before conjugation is between 10 kDa and 2,000 kDa.
43. The capsular saccharide glycoconjugate of any one of paragraphs 38 to 41 comprising a capsular saccharide wherein the weight average molecular weight (Mw) of
10 said capsular saccharide before conjugation is between 50 kDa and 1,000 kDa.
44. The capsular saccharide glycoconjugate of any one of paragraphs 38 to 41 comprising a capsular saccharide wherein the weight average molecular weight (Mw) of said capsular saccharide before conjugation is between 200 kDa and 750 kDa.
45. The capsular saccharide glycoconjugate of any one of paragraphs 38 to 44 having a
15 weight average molecular weight (Mw) of between 250 kDa and 20,000 kDa.
46. The capsular saccharide glycoconjugate of any one of paragraphs 38 to 44 having a weight average molecular weight (Mw) of between 500 kDa and 5,000 kDa.
47. The capsular saccharide glycoconjugate of any one of paragraphs 38 to 44 having a weight average molecular weight (Mw) of between 750 kDa and 2,500 kDa.
- 20 48. The capsular saccharide glycoconjugate of any one of paragraphs 38 to 47 wherein, the degree of conjugation of the capsular saccharide glycoconjugate is between 2 and 15.
49. The capsular saccharide glycoconjugate of any one of paragraphs 38 to 48 wherein the ratio of capsular saccharide to carrier protein in the glycoconjugate (w/w) is between
25 0.5 and 3.0.
50. The capsular saccharide glycoconjugate of any one of paragraphs 38 to 49 wherein the said capsular saccharide glycoconjugate comprises at least one covalent linkage

between the carrier protein and the saccharide for every 4 saccharide repeat units of the saccharide.

51. The capsular saccharide glycoconjugate of any one of paragraphs 38 to 49 wherein the said capsular saccharide glycoconjugate comprises at least one covalent linkage between the carrier protein and the saccharide for every 25 saccharide repeat units of the saccharide.

52. The capsular saccharide glycoconjugate of any one of paragraphs 38 to 49 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.

53. The capsular saccharide glycoconjugate of any one of paragraphs 38 to 56 wherein said carrier protein is CRM₁₉₇.

54. The capsular saccharide glycoconjugate of any one of paragraphs 38 to 53 wherein said carrier protein is SCP.

55. The capsular saccharide glycoconjugate of any one of paragraphs 38 to 53 wherein said carrier protein is an enzymatically inactive SCP.

56. The capsular saccharide glycoconjugate of any one of paragraphs 38 to 53 wherein said carrier protein is an enzymatically inactive SCP from GBS (SCP_B).

57. The capsular saccharide glycoconjugate of any one of paragraphs 38 to 53 wherein said carrier protein is a fragment of an SCP_B.

58. The capsular saccharide glycoconjugate of any one of paragraphs 38 to 53 wherein said carrier protein is a fragment of an SCP which comprises the protease domain, the protease-associated domain (PA domain) and the three fibronectin type III (Fn) domains but does not comprise the export signal presequence, the pro-sequence and the cell wall anchor domain.

59. The capsular saccharide glycoconjugate of any one of paragraphs 38 to 53 wherein said carrier protein is an enzymatically inactive fragment of an SCP, wherein said enzymatically inactive fragment of SCP comprises the protease domain, the protease-associated domain (PA domain) and the three fibronectin type III (Fn) domains but does not comprise the export signal presequence, the pro-sequence and the cell wall anchor domain.

60. The capsular saccharide glycoconjugate of any one of paragraphs 38 to 53 wherein said carrier protein is an enzymatically inactive fragment of SCP which comprises the protease domain, the protease-associated domain (PA domain) and the three fibronectin type III (Fn) domains but does not comprise the export signal presequence, the pro-
5 sequence and the cell wall anchor domain, where said inactivation is accomplished by replacing at least one amino acid of the wild type sequence and wherein said replacement is selected from the group consisting of D130A, H193A, N295A and S512A where the numbers indicate the amino acid residue position in the peptidase according to the numbering of SEQ ID NO: 1 of WO00/34487.
- 10 61. The capsular saccharide glycoconjugate of any one of paragraphs 38 to 53 wherein said carrier protein is an enzymatically inactive fragment of SCP which comprises the protease domain, the protease-associated domain (PA domain) and the three fibronectin type III (Fn) domains but does not comprise the export signal presequence, the pro-
15 sequence and the cell wall anchor domain, where said inactivation is accomplished by replacing at least two amino acids of the wild type sequence wherein said at least two amino acids replacements are D130A and S512A where the numbers indicate the amino acid residue position in the peptidase according to the numbering of SEQ ID NO: 1 of WO00/34487..
- 20 62. The capsular saccharide glycoconjugate of any one of paragraphs 38 to 53 wherein said carrier protein is an enzymatically inactive fragment of SCP consisting of a polypeptide having at least 95% identity with SEQ ID NO: 41.
63. The capsular saccharide glycoconjugate of any one of paragraphs 38 to 53 wherein said carrier protein is an enzymatically inactive fragment of SCP consisting of a polypeptide having at least 95% identity with SEQ ID NO: 42.
- 25 64. The capsular saccharide glycoconjugate of any one of paragraphs 38 to 53 wherein said carrier protein is an enzymatically inactive fragment of SCP which consists of SEQ ID NO: 41.
- 30 65. The capsular saccharide glycoconjugate of any one of paragraphs 38 to 53 wherein said carrier protein is an enzymatically inactive fragment of SCP which consists of SEQ ID NO: 42.
66. The capsular saccharide glycoconjugate of any one of paragraphs 38 to 53 wherein said carrier protein is DT (Diphtheria toxoid).

67. The capsular saccharide glycoconjugate of any one of paragraphs 38 to 53 wherein said carrier protein is TT (tetanus toxoid).

68. The capsular saccharide glycoconjugate of any one of paragraphs 38 to 53 wherein said carrier protein is PD (*H. influenzae* protein D).

5 69. The method of making a capsular saccharide glycoconjugate of any one of paragraphs 1 to 37 or the capsular saccharide glycoconjugate of any one of paragraphs 38 to 68, wherein said capsular saccharide is a capsular saccharide from a pathogenic bacteria.

10 70. The method of making a capsular saccharide glycoconjugate of any one of paragraphs 1 to 37 or the capsular saccharide glycoconjugate of any one of paragraphs 38 to 68, wherein said capsular saccharide is a capsular saccharide from a pathogenic *Streptococcus*, a pathogenic *Staphylococcus*, a pathogenic *Enterococcus*, a pathogenic *Bacillus*, a pathogenic *Corynebacterium*, a pathogenic *Listeria*, a pathogenic *Erysipelothrix*, a pathogenic *Clostridium*, a pathogenic *Haemophilus*, a pathogenic
15 *Neisseria* or a pathogenic *Escherichia*.

71. The method of making a capsular saccharide glycoconjugate of any one of paragraphs 1 to 37 or the capsular saccharide glycoconjugate of any one of paragraphs 38 to 68, wherein said capsular saccharide is a capsular saccharide from *Enterococcus faecalis*, *Escherichia coli*, *Staphylococcus aureus* or *Streptococcus*.

20 72. The method of making a capsular saccharide glycoconjugate of any one of paragraphs 1 to 37 or the capsular saccharide glycoconjugate of any one of paragraphs 38 to 68, wherein said capsular saccharide is a capsular saccharide from *Haemophilus influenzae*, *Neisseria meningitidis*, *S. pneumoniae*, *S. pyogenes*, *S. agalactiae*, Group C & G *Streptococci* or *Escherichia coli*.

25 73. The method of making a capsular saccharide glycoconjugate of any one of paragraphs 1 to 37 or the capsular saccharide glycoconjugate of any one of paragraphs 38 to 68, wherein said capsular saccharide is a capsular saccharide from *Neisseria meningitidis*, *S. pneumoniae*, *S. agalactiae* or *Escherichia coli*.

30 74. The method of making a capsular saccharide glycoconjugate of any one of paragraphs 1 to 37 or the capsular saccharide glycoconjugate of any one of paragraphs 38 to 68, wherein said capsular saccharide is a capsular saccharide from *S. pneumoniae* or *S. agalactiae*.

75. The method of making a capsular saccharide glycoconjugate of any one of paragraphs 1 to 37 or the capsular saccharide glycoconjugate of any one of paragraphs 38 to 68, wherein said capsular saccharide is a capsular saccharide from *S. pneumoniae*.

76. The method of making a capsular saccharide glycoconjugate of any one of paragraphs 1 to 37 or the capsular saccharide glycoconjugate of any one of paragraphs 38 to 68, wherein said capsular saccharide is a capsular saccharide from *Staphylococcus aureus*.

77. The method of making a capsular saccharide glycoconjugate of any one of paragraphs 1 to 37 or the capsular saccharide glycoconjugate of any one of paragraphs 38 to 68, wherein said capsular saccharide is a capsular saccharide from *Enterococcus faecalis*.

78. The method of making a capsular saccharide glycoconjugate of any one of paragraphs 1 to 37 or the capsular saccharide glycoconjugate of any one of paragraphs 38 to 68, wherein said capsular saccharide is a capsular saccharide from *Haemophilus influenzae* type b.

79. The method of making a capsular saccharide glycoconjugate of any one of paragraphs 1 to 37 or the capsular saccharide glycoconjugate of any one of paragraphs 38 to 68, wherein said capsular saccharide is a capsular saccharide from *Neisseria meningitidis*.

80. The method of making a capsular saccharide glycoconjugate of any one of paragraphs 1 to 37 or the capsular saccharide glycoconjugate of any one of paragraphs 38 to 68, wherein said capsular saccharide is a capsular saccharide from *Escherichia coli*.

81. The method of making a capsular saccharide glycoconjugate of any one of paragraphs 1 to 37 or the capsular saccharide glycoconjugate of any one of paragraphs 38 to 68, wherein said capsular saccharide is a capsular saccharide from *Enterococcus faecalis*.

82. The method of making a capsular saccharide glycoconjugate of any one of paragraphs 1 to 37 or the capsular saccharide glycoconjugate of any one of paragraphs 38 to 68, wherein said capsular saccharide is a capsular saccharide from *Streptococcus agalactiae* (Group B streptococcus (GBS)).

83. The method of making a capsular saccharide glycoconjugate of any one of paragraphs 1 to 37 or the capsular saccharide glycoconjugate of any one of paragraphs

38 to 68, wherein said capsular saccharide is a capsular saccharide from GBS type Ia, Ib, II, III, IV, V, VI, VII or VIII.

84. The method of making a capsular saccharide glycoconjugate of any one of paragraphs 1 to 37 or the capsular saccharide glycoconjugate of any one of paragraphs 38 to 68, wherein said capsular saccharide is a capsular saccharide from a *Streptococcus pneumoniae* serotype selected from the group consisting of serotypes 1, 2, 4, 5, 6A, 6B, 6C, 7C, 7F, 8, 9V, 9N, 10A, 10B, 11A, 12F, 14, 15A, 15B, 15C, 16F, 17F, 18C, 19A, 19F, 20, 21, 22A, 22F, 23A, 23B, 23F, 24B, 24F, 27, 29, 31, 33B, 33F, 34, 35B, 35F, 38, 72 and 73.

85. The method of making a capsular saccharide glycoconjugate of any one of paragraphs 1 to 37 or the capsular saccharide glycoconjugate of any one of paragraphs 38 to 68, wherein said capsular saccharide is a capsular saccharide from *Streptococcus pneumoniae* serotype 35B.

86. The method of making a capsular saccharide glycoconjugate of any one of paragraphs 1 to 37 or the capsular saccharide glycoconjugate of any one of paragraphs 38 to 68, wherein said capsular saccharide is a capsular saccharide from *Streptococcus pneumoniae* serotype 29.

87. The method of making a capsular saccharide glycoconjugate of any one of paragraphs 1 to 37 or the capsular saccharide glycoconjugate of any one of paragraphs 38 to 68, wherein said capsular saccharide is a capsular saccharide from *Streptococcus pneumoniae* serotype 15A.

88. The method of making a capsular saccharide glycoconjugate of any one of paragraphs 1 to 37 or the capsular saccharide glycoconjugate of any one of paragraphs 38 to 68, wherein said capsular saccharide is a capsular saccharide from *Streptococcus pneumoniae* serotype 24F.

89. The method of making a capsular saccharide glycoconjugate of any one of paragraphs 1 to 37 or the capsular saccharide glycoconjugate of any one of paragraphs 38 to 68, wherein said capsular saccharide is a capsular saccharide from *Streptococcus pneumoniae* serotype 23A.

90. The method of making a capsular saccharide glycoconjugate of any one of paragraphs 1 to 37 or the capsular saccharide glycoconjugate of any one of paragraphs 38 to 68, wherein said capsular saccharide is a capsular saccharide from *Streptococcus pneumoniae* serotype 23B.

91. The method of making a capsular saccharide glycoconjugate of any one of paragraphs 1 to 37 or the capsular saccharide glycoconjugate of any one of paragraphs 38 to 68, wherein said capsular saccharide is a capsular saccharide from *Streptococcus pneumoniae* serotype 6B.

5 92. The method of making a capsular saccharide glycoconjugate of any one of paragraphs 1 to 37 or the capsular saccharide glycoconjugate of any one of paragraphs 38 to 68, wherein said capsular saccharide is a capsular saccharide from *Streptococcus pneumoniae* serotype 19A.

10 93. The method of making a capsular saccharide glycoconjugate of any one of paragraphs 1 to 37 or the capsular saccharide glycoconjugate of any one of paragraphs 38 to 68, wherein said capsular saccharide is a capsular saccharide from *Streptococcus pneumoniae* serotype 19F.

15 94. The method of making a capsular saccharide glycoconjugate of any one of paragraphs 1 to 37 or the capsular saccharide glycoconjugate of any one of paragraphs 38 to 68, wherein said capsular saccharide is a capsular saccharide from *Streptococcus pneumoniae* serotype 23F.

95. The method of making a capsular saccharide glycoconjugate or the capsular saccharide glycoconjugate of any one of paragraphs 1 to 94, wherein said capsular saccharide is not a capsular saccharide from *Streptococcus pneumoniae* serotype 3.

20 96. An immunogenic composition comprising a capsular saccharide glycoconjugate of any one of paragraphs 38 to 95.

As used herein, the term "about" means within a statistically meaningful range of a value, such as a stated concentration range, time frame, molecular weight, temperature or pH.

25 Such a range can be within an order of magnitude, typically within 20%, more typically within 10%, and even more typically within 5% or within 1% of a given value or range. Sometimes, such a range can be within the experimental error typical of standard methods used for the measurement and/or determination of a given value or range. The allowable variation encompassed by the term "about" will depend upon the particular
30 system under study, and can be readily appreciated by one of ordinary skill in the art. Whenever a range is recited within this application, every number within the range is also contemplated as an embodiment of the disclosure.

The terms "comprising", "comprise" and "comprises" herein are intended by the inventors to be optionally substitutable with the terms "consisting essentially of", "consist essentially

of", "consists essentially of", "consisting of", "consist of" and "consists of", respectively, in every instance.

An "immunogenic amount", an "immunologically effective amount", a "therapeutically effective amount", a "prophylactically effective amount", or "dose", each of which is used interchangeably herein, generally refers to the amount of antigen or immunogenic composition sufficient to elicit an immune response, either a cellular (T cell) or humoral (B cell or antibody) response, or both, as measured by standard assays known to one skilled in the art.

Any whole number integer within any of the ranges of the present document is contemplated as an embodiment of the disclosure.

All references or patent applications cited within this patent specification are incorporated by reference herein.

The invention is illustrated in the accompanying examples. The examples below are carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. The examples are illustrative, but do not limit the invention.

EXAMPLE

Example 1: Conjugation of *S. pneumoniae* serotype 35B Capsular Polysaccharide using click chemistry (see Figure 3)

1. Activation of Serotype 35B Capsular Polysaccharide with azido linker

Serotype 35B capsular polysaccharide (200 mg) was mixed with imidazole (600 mg) and then frozen and lyophilized.

After 3 days lyophilization, the lyophilized polysaccharide was reconstituted with anhydrous DMSO (100 mL). The reaction mixture was then warmed to 45°C, and CDI (100 mg/mL in DMSO), 669 µL (2 MEq) was added. The reaction mixtures were stirred at 45°C for 2 hrs. After the reaction mixtures were cooled to 23°C, WFI 2 mL (2% v/v) was added to quench free CDI and stirred for 30 min at 23°C. Then, 3-Azido-1-propylamine 41 µL (2 MEq) was added and then stirred at 23°C for 20 hrs. After 20 hrs reaction, the reaction mixture was diluted to chilled (at 5°C) with 400 mL 10 mM Sodium Phosphate Buffer (SPB) in saline (pH 7) (5X, v/v). The diluted reaction mixture was then purified by UF/DF using 10K MWCO PES membrane (Millipore Pellicon 2 Mini) against 10 mM SPB in saline (pH 7) (30X, v/v).

2. Activation of CRM₁₉₇ and SCP to alkyne-CRM₁₉₇ with Alkyne NHS esterActivation of CRM₁₉₇:

To the CRM₁₉₇ solution (1000 mg) WFI 57 mL and 0.5 M sodium phosphate buffer (pH 8.3) 50 mL were added. After cooled to 8 °C, 3-Propargyloxy-propanoic acid NHS ester (POPS) (20 mg/mL in DMSO) 3.8 mL (0.5 MEq to lysine on CRM₁₉₇) was added to the reaction mixture dropwise maintaining the reaction temperature at 8 ± 3 °C. After the reaction mixture was stirred for 2 hrs at 8 °C, purified by UF/DF using 10K MWCO PES membrane (Millipore Pellicon 2 Mini) against 100 mM sodium phosphate buffer in saline (pH 7.0) (30X diavolume). After UF/DF, sucrose 23 g (15% v/v) was added.

10 Activation of SCP:

Activation of SCP was similar to activation of CRM₁₉₇ except that the amount of linker was adapted. The final amount of linker was 0.5 MEq to the SCP (0.092 w/w of linker per SCP).

Click Conjugation:

15 Activated azido poly and alkyne CRM₁₉₇ or SCP is conjugated by Cu⁺¹ mediated azide-alkyne cycloaddition reaction, referred as "Click Reaction"

The mixture of 5 mM copper sulfate (CuSO₄) (1 mL) and 25 mM Tris(3-hydroxypropyltriazolylmethyl)amine (THPTA) (1 mL) were added to the mixture of Serotype 35B Capsular Polysaccharide activated with azido linker (see step 1 above) and alkyne-CRM₁₉₇ or alkyne-SCP (see step 2 above) (in 100 mM Sodium Phosphate Buffer (SPB) in saline, pH 7.0) at 23 °C and followed by the addition of 100 mM aminoguanidine (2 mL) and 100 mM sodium ascorbate (2 mL). After the reaction mixture was stirred for 2 hours at 23 °C, the unreacted azido group was capped by propargyl alcohol (1 MEq) for 2 hours at 23 °C and after the first capping, subsequently the unreacted alkyne group was capped by 3-azido-1-propanol (2 MEq) for 2 hours at 23 °C. Then, the reaction mixture was purified by UF/DF using 100K MWCO PES membrane against 10 mM EDTA + 10 mM SPB in saline (pH 7.0) (30X diavolume) and then followed by 5 mM succinate in saline (pH 6.0) (30X diavolume).

Table 1. Attributes of Pn35B Conjugates obtained using click chemistry

Conjugate #	1	2	3
Polysaccharide			
Polysaccharide MW, kDa	816	252	252
Activated polysaccharide attributes			
Degree of Activation (DoA)	~18	~20	~26
Activated Polysaccharide MW, kDa	898	326	306

Activated carrier attributes			
Degree of Activation (DoA)	15	17	~26
Activated carrier MW, kDa	58	58	101
Conjugate attributes			
Carrier protein	CRM ₁₉₇	CRM ₁₉₇	SCP
Yield (%)	56	79	73
SPR Ratio	1.2	0.9	0.8
Free Saccharide, %	6	<5	<5
Conjugate MW, kDa	1729	1093	939

MW: molecular weight; SPR: Saccharide to protein ratio

The click chemistry allows for generating 35B conjugates with very low free saccharide and a good yield. Furthermore, it allows for obtaining 35B conjugates comprising polysaccharides close to the native polysaccharides (no size reduction is observed during the activation step contrary to activation with periodate, see WO 2020/247299).

Example 2: Conjugation of *S. pneumoniae* serotype 29 Capsular Polysaccharide using click chemistry (see Figure 3)

1. Activation of Serotype 29 Capsular Polysaccharide with azido linker

Serotype 29 capsular polysaccharide (200 mg) was mixed with imidazole (600 mg) and then frozen and lyophilized.

After 3 days lyophilization, the lyophilized polysaccharide was reconstituted with anhydrous DMSO (100 mL). The reaction mixture was then warmed to 45°C, and CDI (100 mg/mL in DMSO), 1050 µL (3 MEq) was added. The reaction mixture was stirred (300 rpm) at 45°C for 3 hrs. After the reaction mixture was cooled to 23°C, WFI 2 mL (2% v/v) was added to quench free CDI and stirred for 30 min at 23°C. Then, 3-Azido-1-propylamine 42 µL (2 MEq) was added and followed by triethylamine 60 µL and then stirred at 23°C for 20 hrs. After 20 hrs reaction, the reaction mixture was diluted to chilled (at 5°C) with 400 mL 10 mM Sodium Phosphate Buffer (SPB) in saline (pH 7) (5X, v/v).

The diluted reaction mixture was then purified by UF/DF using 10K MWCO PES membrane (Millipore Pellicon 2 Mini) against 10 mM SPB in saline (pH 7) (30X, v/v).

2. Activation of CRM₁₉₇ to alkyne-CRM₁₉₇ with Alkyne NHS ester

To the CRM₁₉₇ solution (1000 mg) WFI 57 mL and 0.5 M sodium phosphate buffer (pH 8.3) 50 mL were added. After cooled to 8 °C, 3-Propargyloxy-propanoic acid NHS ester (POPS) (20 mg/mL in DMSO) 3.8 mL (0.5 MEq to lysine on CRM₁₉₇) was added to the reaction mixture dropwise maintaining the reaction temperature at 8 ± 3 °C. After the reaction mixture was stirred for 2 hrs at 8 °C, purified by UF/DF using 10K MWCO PES

membrane (Millipore Pellicon 2 Mini) against 100 mM sodium phosphate buffer in saline (pH 7.0) (30X diavolume). After UF/DF, sucrose 23 g (15% v/v) was added.

3. Click Conjugation: Activated azido poly and alkyne CRM₁₉₇ is conjugated by Cu+1 mediated azide-alkyne cycloaddition reaction, referred as "Click Reaction"

- 5 The mixture of 5 mM copper sulfate (CuSO₄) (1 mL) and 25 mM Tris(3-hydroxypropyltriazolylmethyl)amine (THPTA) (1 mL) were added to the mixture of Serotype 29 Capsular Polysaccharide activated with azido linker (see step 1 above) and alkyne-CRM₁₉₇ (see step 2 above) (in 100 mM Sodium Phosphate Buffer (SPB) in saline, pH 7.0) at 23 °C and followed by the addition of 100 mM aminoguanidine (2 mL) and 100 mM sodium ascorbate (2 mL). After the reaction mixture was stirred for 2 hours at 23 °C, the unreacted azido group was capped by propargyl alcohol (1 MEq) for 2 hours at 23 °C and after the first capping, subsequently the unreacted alkyne group was capped by 3-azido-1-propanol (2 MEq) for 2 hours at 23 °C. Then, the reaction mixture was purified by UF/DF using 100K MWCO PES membrane against 10 mM EDTA + 10 mM SPB in saline (pH 7.0) (30X diavolume) and then followed by 5 mM succinate in saline (pH 6.0) (30X diavolume).

Table 2. Attributes of Pn29 Conjugates obtained using click chemistry

Conjugate #	1
Starting polysaccharide	
Polysaccharide MW, kDa	674
Activated polysaccharide attributes	
Degree of Activation (DoA)	~9
Activated Polysaccharide MW, kDa	755
Conjugate attributes	
Carrier Protein	CRM ₁₉₇
Yield (%)	56
SPR Ratio	1.6
Free Saccharide, %	11
Conjugate MW, kDa	1165

MW: molecular weight; SPR: Saccharide to protein ratio

- 20 The click chemistry allows for generating 29 conjugates with low free saccharide and a good yield. Furthermore, it allows for obtaining serotype 29 conjugates comprising polysaccharides close to the native polysaccharides (no size reduction is observed during the activation step contrary to activation with periodarte, see WO 2020/247299).

Example 3. Immunogenicity of serotype 35B conjugate conjugated using click chemistry

The opsonophagocytic activity (OPA) titers for Serotype 35B conjugate to -CRM₁₉₇ or SCP in mice were determined under standard conditions. Click chemistry (Click) was used to generate the tested conjugates (see attributes at Table 1).

Groups of 6-8 weeks old female Swiss Webster mice were immunized (250 µL) with 0.01 µg/animal, 0.05 µg/ animal or 0.1 µg/ animal of test conjugates via the subcutaneous route on week 0. The mice were boosted with the same dose of conjugate on week 3 and then bled at week 5. Serotype-specific OPAs were performed on week 5 sera samples.

The results are presented at table 3.

Table 3 -- OPA titers following vaccination with Serotype 35B antigen conjugated with Click to -CRM₁₉₇ or SCP. Conjugated Serotype 35B was used to vaccinate mice in the presence of adjuvant. Female Swiss-Webster mice, 6-8 weeks old; Doses: 0.01; 0.05 and 0.1 µg/ animal + AlPO₄; Vaccinate: 0 and 3 wk.; exsanguinate wk. 5 Readout: OPA				
	Dose (µg/ animal)	0.01	0.05	0.1
Click-CRM ₁₉₇ (Native) Conjugate #1 of Table 1	Mean (OPA titers)	21	341	2013
	Total # of mice	45	25	46
	# of non-responders	31	5	0
	%of non-responders	69	20	0
Click-CRM ₁₉₇ (Sized) Conjugate #2 of Table 1	Mean (OPA titers)	NA	44	NA
	Total # of mice	NA	25	NA
	# of non-responders	NA	14	NA
	%of non-responders	NA	56	NA
Click-SCP Conjugate #3 of Table 1	Mean (OPA titers)	27	182	752
	Total # of mice	25	25	25
	# of non-responders	13	5	3
	%of non-responders	52	20	12

NA: 'Not Available'

The data of Table 3 indicate that all serotype 35B conjugates elicited dose dependent OPA titers in a murine immunogenicity model.

Example 4: Conjugation of *S. pneumoniae* serotypes 23A and 23B Capsular Polysaccharide using click chemistry (see Figure 3)

Serotypes 23A and 23B capsular polysaccharides have been activated with the azido linker (3-Azido-1-propylamine), using similar processes used for serotypes 35B and 29 (see Examples 1 and 2). Activation of CRM₁₉₇ and SCP to alkyne-CRM₁₉₇ or alkyne-SCP

with Alkyne NHS ester, and click conjugation of the activated azido polysaccharides 23A and 23B with alkyne CRM₁₉₇ or SCP (conjugated by Cu⁺¹ mediated azide-alkyne cycloaddition reaction), were conducted using similar processes of the ones of Examples 1 and 2.

- 5 The attributes of the conjugates which have been obtained are presented at Table 4 and 5.

Table 4. Attributes of Pn23A Conjugates obtained using click chemistry

Conjugate #	1	2
Polysaccharide		
Polysaccharide MW, kDa	196	196
Activated polysaccharide attributes		
Degree of Activation (DoA)	~10-20	~10-20
Activated Polysaccharide MW, kDa	248	248
Activated carrier attributes		
Degree of Activation (DoA)	15	26
Activated carrier MW, kDa	65	99.3
Conjugate attributes		
Carrier protein	CRM ₁₉₇	SCP
Yield (%)	57	77
SPR Ratio	1.2	1
Free Saccharide, %	<5	<5
Conjugate MW, kDa	1469	1412

MW: molecular weight; SPR: Saccharide to protein ratio

10 The click chemistry allows for generating 23A conjugates with very low free saccharide and a good yield.

Table 5. Attributes of Pn23B Conjugates obtained using click chemistry

Conjugate #	1	2
Polysaccharide		
Polysaccharide MW, kDa	157	157
Activated polysaccharide attributes		
Degree of Activation (DoA)	18	18
Activated Polysaccharide MW, kDa	165	165
Activated carrier attributes		
Degree of Activation (DoA)	17	26
Activated carrier MW, kDa	58	101
Conjugate attributes		
Carrier protein	CRM ₁₉₇	SCP
Yield (%)	58	63
SPR Ratio	0.91	0.85
Free Saccharide, %	10.6	11.5
Conjugate MW, kDa	1113	919

MW: molecular weight; SPR: Saccharide to protein ratio

The click chemistry allows for generating 23B conjugates with low % free saccharide and a good yield.

Example 5: Conjugation of *S. pneumoniae* serotype 15A Capsular Polysaccharide using click chemistry (see Figure 3)

- 5 Serotype 15A capsular polysaccharide has been activated with the azido linker (3-Azido-1-propylamine), using similar processes used for serotypes 35B and 29 (see Examples 1 and 2). Activation of CRM₁₉₇ and SCP to alkyne-CRM₁₉₇ or alkyne-SCP with Alkyne NHS ester, and click conjugation of the activated azido polysaccharide 15A with alkyne CRM₁₉₇ or SCP (conjugated by Cu⁺¹ mediated azide-alkyne cycloaddition reaction), were
10 conducted using similar processes of the ones of Examples 1 and 2.

The attributes of the conjugates which have been obtained are presented at Table 6.

Table 6. Attributes of Pn15A Conjugates obtained using click chemistry

Conjugate #	1	2
Polysaccharide		
Polysaccharide MW, kDa	175	229
Activated polysaccharide attributes		
Degree of Activation (DoA)	~15	~17
Activated Polysaccharide MW, kDa	244	320
Activated carrier attributes		
Degree of Activation (DoA)	17	~26
Activated carrier MW, kDa	58	101
Conjugate attributes		
Carrier protein	CRM ₁₉₇	SCP
Yield (%)	25	64
SPR Ratio	1.0	1.1
Free Saccharide, %	6	9
Conjugate MW, kDa	2249	802

MW: molecular weight; SPR: Saccharide to protein ratio

- 15 The click chemistry allows for generating 15A conjugates with low free saccharide and a good yield for SPC.

Example 6: Conjugation of *S. pneumoniae* serotype 24F Capsular Polysaccharide using click chemistry (see Figure 3)

- 20 Serotype 24F capsular polysaccharide has been activated with the azido linker (3-Azido-1-propylamine), using similar processes used for serotypes 35B and 29 (see Examples 1 and 2). Activation of CRM₁₉₇ and SCP to alkyne-CRM₁₉₇ or alkyne-SCP with Alkyne NHS

ester, and click conjugation of the activated azido polysaccharide 24F with alkyne CRM₁₉₇ or SCP (conjugated by Cu⁺¹ mediated azide-alkyne cycloaddition reaction), were conducted using similar processes of the ones of Examples 1 and 2.

The attributes of the conjugates which have been obtained are presented at Table 7.

5 **Table 7. Attributes of Pn24F Conjugates obtained using click chemistry**

Conjugate #	1	2
Polysaccharide		
Polysaccharide MW, kDa	194	194
Activated polysaccharide attributes		
Degree of Activation (DoA)	6~10	6~10
Activated Polysaccharide MW, kDa	282	282
Activated carrier attributes		
Degree of Activation (DoA)	15	20
Activated carrier MW, kDa	65	101
Conjugate attributes		
Carrier protein	CRM ₁₉₇	SCP
Yield (%)	52	82
SPR Ratio	1.1	0.9
Free Saccharide, %	7	<5
Conjugate MW, kDa	1195	2206

MW: molecular weight; SPR: Saccharide to protein ratio

The click chemistry allows for generating 24F conjugates with low free saccharide and a good yield.

Example 7: Conjugation of *S. pneumoniae* serotype 6B Capsular Polysaccharide using click chemistry (see Figure 3)

Serotype 6B capsular polysaccharide has been activated with the azido linker (3-Azido-1-propylamine), using similar processes used for serotypes 35B and 29 (see Examples 1 and 2). Activation of CRM₁₉₇ and SCP to alkyne-CRM₁₉₇ or alkyne-SCP with Alkyne NHS ester, and click conjugation of the activated azido polysaccharide 6B with alkyne CRM₁₉₇ or SCP (conjugated by Cu⁺¹ mediated azide-alkyne cycloaddition reaction), were conducted using similar processes of the ones of Examples 1 and 2.

The attributes of the conjugates which have been obtained are presented at Table 8.

Table 8. Attributes of 6B Conjugates obtained using click chemistry

Conjugate #	1	2
Activated polysaccharide attributes		
Degree of Activation (DoA)	20	20
Activated carrier attributes		
Degree of Activation (DoA)	15	26
Conjugate attributes		
Carrier protein	CRM ₁₉₇	SCP
SPR Ratio	1.20	1.10
Free Saccharide, %	<5	<5
Free Protein, %	<1	<1
Conjugate MW, kDa	1919	1927

MW: molecular weight; SPR: Saccharide to protein ratio

The click chemistry allows for generating 6B conjugates with very low free saccharide and free protein.

5 Example 8: Conjugation of *S. pneumoniae* serotype 19A Capsular Polysaccharide using click chemistry (see Figure 3)

Serotype 19A capsular polysaccharide has been activated with the azido linker (3-Azido-1-propylamine), using similar processes used for serotypes 35B and 29 (see Examples 1 and 2). Activation of CRM₁₉₇ and SCP to alkyne-CRM₁₉₇ or alkyne-SCP with Alkyne NHS ester, and click conjugation of the activated azido polysaccharide 19A with alkyne CRM₁₉₇ or SCP (conjugated by Cu⁺ mediated azide-alkyne cycloaddition reaction), were conducted using similar processes of the ones of Examples 1 and 2.

The attributes of the conjugates which have been obtained are presented at Table 9.

Table 9. Attributes of 19A Conjugates obtained using click chemistry

Conjugate #	1	2
Activated polysaccharide attributes		
Degree of Activation (DoA)	17	17
Activated carrier attributes		
Degree of Activation (DoA)	15	26
Conjugate attributes		
Carrier protein	CRM ₁₉₇	SCP
SPR Ratio	1.50	1.20
Free Saccharide, %	14	<5
Free Protein, %	<1	<1
Conjugate MW, kDa	1360	994

15 MW: molecular weight; SPR: Saccharide to protein ratio

The click chemistry allows for generating 19A conjugates with low free saccharide and very low free protein.

Example 9: Conjugation of *S. pneumoniae* serotype 19F Capsular Polysaccharide using click chemistry (see Figure 3)

Serotype 19F capsular polysaccharide has been activated with the azido linker (3-Azido-1-propylamine), using similar processes used for serotypes 35B and 29 (see Examples 1 and 2). Activation of CRM₁₉₇ and SCP to alkyne-CRM₁₉₇ or alkyne-SCP with Alkyne NHS ester, and click conjugation of the activated azido polysaccharide 19F with alkyne CRM₁₉₇ or SCP (conjugated by Cu⁺¹ mediated azide-alkyne cycloaddition reaction), were conducted using similar processes of the ones of Examples 1 and 2.

The attributes of the conjugates which have been obtained are presented at Table 10.

Table 10. Attributes of 19F Conjugates obtained using click chemistry

Conjugate #	1	2
Activated polysaccharide attributes		
Degree of Activation (DoA)	24	24
Activated carrier attributes		
Degree of Activation (DoA)	15	26
Conjugate attributes		
Carrier protein	CRM ₁₉₇	SCP
SPR Ratio	0.88	0.92
Free Saccharide, %	7	1
Free Protein, %	<1	<1
Conjugate MW, kDa	1775	2099

MW: molecular weight; SPR: Saccharide to protein ratio

The click chemistry allows for generating 19F conjugates with low free saccharide and very low free protein.

Example 10: Conjugation of *S. pneumoniae* serotype 23F Capsular Polysaccharide using click chemistry (see Figure 3)

Serotype 23F capsular polysaccharide has been activated with the azido linker (3-Azido-1-propylamine), using similar processes used for serotypes 35B and 29 (see Examples 1 and 2). Activation of CRM₁₉₇ and SCP to alkyne-CRM₁₉₇ or alkyne-SCP with Alkyne NHS ester, and click conjugation of the activated azido polysaccharide 23F with alkyne CRM₁₉₇ or SCP (conjugated by Cu⁺¹ mediated azide-alkyne cycloaddition reaction), were conducted using similar processes of the ones of Examples 1 and 2.

The attributes of the conjugates which have been obtained are presented at Table 11.

Table 11. Attributes of 23F Conjugates obtained using click chemistry

Conjugate #	1	2
Activated polysaccharide attributes		
Degree of Activation (DoA)	26	28
Activated carrier attributes		
Degree of Activation (DoA)	15	26
Conjugate attributes		
Carrier protein	CRM ₁₉₇	SCP
SPR Ratio	1.20	1.10
Free Saccharide, %	5	8
Free Protein, %	<1	<1
Conjugate MW, kDa	2111	1798

MW: molecular weight; SPR: Saccharide to protein ratio

The click chemistry allows for generating 23F conjugates with low free saccharide and very low free protein.

5 **Example 11. Immunogenicity of serotype 19F conjugates conjugated using click chemistry**

The opsonophagocytic activity (OPA) titers for Serotype 19F conjugate to -CRM₁₉₇ or SCP in mice were determined under standard conditions. Click chemistry (Click) was used to generate the tested conjugates (see attributes at Example 9, Table 10).

- 10 Groups of 6-8 weeks old female Swiss Webster mice were immunized (250 µL) with 0.05 µg/animal or 0.1 µg/animal of test conjugates via the subcutaneous route on week 0. The mice were boosted with the same dose of conjugate on week 3 and then bled at week 5. Serotype-specific OPAs were performed on week 5 sera samples.

The results are presented at table 12.

Table 12 -- OPA titers following vaccination with Serotype 19F antigen conjugated with Click to -CRM₁₉₇ or SCP. Conjugated Serotype 19F was used to vaccinate mice in the presence of adjuvant. Female Swiss-Webster mice, 6-8 weeks old; Doses: 0.1, and 0.05 µg/animal + AIPO₄; Vaccinate: 0 and 3 wk.; exsanguinate wk. 5 Readout: OPA			
	Dose (µg/animal)	0.05	0.1
Click - CRM ₁₉₇ Conjugate #1 of Table 10	Mean	102	143
	Total # of mice	50	50
	# of non responders	8	8
	%of non responders	16	16
Click – SCP Conjugate #2 of Table 10	Mean	306	328
	Total # of mice	50	50
	# of non responders	0	1
	%of non responders	0	2

Example 12. Immunogenicity of serotype 29 conjugates conjugated using click chemistry

The opsonophagocytic activity (OPA) titers for Serotype 29 conjugate to -CRM₁₉₇ in mice were determined under standard conditions. Click chemistry (Click) was used to generate the tested conjugate (see attributes at Example 2, Table 2).

Groups of 6-8 weeks old female Swiss Webster mice were immunized (250 µL) with 0.01 µg/animal, 0.05 µg/animal or 0.1 µg/animal of test conjugates via the subcutaneous route on week 0. The mice were boosted with the same dose of conjugate on week 3 and then bled at week 5. Serotype-specific OPAs were performed on week 5 sera samples.

The results are presented at table 13.

Table 13 -- OPA titers following vaccination with Serotype 29 antigen conjugated to -CRM₁₉₇, with Click chemistry. Conjugated Serotype 29 was used to vaccinate mice in the presence of adjuvant. Female Swiss-Webster mice, 6-8 weeks old; Doses: 0.01; 0.05 and 0.1 µg/animal + AIPO4; Vaccinate: 0 and 3 wk.; exsanguinate wk. 5 Readout: OPA				
	Dose (µg/animal)	0.01	0.05	0.1
Click-CRM ₁₉₇	Mean (OPA titers)	28	1332	2271
	Total # of mice	25	25	25
	# of non-responders	12	1	0
	%of non-responders	48	4	0

The data of Table 13 indicate that the serotype 29 conjugate elicited dose dependent OPA titers in a murine immunogenicity model.

Example 13. Immunogenicity of serotype 19A conjugates conjugated using click chemistry

The opsonophagocytic activity (OPA) titers for Serotype 19A conjugate to -CRM₁₉₇ or SCP in mice were determined under standard conditions. Click chemistry (Click) was used to generate the tested conjugates (see attributes at Example 8, Table 9).

Groups of 6-8 weeks old female Swiss Webster mice were immunized (250 µL) with 0.05 µg/animal or 0.1 µg/animal of test conjugates via the subcutaneous route on week 0. The mice were boosted with the same dose of conjugate on week 3 and then bled at week 5. Serotype-specific OPAs were performed on week 5 sera samples.

The results are presented at table 14.

Table 14 -- OPA titers following vaccination with Serotype 19A antigen conjugated with Click to -CRM₁₉₇ or SCP. Conjugated Serotype 19A was used to vaccinate mice in the presence of adjuvant. Female Swiss-Webster mice, 6-8 weeks old; Doses: 0.05 and 0.1 µg/animal + AIPO4; Vaccinate: 0 and 3 wk.; exsanguinate wk. 5 Readout: OPA			
	Dose (µg/animal)	0.05	0.1
Click-CRM ₁₉₇ Conjugate #1 of Table 9	Mean	25	40
	Total # of mice	50	49
	# of non responders	24	19
	%of non responders	48	39
Click-SCP Conjugate #2 of Table 9	Mean	126	327
	Total # of mice	50	50
	# of non responders	4	2
	%of non responders	8	4

Example 14. Immunogenicity of serotype 6B conjugates conjugated using click chemistry

The opsonophagocytic activity (OPA) titers for Serotype 6B conjugate to -CRM₁₉₇ or SCP in mice were determined under standard conditions. Click chemistry (Click) was used to generate the tested conjugates (see attributes at Example 7, Table 8).

Groups of 6-8 weeks old female Swiss Webster mice were immunized (250 µL) with 0.05 µg/animal, 0.1 µg/animal or 0.2 µg/animal of test conjugates via the subcutaneous route on week 0. The mice were boosted with the same dose of conjugate on week 3 and then bled at week 5. Serotype-specific OPAs were performed on week 5 sera samples.

The results are presented at table 15.

Table 15 -- OPA titers following vaccination with Serotype 6B antigen conjugated with Click to -CRM₁₉₇ or SCP. Conjugated Serotype 6B was used to vaccinate mice in the presence of adjuvant. Female Swiss-Webster mice, 6-8 weeks old; Doses: 0.2, 0.1, and 0.05 µg/animal + AIPO4; Vaccinate: 0 and 3 wk.; exsanguinate wk. 5 Readout: OPA				
	Dose (µg/animal)	0.05	0.1	0.2
Click - CRM ₁₉₇ Conjugate #1 of Table 8	Mean	76	255	217
	Total # of mice	50	50	50
	# of non responders	15	9	7
	%of non responders	30	18	14
Click – SCP Conjugate #2 of Table 8	Mean	54	70	202
	Total # of mice	48	50	49
	# of non responders	18	16	7
	%of non responders	38	32	14

Example 15. Immunogenicity of serotype 15A conjugates conjugated using click chemistry

The opsonophagocytic activity (OPA) titers for Serotype 15A conjugate to -CRM₁₉₇ or SCP in mice were determined under standard conditions. Click chemistry (Click) was used to generate the tested conjugates (see attributes at Example 5, Table 6).

Groups of 6-8 weeks old female Swiss Webster mice were immunized (250 µL) with 0.01 µg/animal, 0.1 µg/animal or 1.0 µg/animal of test conjugates via the subcutaneous route on week 0. The mice were boosted with the same dose of conjugate on week 3 and then bled at week 5. Serotype-specific OPAs were performed on week 5 sera samples.

The results are presented at table 16.

Table 16 -- OPA titers following vaccination with Serotype 15A antigen conjugated with Click to -CRM₁₉₇ or SCP. Conjugated Serotype 15A was used to vaccinate mice in the presence of adjuvant. Female Swiss-Webster mice, 6-8 weeks old; Doses: 1.0, 0.1, and 0.01 µg/animal + AIPO4; Vaccinate: 0 and 3 wk.; exsanguinate wk. 5 Readout: OPA				
	Dose (µg/animal)	<u>0.01</u>	<u>0.1</u>	<u>1.0</u>
Click - CRM ₁₉₇ Conjugate #1 of Table 6	Mean	135	6457	16624
	Total # of mice	23	25	25
	# of non responders	5	0	0
	%of non responders	22	0	0
Click – SCP Conjugate #2 of Table 6	Mean	463	3294	5578
	Total # of mice	25	25	25
	# of non responders	2	0	1
	%of non responders	8	0	4

All publications and patent applications mentioned in the specification are indicative of the level of those skilled in the art to which this invention pertains. All publications and patent applications are hereby incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, certain changes and modifications may be practiced within the scope of the appended claims.

Claims

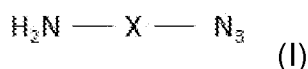
1. A method of making a capsular saccharide glycoconjugate, comprising the steps of:

- 5 (a) reacting an isolated capsular saccharide with a carbonic acid derivative and an azido linker in an aprotic solvent to produce an activated azido saccharide,
 (b) reacting a carrier protein with an agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group where the NHS moiety reacts with the amino groups to form an amide linkage thereby obtaining an alkyne functionalized carrier protein,
 10 (c) reacting the activated azido saccharide of step (a) with the activated alkyne-carrier protein of step (b) by Cu^{+1} mediated azide-alkyne cycloaddition reaction to form a glycoconjugate.

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

- 15 3. The method of any one of claims 1-2 wherein, said carbonic acid derivative is selected from the group consisting of 1,1'-carbonyldiimidazole (CDI), 1,1'-carbonyl-di-(1,2,4-triazole) (CDT), disuccinimidyl carbonate (DSC) and N-hydroxysuccinimidyl chloroformate.

4. The method of any one of claims 1-3 wherein said azido linker is a compound of formula
 20 (I),



wherein X is selected from the group consisting of $\text{CH}_2(\text{CH}_2)_n$, $(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$, $\text{NHCO}(\text{CH}_2)_n$, $\text{NHCO}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$, $\text{OCH}_2(\text{CH}_2)_n$ and $\text{O}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$; where n is selected from 1 to 10 and m is selected from 1 to 4.

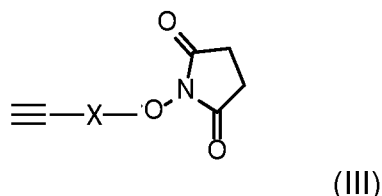
- 25 5. The method of any one of claims 1-3 wherein said azido linker is a compound of formula (II),



6. The method of any one of claims 1-5 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.
 30

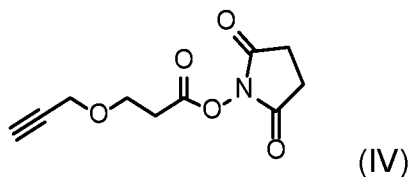
7. The method of any one of claims 1-5 wherein, said agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group is an agent bearing an N-Hydroxysuccinimide (NHS) moiety and a cycloalkyne.

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



where X is selected from the group consisting of $\text{CH}_2\text{O}(\text{CH}_2)_n\text{CH}_2\text{C}=\text{O}$ and $\text{CH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_m(\text{CH}_2)_n\text{CH}_2\text{C}=\text{O}$, where n is selected from 0 to 10 and m is selected from 0 to 4.

9. The method of any one of claims 1-5 wherein, said agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group is a compound of formula (IV):



10. The method of any one of claims 1-9 wherein, step a) comprises reacting the capsular saccharide with a carbonic acid derivative followed by reacting the carbonic acid derivative-activated capsular saccharide with an azido linker in an aprotic solvent to produce an activated azido capsular saccharide.

11. The method of any one of claims 1-10 wherein, at step a) the isolated capsular saccharide is reacted with CDI in an aprotic solvent comprising 0.1% to 1% (v/v) water.

12. The method of any one of claims 1-11 wherein at step a) carbonic acid derivative activation is followed by the addition of water.

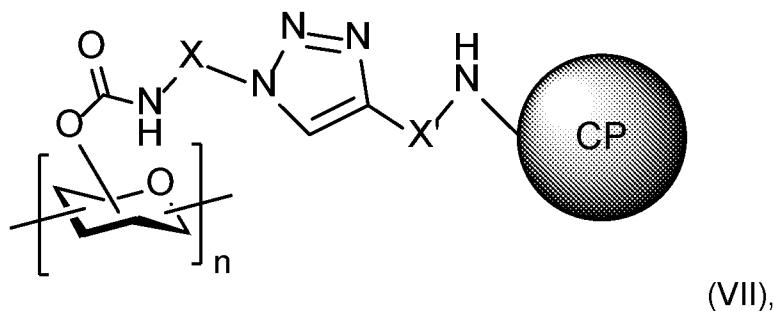
13. The method of any one of claims 1-12 wherein step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 0.01-10 molar equivalent to the amount of saccharide Repeat Unit of the activated saccharide.

14. The method of any one of claims 1-13 wherein the conjugation reaction c) is carried out in aqueous buffer in the presence of copper (I) as catalyst.

15. The method of any one of claims 1-14 wherein the method further comprises the step of purifying the glycoconjugate after it is produced.

16. A capsular saccharide glycoconjugate produced according to any one of the methods of claims 1 to 15.

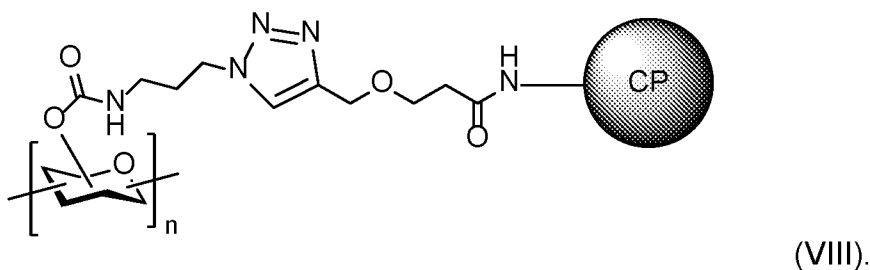
- 5 17. A capsular saccharide glycoconjugate comprising a capsular saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (VII):



- 10 wherein X is selected from the group consisting of $\text{CH}_2(\text{CH}_2)_{n'}$, $(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$, $\text{NHCO}(\text{CH}_2)_{n'}$, $\text{NHCO}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$, $\text{OCH}_2(\text{CH}_2)_{n'}$ and $\text{O}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$; 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 $\text{CH}_2\text{O}(\text{CH}_2)_{n''}\text{CH}_2\text{C}=\text{O}$, $\text{CH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_{m'}(\text{CH}_2)_{n''}\text{CH}_2\text{C}=\text{O}$, where n'' is selected from 0 to 10 and m' is selected from 0 to 4.

- 15 18. A capsular saccharide glycoconjugate comprising a capsular saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (VII), wherein X is $\text{CH}_2(\text{CH}_2)_{n'}$, where n' is 2 and wherein X' is $\text{CH}_2\text{O}(\text{CH}_2)_{n''}\text{CH}_2\text{C}=\text{O}$ where n'' is 1.

- 20 19. A capsular saccharide glycoconjugate comprising a capsular saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (VIII),



- 25 20. The capsular saccharide glycoconjugate of any one of claims 16 to 19 comprising a capsular saccharide wherein the weight average molecular weight (Mw) of said capsular saccharide before conjugation is between 10 kDa and 2,000 kDa.

21. The capsular saccharide glycoconjugate of any one of claims 16 to 20 having a weight average molecular weight (Mw) of between 250 kDa and 20,000 kDa.
22. The capsular saccharide glycoconjugate of any one of claims 16 to 21 wherein, the degree of conjugation of the capsular saccharide glycoconjugate is between 2 and 15.
- 5 23. The capsular saccharide glycoconjugate of any one of claims 16 to 22 wherein the ratio of capsular saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 3.0.
24. The capsular saccharide glycoconjugate of any one of claims 16 to 23 wherein the said capsular saccharide glycoconjugate comprises at least one covalent linkage
10 between the carrier protein and the saccharide for every 5 to 10 saccharide repeat units of the saccharide.
25. The capsular saccharide glycoconjugate of any one of claims 16 to 24 wherein said carrier protein is CRM₁₉₇, SCP, DT (Diphtheria toxoid) TT, (tetanus toxoid) or PD (*H. influenzae* protein D).
- 15 26. The method of making a capsular saccharide glycoconjugate of any one of claims 1 to 15 or the capsular saccharide glycoconjugate of any one of claims 16 to 25, wherein said capsular saccharide is a capsular saccharide from a pathogenic bacteria.
27. The method of making a capsular saccharide glycoconjugate of any one of claims 1 to 15 or the capsular saccharide glycoconjugate of any one of claims 16 to 25, wherein
20 said capsular saccharide is a capsular saccharide from *S. pneumoniae*.
28. The method of making a capsular saccharide glycoconjugate or the capsular saccharide glycoconjugate of any one of claims 1 to 27, wherein said capsular saccharide is not a capsular saccharide from *Streptococcus pneumoniae* serotype 3.
29. An immunogenic composition comprising a capsular saccharide glycoconjugate of
25 any one of claims 16 to 28.

FIG. 1



FIG. 2



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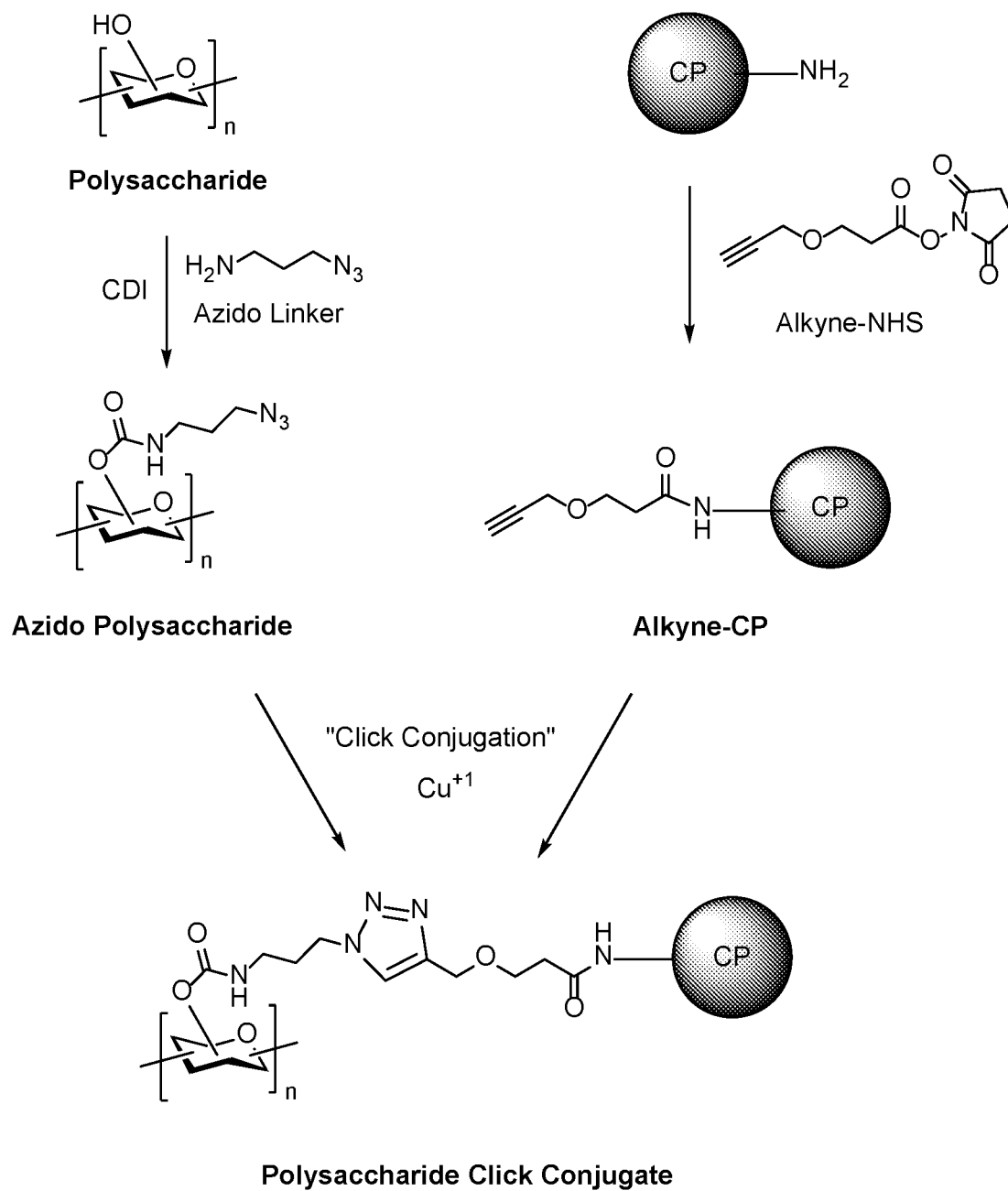


FIG. 3

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2023/050202

A. CLASSIFICATION OF SUBJECT MATTER

INV. **A61K47/64** **A61K39/09**
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, CHEM ABS Data, COMPENDEX, EMBASE, INSPEC, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO 2022/249106 A2 (PFIZER [US]) 1 December 2022 (2022-12-01) examples 5,7-11 claims figure 2 ----- -/--	1-29



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

30 March 2023

Date of mailing of the international search report

14/04/2023

Name and mailing address of the ISA/

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Authorized officer

Villard, Anne-Laure

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2023/050202

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	STEFANO CROTTI ET AL: "Defined Conjugation of Glycans to the Lysines of CRM197 Guided by their Reactivity Mapping", CHEMBIOCHEM, JOHN WILEY & SONS, INC, HOBOKEN, USA, vol. 15, no. 6, 11 March 2014 (2014-03-11), pages 836-843, XP072156559, ISSN: 1439-4227, DOI: 10.1002/CBIC.201300785 page 836, left-hand column, paragraph 1 page 837, left-hand column, paragraph 2 - page 838, right-hand column, paragraph 1 page 838, scheme 2 -----	1-29
Y	WO 2018/126229 A2 (SUTROVAX INC [US]) 5 July 2018 (2018-07-05) paragraphs [0022], [0024], [0025], [0028], [0179], [0215] -----	1-29
A	FRANCESCO BERTI ET AL: "Antimicrobial glycoconjugate vaccines: an overview of classic and modern approaches for protein modification", CHEMICAL SOCIETY REVIEWS, vol. 47, no. 24, 1 January 2018 (2018-01-01), pages 9015-9025, XP055702534, UK ISSN: 0306-0012, DOI: 10.1039/C8CS00495A page 9017; table 1 page 9018, section 3.2 figures 1,2,3E -----	1-29

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2023/050202

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
 - a. ☒ forming part of the international application as filed.
 - b. ☐ furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

☐ accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.
2. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/IB2023/050202

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2022249106 A2	01-12-2022	US 2022387613 A1 WO 2022249106 A2	08-12-2022 01-12-2022
WO 2018126229 A2	05-07-2018	AU 2017388891 A1 BR 112019013475 A2 CA 3048981 A1 CN 110366428 A EP 3562503 A2 IL 267709 A JP 7186166 B2 JP 2020504760 A JP 2023022188 A KR 20190103256 A MA 47223 A PH 12019501552 A1 US 2018333484 A1 WO 2018126229 A2	04-07-2019 27-02-2020 05-07-2018 22-10-2019 06-11-2019 29-08-2019 08-12-2022 13-02-2020 14-02-2023 04-09-2019 06-11-2019 01-06-2020 22-11-2018 05-07-2018



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权利要求书3页 说明书105页

序列表(电子公布) 附图3页

(54) 发明名称

包含缀合的荚膜糖抗原的免疫原性组合物
及其用途

(57) 摘要

本发明涉及新的经缀合的荚膜糖抗原(糖缀合物)、包含所述糖缀合物的免疫原性组合物及其用途。

1. 一种制备荚膜糖糖缀合物的方法, 该方法包括以下步骤:

(a) 使经分离的荚膜糖与碳酸衍生物及叠氮基接头在非质子性溶剂中反应以产生经活化的叠氮基糖,

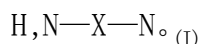
(b) 使载体蛋白与带有N-羟基琥珀酰亚胺 (NHS) 部分及炔烃基的试剂反应, 其中所述 NHS 部分与氨基反应以形成酰胺键, 从而获得经炔烃官能化的载体蛋白,

(c) 通过 Cu^{+1} 介导的叠氮化物-炔烃环加成反应使步骤 (a) 的经活化的叠氮基糖与步骤 (b) 的经活化炔烃-载体蛋白发生反应以形成糖缀合物。

2. 权利要求2所述的方法, 其中在活化步骤 (a) 之前, 将所述分离的荚膜糖尺寸设定为 200kDa 与 800kDa 之间的重量平均分子量。

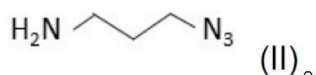
3. 权利要求1-2任一项所述的方法, 其中所述碳酸衍生物选自由以下组成的组: 1,1'-羰基二咪唑 (CDI)、1,1'-羰基-二-(1,2,4-三唑) (CDT)、二琥珀酰亚胺碳酸酯 (DSC) 及 N-羟基琥珀酰亚胺氯甲酸酯。

4. 权利要求1-3任一项所述的方法, 其中所述叠氮基接头为式 (I) 的化合物,



其中 X 选自由以下组成的组: $\text{CH}_2(\text{CH}_2)_n$ 、 $(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$ 、 $\text{NHC}(\text{CH}_2)_n$ 、 $\text{NHC}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$ 、 $0\text{CH}_2(\text{CH}_2)_n$ 和 $0(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$; 其中 n 选自 1 至 10 且 m 选自 1 至 4。

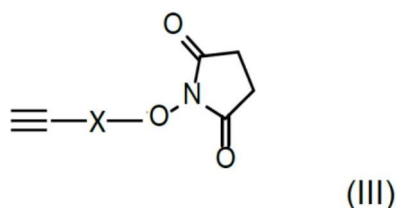
5. 权利要求1-3任一项所述的方法, 其中所述叠氮基接头为式 (II) 的化合物,



6. 权利要求1-5任一项所述的方法, 其中所述带有N-羟基琥珀酰亚胺 (NHS) 部分及炔烃基的试剂为带有N-羟基琥珀酰亚胺 (NHS) 部分及末端炔烃的试剂。

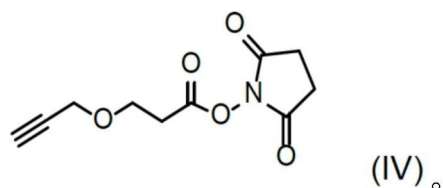
7. 权利要求1-5任一项所述的方法, 其中所述带有N-羟基琥珀酰亚胺 (NHS) 部分及炔烃基的试剂为带有N-羟基琥珀酰亚胺 (NHS) 部分及环炔烃的试剂。

8. 权利要求1-5任一项所述的方法, 其中所述带有N-羟基琥珀酰亚胺 (NHS) 部分及炔烃基的试剂为式 (III) 的化合物,



其中 X 选自由以下组成的组: $\text{CH}_2\text{O}(\text{CH}_2)_n\text{CH}_2\text{C}=\text{O}$ 及 $\text{CH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_m(\text{CH}_2)_n\text{CH}_2\text{C}=\text{O}$, 其中 n 选自 0 至 10 且 m 选自 0 至 4。

9. 权利要求1-5任一项所述的方法, 其中所述带有N-羟基琥珀酰亚胺 (NHS) 部分及炔烃基的试剂为式 (IV) 的化合物:



10. 权利要求1-9任一项所述的方法, 其中步骤 a) 包括在非质子性溶剂中, 使荚膜糖与

碳酸衍生物反应,随后使经碳酸衍生物活化的荚膜糖与叠氮基接头反应,以产生经活化的叠氮基荚膜糖。

11. 权利要求1-10任一项所述的方法,其中在步骤a)处,经分离的荚膜糖与CDI在包含0.1%至1% (v/v) 水的非质子性溶剂中反应。

12. 权利要求1-11任一项所述的方法,其中在步骤a)处,在碳酸衍生物活化之后添加水。

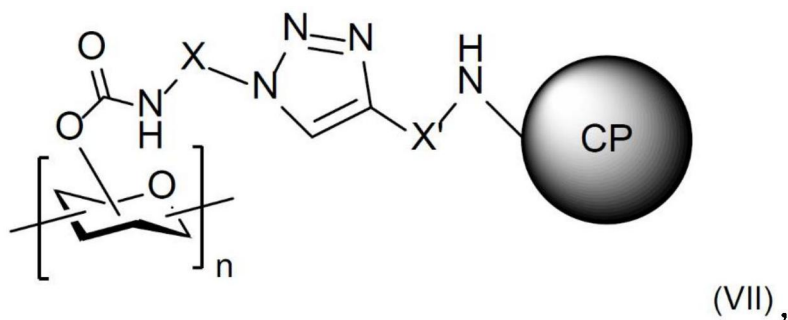
13. 权利要求1-12任一项所述的方法,其中步骤a)进一步包括使经碳酸衍生物活化的糖与相对于经活化糖的糖重复单元的量在0.01至10摩尔当量之间的量的叠氮基接头反应。

14. 权利要求1-13任一项所述的方法,其中所述缀合反应c)在水性缓冲液中于作为催化剂的铜(I)的存在下进行。

15. 权利要求1-14任一项所述的方法,其中所述方法进一步包括在产生糖缀合物之后,纯化该糖缀合物的步骤。

16. 一种荚膜糖糖缀合物,其根据权利要求1-15任一项所述的方法产生。

17. 一种荚膜糖糖缀合物,其包含经由间隔子与载体蛋白(CP)共价缀合的荚膜糖且具有通式(VII):

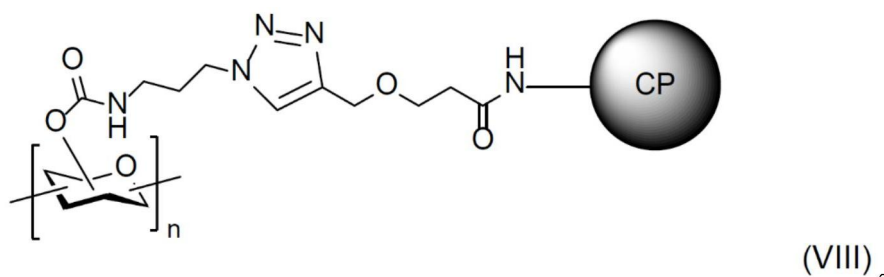


其中X选自由以下组成的组: $\text{CH}_2(\text{CH}_2)_{n'}$ 、 $(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$ 、 $\text{NHCO}(\text{CH}_2)_{n'}$ 、 $\text{NHCO}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$ 、 $\text{OCH}_2(\text{CH}_2)_{n'}$ 及 $\text{O}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$;其中 n' 选自1至10且 m 选自1至4,

且其中 X' 选自由以下组成的组: $\text{CH}_2\text{O}(\text{CH}_2)_{n''}\text{CH}_2\text{C}=\text{O}$ 、 $\text{CH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_m(\text{CH}_2)_{n''}\text{CH}_2\text{C}=\text{O}$,其中 n'' 选自0至10且 m' 选自0至4。

18. 一种荚膜糖糖缀合物,其包含经由间隔子与载体蛋白(CP)共价缀合的荚膜糖且具有通式(VII),其中X为 $\text{CH}_2(\text{CH}_2)_{n'}$,其中 n' 为2,且其中 X' 为 $\text{CH}_2\text{O}(\text{CH}_2)_{n''}\text{CH}_2\text{C}=\text{O}$,其中 n'' 为1。

19. 一种荚膜糖糖缀合物,其包含经由间隔子与载体蛋白(CP)共价缀合的荚膜糖且具有通式(VIII),



20. 权利要求16至19任一项所述的荚膜糖糖缀合物,其包含荚膜糖,其中所述荚膜糖在缀合前的重量平均分子量(Mw)在10kDa与2,000kDa之间。

21. 权利要求16至20任一项所述的荚膜糖糖缀合物,其具有250kDa与20,000kDa之间的

重量平均分子量(Mw)。

22. 权利要求16至21任一项所述的荚膜糖糖缀合物,其中所述荚膜糖糖缀合物的缀合程度在2与15之间。

23. 权利要求16至22任一项所述的荚膜糖糖缀合物,其中所述糖缀合物中荚膜糖与载体蛋白的比率(w/w)在0.5与3.0之间。

24. 权利要求16至23任一项所述的荚膜糖糖缀合物,其中所述荚膜糖糖缀合物针对糖的每5至10个糖重复单元,在载体蛋白与糖之间包含至少一个共价连接。

25. 权利要求16至24任一项所述的荚膜糖糖缀合物,其中所述载体蛋白为CRM₁₉₇、SCP、DT(白喉类毒素)、TT(破伤风类毒素)或PD(流感嗜血杆菌(*H. influenzae*)蛋白D)。

26. 权利要求1至15中任一项所述的制备荚膜糖糖缀合物的方法或权利要求16至25中任一项所述的荚膜糖糖缀合物,其中所述荚膜糖为来自病原性细菌的荚膜糖。

27. 权利要求1至15任一项所述的制备荚膜糖糖缀合物的方法或权利要求16至25中任一项所述的荚膜糖糖缀合物,其中所述荚膜糖为来自肺炎链球菌(*S. pneumoniae*)的荚膜糖。

28. 权利要求1至27任一项所述的制备荚膜糖糖缀合物的方法或荚膜糖糖缀合物,其中所述荚膜糖不是来自肺炎链球菌血清型3的荚膜糖。

29. 一种免疫原性组合物,其包含权利要求16至28任一项所述的荚膜糖糖缀合物。

包含缀合的荚膜糖抗原的免疫原性组合物及其用途

技术领域

[0001] 本发明涉及新的经缀合荚膜糖抗原(糖缀合物)、包含所述糖缀合物之免疫原性组合物及其用途。本发明之免疫原性组合物通常包含糖缀合物,其中糖来源于细菌荚膜多糖抗原,尤其为来源于病原性细菌之荚膜多糖。本发明还涉及使用所述糖缀合物对人类个体尤其婴儿及老年人进行疫苗接种以抵抗感染。

背景技术

[0002] 革兰氏阳性细菌及革兰氏阴性细菌均可能产生细胞外隔室,即荚膜,它覆盖细菌细胞且通常会阻止潜在的细胞表面抗原与其同源抗体发生反应。荚膜存在于数种具有医疗重要性之细菌中,尤其是肠道外及侵袭性菌株。细菌荚膜主要由多糖构成。多糖在细胞周围形成凝胶状物质。荚膜多糖为重要的免疫原,由此使得其成为疫苗设计中之重要组分。荚膜多糖已被证明可用于诱发免疫反应,尤其当与载体蛋白连接时。

[0003] 数十年来,已成功地利用通过将弱免疫原性分子缀合至“载体”分子来提高这些弱免疫原性分子之免疫原性的方法(参见例如Goebel等人(1939)J.Exp.Med.69:53)。举例而言,已描述了许多免疫原性组合物,其中经纯化之荚膜聚合物已与载体蛋白缀合,以通过采用此“载体效应”来产生更高效的免疫原性组合物(Schneerson等人(1984)Infect.Immun.45:582-591)。还已证明,缀合可避免在用游离多糖进行免疫时通常在婴儿中观测到的不良抗体反应(Anderson等人(1985)J.Pediatr.107:346;Insel等人(1986)J.Exp.Med.158:294)。

[0004] 已成功使用各种交联或偶联试剂,诸如同型双官能、异型双官能或零长度交联剂来产生缀合物。目前许多方法可供用于将免疫原性分子(诸如糖、蛋白质及肽)与肽或蛋白质载体偶联。大多数方法产生胺、酰胺、氨基甲酸乙酯、异硫脲或二硫键,或在一些情况下产生硫醚。使用将反应性位点引入载体及/或免疫原性分子上之反应性氨基酸分子之侧链中的交联剂或偶联剂的不足之处在于,反应性位点在未中和的情况下会在体外与任何非所需分子自由反应(因此可能不利地影响缀合物之功能性或稳定性),或在体内与任何非所需分子自由反应(因此在使用制剂进行免疫接种之个人或动物中引起不良事件的潜在风险)。可利用各种已知的化学反应来使此类过量的反应性位点发生反应或对其进行“封端”,以便使此等位点失活,但此等反应可能在其他方面破坏缀合物之功能性。

[0005] 因此,仍需要新的经适当封端之糖缀合物及制备所述缀合物之方法,使得功能性得以保留且缀合物仍能够诱发所需免疫反应。

[0006] 本发明人已发现用以产生糖缀合物的有效新方法。该方法允许产生游离糖含量极低且产率良好的缀合物。此外,本发明人已发现,一些肺炎链球菌(*Streptococcus pneumoniae*)血清型(例如35B及29多糖)由于其独特结构而在产生缀合物方面造成特定挑战。需要免疫原性肺炎链球菌血清型多糖-载体蛋白缀合物及其经改良的制备方法。

[0007] 发明概述

[0008] 在一方面中,本发明涉及一种制备荚膜糖糖缀合物之方法,其包含以下步骤:

[0009] (a) 使经分离荚膜糖与碳酸衍生物及叠氮基接头在非质子性溶剂中反应以产生经活化叠氮基糖,

[0010] (b) 使载体蛋白与带有N-羟基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应,其中NHS部分与氨基反应以形成酰胺键,从而获得经炔烃官能化载体蛋白,

[0011] (c) 通过 Cu^{+1} 介导之叠氮化物-炔烃环加成反应使步骤(a)之经活化叠氮基糖与步骤(b)之经活化炔烃-载体蛋白发生反应以形成糖缀合物。

[0012] 在一方面中,经分离糖在活化步骤(a)前系经尺寸设定。

[0013] 在一方面中,碳酸衍生物系选自由以下组成的组:1,1'-羰基二咪唑(CDI)、1,1'-羰基-二-(1,2,4-三唑)(CDT)、二琥珀酰亚胺碳酸酯(DSC)及N-羟基琥珀酰亚胺氯甲酸酯。

[0014] 在一方面中,该叠氮基接头为式(I)化合物,

[0015] $\text{H}_2\text{N}-\text{X}-\text{N}_3$ (I)

[0016] 其中X系选自由以下组成的组: $\text{CH}_2(\text{CH}_2)_n$ 、 $(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$ 、 $\text{NHC}(\text{CH}_2)_n$ 、 $\text{NHC}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$ 、 $\text{OCH}_2(\text{CH}_2)_n$ 及 $\text{O}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$;其中n系选自1至10且m系选自1至4。

[0017] 在一方面中,该带有N-羟基琥珀酰亚胺(NHS)部分及炔烃基之试剂为带有N-羟基琥珀酰亚胺(NHS)部分及末端炔烃之试剂。

[0018] 在一方面中,步骤a)包含在非质子性溶剂中,使荚膜糖与碳酸衍生物反应,随后使经碳酸衍生物活化之荚膜糖与叠氮基接头反应,以产生经活化叠氮基荚膜糖。

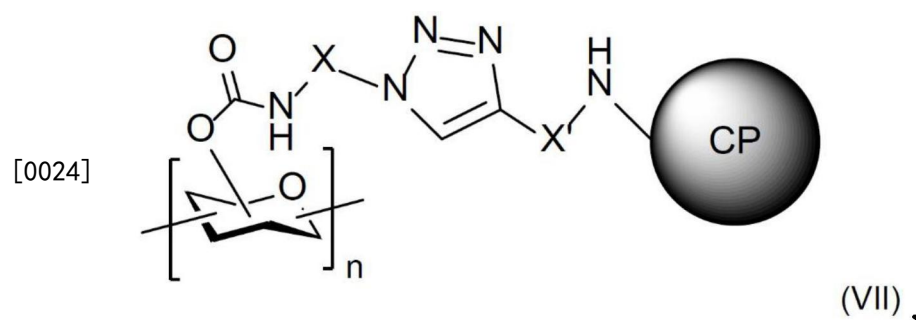
[0019] 在一方面中,缀合反应c)系在水性缓冲液中在作为催化剂之铜(I)存在下进行。

[0020] 在一方面中,在步骤c)之后,该方法进一步包含用叠氮基封端剂对缀合物中保留之未反应叠氮基进行封端的步骤。

[0021] 在一方面中,在步骤c)之后,该方法进一步包含用炔烃基封端剂对缀合物中保留之未反应炔烃基进行封端的步骤。

[0022] 在一方面中,本发明涉及一种根据所述方法产生之荚膜糖糖缀合物。

[0023] 在另一方面中,本发明涉及一种荚膜糖糖缀合物,其包含经由间隔子与载体蛋白(CP)共价缀合之荚膜糖且具有通式(VII):

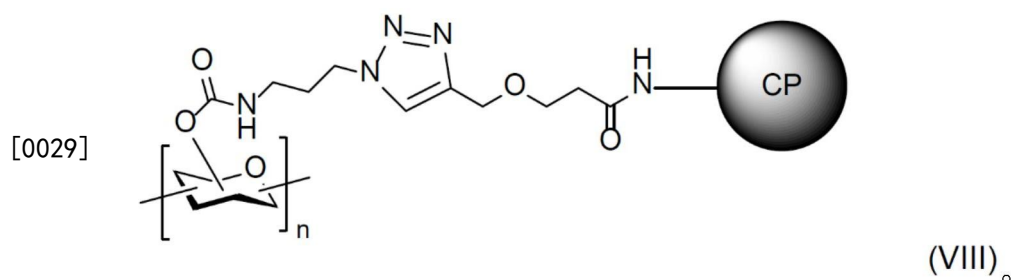


[0025] 其中X系选自由以下组成的组: $\text{CH}_2(\text{CH}_2)_n$ 、 $(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$ 、 $\text{NHC}(\text{CH}_2)_n$ 、 $\text{NHC}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$ 、 $\text{OCH}_2(\text{CH}_2)_n$ 及 $\text{O}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$;其中n'系选自1至10且m系选自1至4,

[0026] 且其中X'系选自由以下组成的组: $\text{CH}_2\text{O}(\text{CH}_2)_n\text{CH}_2\text{C}=\text{O}$ 、 $\text{CH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_m$ 、 $(\text{CH}_2)_n\text{CH}_2\text{C}=\text{O}$,其中n''系选自0至10且m'系选自0至4。

[0027] 在特定方面中,本发明涉及一种荚膜糖糖缀合物,其包含经由间隔子与载体蛋白(CP)共价缀合之荚膜糖且具有通式(VII),其中X为 $\text{CH}_2(\text{CH}_2)_n$,其中n'为2,且其中X'为 $\text{CH}_2\text{O}(\text{CH}_2)_n\text{CH}_2\text{C}=\text{O}$,其中n''为1。

[0028] 在一特定方面中,本发明涉及一种荚膜糖糖缀合物,其包含经由间隔子与载体蛋白(CP)共价缀合之荚膜糖且具有通式(VIII),



附图说明

[0030] 图1展示肺炎链球菌血清型35B荚膜多糖之重复多糖结构。

[0031] 图2展示肺炎链球菌血清型29荚膜多糖之重复多糖结构。

[0032] 图3展示使用点击化学且使用3-叠氮基-1-丙胺作为叠氮基接头来制备的本发明之糖缀合物之通用制备流程。CP=载体蛋白, CDI=1,1'-羰基二咪唑。

[0033] 发明详述

[0034] 本发明人已研发出新的多糖-载体蛋白缀合物及制备此等缀合物之方法。已发现用以产生糖缀合物之方法允许生产游离糖含量极低且产率良好的糖缀合物。

[0035] 此外,本发明人已发现肺炎链球菌血清型35B及29多糖在产生缀合物方面造成特定挑战。已发现肺炎链球菌血清型35B及29多糖在用高碘酸盐活化期间裂解,高碘酸盐为常用还原氨基化方法中使用的典型氧化剂。看起来,高碘酸盐氧化在血清型35B及29多糖之主链上发生且使甘露糖醇或核糖醇裂解,引起尺寸减小。此情形导致若干问题,包括难以获得某一尺寸之经活化之血清型35B及29多糖。用高碘酸盐进行活化导致多糖Mw减小,从而大幅度限制了有效活化范围。

[0036] WO 2020/247299提出,据称为了改良血清型35B缀合物属性,可减少高碘酸盐之量且使用某一温度范围来进行缀合反应。然而,已发现此类溶液会导致低产率及/或较高含量之游离糖。本发明人已研发出新的肺炎链球菌血清型35B及29多糖-载体蛋白缀合物及制备此等缀合物之经改良方法,其不具有此等缺陷。

[0037] 1. 本发明之糖缀合物

[0038] 本发明部分针对经缀合细菌荚膜糖抗原(也称为糖缀合物)。出于本发明之目的,术语“糖缀合物”指示与载体蛋白共价连接之荚膜糖(尤其细菌荚膜糖)。

[0039] 1.1 本发明之荚膜糖

[0040] 在整个本说明书中,术语“糖”可指示多糖或寡糖且包括两者。在一个实施方案中,本发明之糖可为寡糖。寡糖具有低数目之重复单元(通常5至15个重复单元)且通常以合成方式或通过多糖之水解得到。然而优选地,本发明之所有糖及本发明之免疫原性组合物中之所有糖为多糖。高分子量多糖由于存在于抗原表面上之表位而能够诱导某些抗体免疫反应。优选地,考虑高分子量荚膜多糖之分离及纯化以用于本发明之缀合物、组合物及方法中。因此,在本发明之一优选实施方案中,糖为多糖。

[0041] 优选地,本发明中所使用之糖为细菌荚膜糖(本文中还称为“荚膜糖”)。具有医疗重要性之若干细菌中存在荚膜。细菌荚膜主要由多糖构成。荚膜糖是通过本领域技术人员

已知之标准技术制备的。

[0042] 在本发明之一最优实施方案中,糖为肺炎链球菌荚膜多糖。

[0043] 在一个实施方案中,本发明中所使用之荚膜糖为合成碳水化合物。

[0044] 然而,在一个优选实施方案中,根据本发明之细菌荚膜糖来源可为细菌细胞。可用作荚膜糖来源之细菌菌株可获自现有培养物保藏中心(诸如来自美国典型培养物保藏中心(ATCC,Manassas,VA USA)或链球菌参考实验室(疾病控制与预防中心,Atlanta,GA USA))或临床标本。

[0045] 细菌荚膜糖可使用本领域技术人员已知之分离程序自细菌直接获得(参见例如US2006/0228380、US2006/0228381、US2007/0184071、US2007/0184072、US2007/0231340及US2008/0102498及W02008/118752中所公开之方法)。其还可使用本领域技术人员已知之合成方案产生。

[0046] 在细菌荚膜糖自细菌直接获得的情况下,细菌细胞可在培养基中生长。在产生荚膜糖之细菌细胞发酵之后,细菌细胞可溶解以产生细胞溶解产物。荚膜糖接着可使用本领域中已知之纯化技术自细胞溶解产物分离,包括使用离心、深层过滤、沉淀、超滤、用活性炭处理、渗滤及/或柱层析(参见例如US2006/0228380、US2006/0228381及W02008/118752)。经纯化之荚膜糖随后可用于制备免疫原性缀合物。

[0047] 通过纯化获得的经分离荚膜糖可通过不同参数表征,包括例如重量平均分子量(Mw)。多糖之分子量可通过大小排阻层析(SEC)与多角度激光散射检测器(MALLS)之组合来测量。

[0048] 在一个实施方案中,制备本发明之糖缀合物或本发明之糖缀合物之一部分的方法中使用的荚膜糖为来自病原性细菌之荚膜糖。优选地,本发明中所使用之荚膜糖为来自病原性链球菌属(*Streptococcus*)、病原性葡萄球菌属(*Staphylococcus*)、病原性肠球菌属(*Enterococcus*)、病原性芽孢杆菌属(*Bacillus*)、病原性棒状杆菌属(*Corynebacterium*)、病原性利斯特菌属(*Listeria*)、病原性丹毒丝菌属(*Erysipelothrix*)、病原性梭菌属(*Clostridium*)、病原性嗜血杆菌属(*Haemophilus*)、病原性奈瑟氏菌属(*Neisseria*)或病原性埃希氏菌属(*Escherichia*)之荚膜糖。更优选地,本发明中所使用之荚膜糖为来自病原性链球菌属、病原性奈瑟氏菌属或病原性埃希氏菌属之荚膜糖。

[0049] 在一个实施方案中,本发明中所使用之荚膜糖为来自以下之荚膜糖:嗜水气单胞菌(*Aeromonas hydrophila*)及其他菌种;炭疽芽孢杆菌(*Bacillus anthracis*);蜡样芽孢杆菌(*Bacillus cereus*);梭菌属之产生肉毒杆菌(*Botulinum*)神经毒素之菌种;流产布鲁氏菌(*Brucella abortus*);马耳他布鲁氏菌(*Brucella melitensis*);猪布鲁氏菌(*Brucella suis*);鼻疽伯克霍尔德菌(*Burkholderia mallei*)(官方名称鼻疽假单胞菌(*Pseudomonas mallei*));类鼻疽伯克霍尔德菌(*Burkholderia pseudomallei*)(以前称为类鼻疽假单胞菌(*Pseudomonas pseudomallei*));空肠弯曲杆菌(*Campylobacter jejuni*);鹦鹉热衣原体(*Chlamydia psittaci*);沙眼衣原体(*Chlamydia trachomatis*)、肉毒梭菌(*Clostridium botulinum*);艰难梭菌(*Clostridium difficile*);产气荚膜梭菌(*Clostridium perfringens*);粗球孢子菌(*Coccidioides immitis*);波萨达斯球孢子菌(*Coccidioides posadasii*);反刍类考德里氏体(*Cowdria ruminantium*)(水胸病(Heartwater));伯氏考克斯氏体(*Coxiella burnetii*);粪肠球菌(*Enterococcus*

faecalis);致泻性大肠杆菌(Enterovirulent *Escherichia coli*)群(EEC群),诸如肠毒性大肠杆菌(ETEC)、肠病原性大肠杆菌(EPEC)、O157:H7肠出血性大肠杆菌(EHEC)及肠侵袭性大肠杆菌(EIEC);埃里希氏体属(*Ehrlichia*)菌种,诸如查菲埃里希氏体(*Ehrlichia chajfeensis*);土拉热弗朗西斯氏菌(*Francisella tularensis*);嗜肺军团菌(*Legionella pneumophila*);非洲柑橘黄龙病菌(*Liberobacter africanus*);亚洲柑橘黄龙病菌(*Liberobacter asiaticus*);单核细胞增生利斯特氏菌(*Listeria monocytogenes*);其他肠道菌属(miscellaneous enterics),诸如克雷伯氏菌属(*Klebsiella*)、肠杆菌属(*Enterobacter*)、变形杆菌属(*Proteus*)、柠檬酸杆菌属(*Citrobacter*)、产气杆菌属(*Aerobacter*)、普罗威登斯菌属(*Providencia*)及沙雷氏菌属(*Serratia*);牛分枝杆菌(*Mycobacterium bovis*);结核分枝杆菌(*Mycobacterium tuberculosis*);山羊支原体(*Mycoplasma capricolum*);草状支原体草状亚种(*Mycoplasma mycoides ssp mycoides*);菲律宾露菌(*Peronosclerosporaphilippinensis*);豆薯层锈菌(*Phakopsora pachyrhizi*);类志贺邻单胞菌(*Plesiomonas shigelloides*);青枯雷尔氏菌生理小种3生物变种2(*Ralstonia solanacearum* race 3, biovar2);普氏立克次氏体(*Rickettsia prowazekii*);立氏立克次氏体(*Rickettsia rickettsii*);沙门氏菌属菌种(*Salmonella* spp.);玉米褐条霜霉病菌(*Schlerophthora rayssiae* var *zeae*);志贺氏菌属菌种(*Shigella* spp.);金黄色葡萄球菌(*Staphylococcus aureus*);链球菌;马铃薯癌肿病菌(*Synchytrium endobioticum*);非O1霍乱弧菌(*Vibrio cholerae* non-O1);O1霍乱弧菌;副溶血性弧菌(*Vibrio parahaemolyticus*)及其他弧菌;创伤弧菌(*Vibrio vulnificus*);水稻黄单胞菌(*Xanthomonas oryzae*);苛求木杆菌(*Xylella fastidiosa*) (柑桔杂色褪绿病菌株(citrus variegated chlorosis strain));小肠结肠炎耶尔森氏菌(*Yersinia enterocolitica*)及假结核耶尔森氏菌(*Yersinia pseudotuberculosis*);或鼠疫耶尔森氏菌(*Yersinia pestis*)。优选地,本发明中所使用之荚膜糖为来自粪肠球菌、大肠杆菌、金黄色葡萄球菌或链球菌之荚膜糖。

[0050] 在一个实施方案中,本发明中所使用之荚膜糖为来自流感嗜血杆菌(*Haemophilus influenzae*)、脑膜炎奈瑟氏菌(*Neisseria meningitidis*)、肺炎链球菌、酿脓链球菌(*S.pyogenes*)、无乳链球菌(*S.agalactiae*)、C组及G组链球菌或大肠杆菌之荚膜糖。更优选地,本发明中所使用之荚膜糖为来自脑膜炎奈瑟氏菌、肺炎链球菌、无乳链球菌或大肠杆菌之荚膜糖。甚至更优选地,本发明中所使用之荚膜糖为来自肺炎链球菌或无乳链球菌之荚膜糖。甚至更优选地,本发明中所使用之荚膜糖为来自肺炎链球菌之荚膜糖。

[0051] 在一个实施方案中,本发明中所使用之荚膜糖为来自金黄色葡萄球菌之荚膜糖。在一个实施方案中,本发明中所使用之荚膜糖为来自金黄色葡萄球菌5型或金黄色葡萄球菌8型之荚膜糖。

[0052] 在一个实施方案中,本发明中所使用之荚膜糖为来自粪肠球菌之荚膜糖。在另一个实施方案中,本发明中所使用之荚膜糖为来自流感嗜血杆菌b型之荚膜糖。

[0053] 在另一个实施方案中,本发明中所使用之荚膜糖为来自脑膜炎奈瑟氏菌之荚膜糖。在一个实施方案中,本发明中所使用之荚膜糖为来自脑膜炎奈瑟氏菌血清群A(MenA)、脑膜炎奈瑟氏菌血清群W135(MenW135)、脑膜炎奈瑟氏菌血清群Y(MenY)、脑膜炎奈瑟氏菌血清群X(MenX)或脑膜炎奈瑟氏菌血清群C(MenC)之荚膜糖。在一个实施方案中,本发明中

所使用之荚膜糖为来自脑膜炎奈瑟氏菌血清群A (MenA) 之荚膜糖。在一个实施方案中,本发明中所使用之荚膜糖为来自脑膜炎奈瑟氏菌血清群W135 (MenW135) 之荚膜糖。在一个实施方案中,本发明中所使用之荚膜糖为来自脑膜炎奈瑟氏菌血清群Y (MenY) 之荚膜糖。在一个实施方案中,本发明中所使用之荚膜糖为来自脑膜炎奈瑟氏菌血清群C (MenC) 之荚膜糖。在一个实施方案中,本发明中所使用之荚膜糖为来自脑膜炎奈瑟氏菌血清群X (MenX) 之荚膜糖。

[0054] 在另一个实施方案中,本发明中所使用之荚膜糖为来自大肠杆菌之荚膜糖。在另一个实施方案中,本发明中所使用之荚膜糖为来自粪肠球菌之荚膜糖。

[0055] 在另一个实施方案中,本发明中所使用之荚膜糖为来自无乳链球菌 (B组链球菌 (GBS)) 之荚膜糖。在一些实施方案中,本发明中所使用之荚膜糖为来自GBS Ia、Ib、II、III、IV、V、VI、VII或VIII型之荚膜糖。在一些实施方案中,本发明中所使用之荚膜糖为来自GBS Ia、Ib、II、III或V型之荚膜糖。

[0056] 在另一个实施方案中,本发明中所使用之荚膜糖为来自大肠杆菌之荚膜糖。在一个实施方案中,本发明中所使用之荚膜糖为来自致泻性大肠杆菌群 (EEC群), 诸如肠毒性大肠杆菌 (ETEC)、肠病原性大肠杆菌 (EPEC)、O157:H7肠出血性大肠杆菌 (EHEC) 或肠侵袭性大肠杆菌 (EIEC) 之大肠杆菌部分之荚膜糖。在一个实施方案中,本发明中所使用之荚膜糖为来自泌尿道致病性大肠杆菌 (UPEC) 之荚膜糖。

[0057] 在一个实施方案中,本发明中所使用之荚膜糖为来自选自由血清型O157:H7、O26:H11、O111:H- 及O103:H2组成的组之大肠杆菌血清型的荚膜糖。在一个实施方案中,本发明中所使用之荚膜糖为来自选自由血清型O6:K2:H1及O18:K1:H7组成的组之大肠杆菌血清型的荚膜糖。在一个实施方案中,本发明中所使用之荚膜糖为来自选自由血清型O45:K1、O17:K52:H18、O19:H34及O7:K1组成的组之大肠杆菌血清型的荚膜糖。在一个实施方案中,本发明中所使用之荚膜糖为来自大肠杆菌血清型O104:H4之荚膜糖。在一个实施方案中,本发明中所使用之荚膜糖为来自大肠杆菌血清型O1:K12:H7之荚膜糖。在一个实施方案中,本发明中所使用之荚膜糖为来自大肠杆菌血清型O127:H6之荚膜糖。在一个实施方案中,本发明中所使用之荚膜糖为来自大肠杆菌血清型O139:H28之荚膜糖。在一个实施方案中,本发明中所使用之荚膜糖为来自大肠杆菌血清型O128:H2之荚膜糖。

[0058] 在一个优选实施方案中,本发明中所使用之荚膜糖为来自肺炎链球菌之荚膜糖。优选地,本发明中所使用之荚膜糖为来自选自由以下组成的组之肺炎链球菌血清型的荚膜糖:血清型1、2、4、5、6A、6B、6C、7C、7F、8、9V、9N、10A、10B、11A、12F、14、15A、15B、15C、16F、17F、18C、19A、19F、20、21、22A、22F、23A、23B、23F、24B、24F、27、29、31、33B、33F、34、35B、35F、38、72及73。

[0059] 在一个优选实施方案中,本发明中所使用之荚膜糖不为来自肺炎链球菌血清型3之荚膜糖。

[0060] 在一个实施方案中,本发明中所使用之荚膜糖为来自肺炎链球菌血清型1之荚膜糖。在一个实施方案中,本发明中所使用之荚膜糖为来自肺炎链球菌血清型2之荚膜糖。在一个实施方案中,本发明中所使用之荚膜糖为来自肺炎链球菌血清型4之荚膜糖。在一个实施方案中,本发明中所使用之荚膜糖为来自肺炎链球菌血清型5之荚膜糖。在一个实施方案中,本发明中所使用之荚膜糖为来自肺炎链球菌血清型6A之荚膜糖。在一个实施方案中,本

[illegible]

[0061] 在一个优选实施方案中,本发明中所使用之荚膜糖为来自肺炎链球菌血清型35B之荚膜糖。在另一个优选实施方案中,本发明中所使用之荚膜糖为来自肺炎链球菌血清型

29-萘膜糖。

[0062] 在一个优选实施方案中,本发明中所使用之(在进一步处理之前经纯化之)萘膜糖具有50kDa与5000kDa之间的重量平均分子量。在一个优选实施方案中,本发明中所使用之萘膜糖具有500kDa与5000kDa之间的重量平均分子量。在另一个优选实施方案中,本发明中所使用之萘膜糖具有1000kDa与5000kDa之间的重量平均分子量。

[0063] 考虑以上范围中之任一者内之任何全数整数作为本发明之一实施方案。

[0064] 优选地,为了产生具有有利可过滤性特性、免疫原性及/或产率之糖缀合物,糖在与载体蛋白缀合前,将尺寸设定为目标分子量范围。

[0065] 有利地,经纯化萘膜糖之尺寸减小,同时保留多糖结构之关键特征。可采用机械或化学尺寸设定。

[0066] 在一个实施方案中,经纯化萘膜糖之尺寸通过化学水解减小。化学水解可使用弱酸(例如乙酸、甲酸、丙酸)进行。在一个实施方案中,化学水解系使用甲酸进行。在一个实施方案中,化学水解系使用丙酸进行。在一个优选实施方案中,化学水解系使用乙酸进行。化学水解还可使用经稀释之强酸(诸如稀盐酸、稀硫酸、稀磷酸、稀硝酸或稀高氯酸)进行。在一个实施方案中,化学水解系使用稀盐酸进行。在一个实施方案中,化学水解系使用稀硫酸进行。在一个实施方案中,化学水解系使用稀磷酸进行。在一个实施方案中,化学水解系使用稀硝酸进行。在一个实施方案中,化学水解系使用稀高氯酸进行。

[0067] 经纯化萘膜糖之尺寸还可通过机械均质化减小。在一个实施方案中,经纯化萘膜糖之尺寸通过高压均质化减小。高压均质化通过经由具有足够小尺寸之流道泵送工艺流来实现高剪切速率。剪切速率通过使用较大的所施加均质化压力而增加,且暴露时间可通过使进料流再循环通过均质器而增加。

[0068] 高压均质化方法可适用于减小经纯化萘膜糖之尺寸,同时保留糖之结构特征。

[0069] 在一个优选实施方案中,经分离萘膜糖之尺寸设定为10kDa与1000kDa之间的重量平均分子量。在一个实施方案中,经分离萘膜糖之尺寸设定为50kDa与500kDa之间的重量平均分子量。在一个实施方案中,经分离萘膜糖之尺寸设定为50kDa与400kDa之间的重量平均分子量。在一个实施方案中,经分离萘膜糖之尺寸设定为50kDa与250kDa之间的重量平均分子量。

[0070] 在一个实施方案中,经分离萘膜糖之尺寸设定为250kDa与1000kDa之间的重量平均分子量。在一个实施方案中,经分离萘膜糖之尺寸设定为250kDa与500kDa之间的重量平均分子量。在一个实施方案中,经分离萘膜糖之尺寸设定为250kDa与400kDa之间的重量平均分子量。在一个优选实施方案中,经分离萘膜糖之尺寸设定为200kDa与800kDa之间的重量平均分子量。

[0071] 在一个实施方案中,经分离萘膜糖之尺寸设定为约250kDa之重量平均分子量。在一个实施方案中,经分离萘膜糖之尺寸设定为约300kDa之重量平均分子量。在一个实施方案中,经分离萘膜糖之尺寸设定为约350kDa之重量平均分子量。在一个实施方案中,经分离萘膜糖之尺寸设定为约400kDa之重量平均分子量。在一个实施方案中,经分离萘膜糖之尺寸设定为约450kDa之重量平均分子量。在一个实施方案中,经分离萘膜糖之尺寸设定为约500kDa之重量平均分子量。在一个实施方案中,经分离萘膜糖之尺寸设定为约550kDa之重量平均分子量。在一个实施方案中,经分离萘膜糖之尺寸设定为约600kDa之重量平均分子

量。在一个实施方案中,经分离荚膜糖之尺寸设定为约700kDa之重量平均分子量。在一个实施方案中,经分离荚膜糖之尺寸设定为约800kDa之重量平均分子量。在一个实施方案中,经分离荚膜糖之尺寸设定为约900kDa之重量平均分子量。在一个实施方案中,经分离荚膜糖之尺寸设定为约1000kDa之重量平均分子量。

[0072] 在一个实施方案中,经分离荚膜糖未经尺寸设定。

[0073] 上文所描述之经分离荚膜糖可经活化(例如经化学活化),使得其能够(例如与接头)反应且随后并入糖缀合物中,如本文进一步所描述。

[0074] 出于本发明之目的,术语‘糖缀合物’指示与载体蛋白共价连接之糖。

[0075] 一般而言,糖与载体之共价缀合会增强糖之免疫原性,因为共价缀合将所述糖自T非依赖性抗原转化为T依赖性抗原,由此引起免疫记忆之引发。缀合尤其适用于儿童疫苗。

[0076] 1.2 本发明之荚膜糖糖缀合物

[0077] 在一些实施方案中,本发明之糖缀合物包含荚膜糖,其中该多糖在缀合前的重量平均分子量(Mw)在10kDa与2,000kDa之间。

[0078] 糖在缀合前的重量平均分子量(Mw)系指糖活化之前(还即在最终尺寸设定步骤之后但在糖与活化剂反应之前)的Mw。在本发明之上下文中,糖之Mw未由活化步骤实质上改变,且并入缀合物中的糖之Mw与活化之前测量的糖之Mw相似。在一个实施方案中,糖系用碳酸衍生物(例如CDI或CDT)与叠氮基接头之组合活化(参见下文部分1.3)。在一个实施方案中,糖系用CDI与叠氮基接头之组合活化(参见下文部分1.3)。在一个实施方案中,糖系用CDT与叠氮基接头之组合活化(参见下文部分1.3)。

[0079] 在一个实施方案中,本发明之糖缀合物包含荚膜糖,其中该糖在缀合前的重量平均分子量(Mw)在50kDa与1,000kDa之间。在一个实施方案中,重量平均分子量(Mw)在50kDa与750kDa之间。在一个实施方案中,重量平均分子量(Mw)在50kDa与500kDa之间。在一个实施方案中,重量平均分子量(Mw)在50kDa与250kDa之间。在一个实施方案中,重量平均分子量(Mw)在50kDa与200kDa之间。在一个实施方案中,重量平均分子量(Mw)在50kDa与150kDa之间。在一个实施方案中,重量平均分子量(Mw)在50kDa与100kDa之间。

[0080] 在一个实施方案中,本发明之糖缀合物包含荚膜糖,其中该糖在缀合前的重量平均分子量(Mw)在75kDa与1,000kDa之间。在一个实施方案中,重量平均分子量(Mw)在75kDa与750kDa之间。在一个实施方案中,重量平均分子量(Mw)在75kDa与500kDa之间。在一个实施方案中,重量平均分子量(Mw)在75kDa与250kDa之间。在一个实施方案中,重量平均分子量(Mw)在75kDa与200kDa之间。在一个实施方案中,重量平均分子量(Mw)在75kDa与150kDa之间。在一个实施方案中,重量平均分子量(Mw)在75kDa与100kDa之间。

[0081] 在一个实施方案中,本发明之糖缀合物包含荚膜糖,其中该糖在缀合前的重量平均分子量(Mw)在100kDa与1,000kDa之间。在一个实施方案中,重量平均分子量(Mw)在100kDa与750kDa之间。在一个实施方案中,重量平均分子量(Mw)在100kDa与500kDa之间。在一个实施方案中,重量平均分子量(Mw)在100kDa与250kDa之间。在一个实施方案中,重量平均分子量(Mw)在100kDa与200kDa之间。在一个实施方案中,重量平均分子量(Mw)在100kDa与150kDa之间。

[0082] 在一个实施方案中,本发明之糖缀合物包含荚膜糖,其中该糖在缀合前的重量平均分子量(Mw)在150kDa与1,000kDa之间。在一个实施方案中,重量平均分子量(Mw)在

150kDa与750kDa之间。在一个实施方案中,重量平均分子量(Mw)在150kDa与500kDa之间。在一个实施方案中,重量平均分子量(Mw)在150kDa与250kDa之间。在一个实施方案中,重量平均分子量(Mw)在150kDa与200kDa之间。

[0083] 在一个实施方案中,本发明之糖缀合物包含荚膜糖,其中该糖在缀合前的重量平均分子量(Mw)在200kDa与1,000kDa之间。在一个实施方案中,重量平均分子量(Mw)在200kDa与750kDa之间。在一个实施方案中,重量平均分子量(Mw)在200kDa与500kDa之间。在一个实施方案中,重量平均分子量(Mw)在200kDa与300kDa之间。在一个实施方案中,重量平均分子量(Mw)在200kDa与250kDa之间。

[0084] 在一个实施方案中,本发明之糖缀合物包含荚膜糖,其中该糖在缀合前的重量平均分子量(Mw)在500kDa与1,000kDa之间。在一个实施方案中,重量平均分子量(Mw)在500kDa与750kDa之间。在一个实施方案中,重量平均分子量(Mw)在500kDa与700kDa之间。在一个实施方案中,重量平均分子量(Mw)在500kDa与600kDa之间。

[0085] 考虑以上范围中之任一者内之任何全数整数作为本发明之一实施方案。

[0086] 在一个实施方案中,本发明之糖缀合物包含荚膜糖,其中该糖在缀合前的重量平均分子量(Mw)为约1,000kDa。在一个实施方案中,重量平均分子量(Mw)为约750kDa。在一个实施方案中,重量平均分子量(Mw)为约700kDa。在一个实施方案中,重量平均分子量(Mw)为约600kDa。在一个实施方案中,重量平均分子量(Mw)为约500kDa。在一个实施方案中,重量平均分子量(Mw)为约400kDa。在一个实施方案中,重量平均分子量(Mw)为约300kDa。在一个实施方案中,重量平均分子量(Mw)为约200kDa。在一个实施方案中,重量平均分子量(Mw)为约150kDa。在一个实施方案中,重量平均分子量(Mw)为约125kDa。在一个实施方案中,重量平均分子量(Mw)为约100kDa。

[0087] 在一些实施方案中,本发明之糖缀合物具有250kDa与20,000kDa之间的重量平均分子量(Mw)。在其他实施方案中,本发明之糖缀合物具有500kDa与15,000kDa之间的重量平均分子量(Mw)。在又其他实施方案中,本发明之糖缀合物具有500kDa与10,000kDa之间的重量平均分子量(Mw)。

[0088] 在其他实施方案中,本发明之糖缀合物具有500kDa与10,000kDa之间的重量平均分子量(Mw)。在其他实施方案中,本发明之糖缀合物具有500kDa与7,500kDa之间的重量平均分子量(Mw)。在另其他实施方案中,本发明之糖缀合物具有500kDa与5,000kDa之间的重量平均分子量(Mw)。在另其他实施方案中,本发明之糖缀合物具有500kDa与2,500kDa之间的重量平均分子量(Mw)。在另其他实施方案中,本发明之糖缀合物具有500kDa与2,000kDa之间的重量平均分子量(Mw)。在另其他实施方案中,本发明之糖缀合物具有500kDa与1,500kDa之间的重量平均分子量(Mw)。在另其他实施方案中,本发明之糖缀合物具有500kDa与1,000kDa之间的重量平均分子量(Mw)。在另其他实施方案中,本发明之糖缀合物具有500kDa与750kDa之间的重量平均分子量(Mw)。

[0089] 在其他实施方案中,本发明之糖缀合物具有750kDa与10,000kDa之间的重量平均分子量(Mw)。在其他实施方案中,本发明之糖缀合物具有750kDa与7,500kDa之间的重量平均分子量(Mw)。在另其他实施方案中,本发明之糖缀合物具有750kDa与5,000kDa之间的重量平均分子量(Mw)。在另其他实施方案中,本发明之糖缀合物具有750kDa与2,500kDa之间的重量平均分子量(Mw)。在另其他实施方案中,本发明之糖缀合物具有750kDa与2,000kDa

之间的重量平均分子量 (Mw)。在另其他实施方案中,本发明之糖缀合物具有750kDa与1,500kDa之间的重量平均分子量 (Mw)。在另其他实施方案中,本发明之糖缀合物具有750kDa与1,000kDa之间的重量平均分子量 (Mw)。

[0090] 在其他实施方案中,本发明之糖缀合物具有1,000kDa与10,000kDa之间的重量平均分子量 (Mw)。在其他实施方案中,本发明之糖缀合物具有1,000kDa与7,500kDa之间的重量平均分子量 (Mw)。在另其他实施方案中,本发明之糖缀合物具有1,000kDa与5,000kDa之间的重量平均分子量 (Mw)。在另其他实施方案中,本发明之糖缀合物具有1,000kDa与2,500kDa之间的重量平均分子量 (Mw)。在另其他实施方案中,本发明之糖缀合物具有1,000kDa与2,000kDa之间的重量平均分子量 (Mw)。在另其他实施方案中,本发明之糖缀合物具有1,000kDa与1,500kDa之间的重量平均分子量 (Mw)。

[0091] 在其他实施方案中,本发明之糖缀合物具有2,000kDa与10,000kDa之间的重量平均分子量 (Mw)。在其他实施方案中,本发明之糖缀合物具有2,000kDa与7,500kDa之间的重量平均分子量 (Mw)。在另其他实施方案中,本发明之糖缀合物具有2,000kDa与5,000kDa之间的重量平均分子量 (Mw)。在另其他实施方案中,本发明之糖缀合物具有2,000kDa与4,000kDa之间的重量平均分子量 (Mw)。在另其他实施方案中,本发明之糖缀合物具有2,000kDa与3,000kDa之间的重量平均分子量 (Mw)。在另其他实施方案中,本发明之糖缀合物具有2,000kDa与3,500kDa之间的重量平均分子量 (Mw)。

[0092] 在另其他实施方案中,本发明之糖缀合物具有2,250kDa与3,500kDa之间的重量平均分子量 (Mw)。

[0093] 在优选实施方案中,本发明之糖缀合物具有1,000kDa与2,500kDa之间的重量平均分子量 (Mw)。

[0094] 考虑以上范围中之任一者内之任何全数整数作为本发明之一实施方案。

[0095] 在其他实施方案中,本发明之糖缀合物具有约10,000kDa之重量平均分子量 (Mw)。在其他实施方案中,本发明之糖缀合物具有约9,000kDa之重量平均分子量 (Mw)。在其他实施方案中,本发明之糖缀合物具有约8,000kDa之重量平均分子量 (Mw)。在另其他实施方案中,本发明之糖缀合物具有约7,500kDa之重量平均分子量 (Mw)。在另其他实施方案中,本发明之糖缀合物具有约6,000kDa之重量平均分子量 (Mw)。在另其他实施方案中,本发明之糖缀合物具有约5,000kDa之重量平均分子量 (Mw)。在另其他实施方案中,本发明之糖缀合物具有约4,000kDa之重量平均分子量 (Mw)。在另其他实施方案中,本发明之糖缀合物具有约3,500kDa之重量平均分子量 (Mw)。在另其他实施方案中,本发明之糖缀合物具有约3,250kDa之重量平均分子量 (Mw)。在另其他实施方案中,本发明之糖缀合物具有约3,000kDa之重量平均分子量 (Mw)。在另其他实施方案中,本发明之糖缀合物具有约2,500kDa之重量平均分子量 (Mw)。在另其他实施方案中,本发明之糖缀合物具有约2,000kDa之重量平均分子量 (Mw)。在另其他实施方案中,本发明之糖缀合物具有约1,500kDa之重量平均分子量 (Mw)。在另其他实施方案中,本发明之糖缀合物具有约1,000kDa之重量平均分子量 (Mw)。在另其他实施方案中,本发明之糖缀合物具有约750kDa之重量平均分子量 (Mw)。

[0096] 表征本发明之糖缀合物之另一方式为通过在载体蛋白(例如CRM₁₉₇或SCP)中变得与糖缀合之赖氨酸残基之数目来表征,此可表征为缀合之赖氨酸之范围(缀合程度)。载体蛋白由于与糖之共价连接而发生的赖氨酸修饰之证据可通过使用本领域技术人员已知之

常规方法的氨基酸分析来获得。与用于产生缀合物物质之载体蛋白起始物质相比,缀合引起所回收之赖氨酸残基之数目减少。在一个优选实施方案中,本发明之糖缀合物之缀合程度在2与15之间。在一个实施方案中,本发明之糖缀合物之缀合程度在2与13之间。在一个实施方案中,本发明之糖缀合物之缀合程度在2与10之间。在一个实施方案中,本发明之糖缀合物之缀合程度在2与8之间。在一个实施方案中,本发明之糖缀合物之缀合程度在2与6之间。在一个实施方案中,本发明之糖缀合物之缀合程度在2与5之间。在一个实施方案中,本发明之糖缀合物之缀合程度在2与4之间。在一个实施方案中,本发明之糖缀合物之缀合程度在3与15之间。在一个实施方案中,本发明之糖缀合物之缀合程度在3与13之间。在一个实施方案中,本发明之糖缀合物之缀合程度在3与10之间。在一个实施方案中,本发明之糖缀合物之缀合程度在3与8之间。在一个实施方案中,本发明之糖缀合物之缀合程度在3与6之间。在一个实施方案中,本发明之糖缀合物之缀合程度在3与5之间。在一个实施方案中,本发明之糖缀合物之缀合程度在3与4之间。在一个实施方案中,本发明之糖缀合物之缀合程度在5与15之间。在一个实施方案中,本发明之糖缀合物之缀合程度在5与10之间。在一个实施方案中,本发明之糖缀合物之缀合程度在8与15之间。在一个实施方案中,本发明之糖缀合物之缀合程度在8与12之间。在一个实施方案中,本发明之糖缀合物之缀合程度在10与15之间。在一个实施方案中,本发明之糖缀合物之缀合程度在10与12之间。

[0097] 在一个实施方案中,本发明之糖缀合物之缀合程度为约2。在一个实施方案中,本发明之糖缀合物之缀合程度为约3。在一个实施方案中,本发明之糖缀合物之缀合程度为约4。在一个实施方案中,本发明之糖缀合物之缀合程度为约5。在一个实施方案中,本发明之糖缀合物之缀合程度为约6。在一个实施方案中,本发明之糖缀合物之缀合程度为约7。在一个实施方案中,本发明之糖缀合物之缀合程度为约8。在一个实施方案中,本发明之糖缀合物之缀合程度为约9。在一个实施方案中,本发明之糖缀合物之缀合程度为约10、约11。在一个实施方案中,本发明之糖缀合物之缀合程度为约12。在一个实施方案中,本发明之糖缀合物之缀合程度为约13。在一个实施方案中,本发明之糖缀合物之缀合程度为约14。在一个实施方案中,本发明之糖缀合物之缀合程度为约15。在一个优选实施方案中,本发明之糖缀合物之缀合程度在4与7之间。在一些此类实施方案中,载体蛋白为CRM₁₉₇。在其他此类实施方案中,载体蛋白为SCP。

[0098] 本发明之糖缀合物还可由糖与载体蛋白之比率(重量/重量)表征。在一些实施方案中,糖缀合物中糖与载体蛋白之比率(w/w)在0.5与3.0之间。在其他实施方案中,糖与载体蛋白之比率(w/w)在0.5与2.0之间。在其他实施方案中,糖与载体蛋白之比率(w/w)在0.5与1.5之间。在其他实施方案中,糖与载体蛋白之比率(w/w)在0.8与1.2之间。在其他实施方案中,糖与载体蛋白之比率(w/w)在0.5与1.0之间。在其他实施方案中,糖与载体蛋白之比率(w/w)在1.0与1.5之间。在其他实施方案中,糖与载体蛋白之比率(w/w)在1.0与2.0之间。在其他实施方案中,糖与载体蛋白之比率(w/w)在0.8与1.2之间。在一个优选实施方案中,缀合物中糖与载体蛋白之比率在0.9与1.1之间。

[0099] 在一个实施方案中,糖与载体蛋白之比率(w/w)为约0.5。在其他实施方案中,糖与载体蛋白之比率(w/w)为约0.6。在其他实施方案中,糖与载体蛋白之比率(w/w)为约0.7。在其他实施方案中,糖与载体蛋白之比率(w/w)为约0.8。在其他实施方案中,糖与载体蛋白之比率(w/w)为约0.9。在其他实施方案中,糖与载体蛋白之比率(w/w)为约1.0。在其他实施方

案中,糖与载体蛋白之比率(w/w)为约1.1。在其他实施方案中,糖与载体蛋白之比率(w/w)为约1.2。在其他实施方案中,糖与载体蛋白之比率(w/w)为约1.3。在其他实施方案中,糖与载体蛋白之比率(w/w)为约1.4。在其他实施方案中,糖与载体蛋白之比率(w/w)为约1.5。在其他实施方案中,糖与载体蛋白之比率(w/w)为约1.6。在其他实施方案中,糖与载体蛋白之比率(w/w)为约1.7。在其他实施方案中,糖与载体蛋白之比率(w/w)为约1.8。在其他实施方案中,糖与载体蛋白之比率(w/w)为约1.9。在其他实施方案中,糖与载体蛋白之比率(w/w)为约2.0。在其他实施方案中,糖与载体蛋白之比率(w/w)为约2.1。在其他实施方案中,糖与载体蛋白之比率(w/w)为约2.2。在其他实施方案中,糖与载体蛋白之比率(w/w)为约2.5。在其他实施方案中,糖与载体蛋白之比率(w/w)为约2.8。在其他实施方案中,糖与载体蛋白之比率(w/w)为约3.0。在一些此类实施方案中,载体蛋白为CRM₁₉₇。在其他此类实施方案中,载体蛋白为SCP。

[0100] 本发明之糖缀合物还可通过载体蛋白与糖之间的共价连接之数目表征,其随糖之重复单元而变化。在一个实施方案中,本发明之糖缀合物针对糖之每4个糖重复单元在载体蛋白与糖之间包含至少一个共价连接。在另一个实施方案中,载体蛋白与糖之间的共价连接在糖之每10个糖重复单元中出现至少一次。在另一个实施方案中,载体蛋白与糖之间的共价连接在糖之每15个糖重复单元中出现至少一次。在另一个实施方案中,载体蛋白与糖之间的共价连接在糖之每25个糖重复单元中出现至少一次。在另一个实施方案中,载体蛋白与糖之间的共价连接在糖之每50个糖重复单元中出现至少一次。在又另一个实施方案中,载体蛋白与糖之间的共价连接在糖之每100个糖重复单元中出现至少一次。

[0101] 在其他实施方案中,本发明之糖缀合物针对糖之每5至10个糖重复单元在载体蛋白与糖之间包含至少一个共价连接。

[0102] 在其他实施方案中,本发明之糖缀合物针对糖之每2至7个糖重复单元在载体蛋白与糖之间包含至少一个共价连接。

[0103] 在其他实施方案中,本发明之糖缀合物针对糖之每6至11个糖重复单元在载体蛋白与糖之间包含至少一个共价连接。

[0104] 在其他实施方案中,本发明之糖缀合物针对糖之每9至14个糖重复单元在载体蛋白与糖之间包含至少一个共价连接。

[0105] 在其他实施方案中,本发明之糖缀合物针对糖之每10至20个糖重复单元在载体蛋白与糖之间包含至少一个共价连接。

[0106] 在其他实施方案中,本发明之糖缀合物针对糖之每4至25个糖重复单元在载体蛋白与糖之间包含至少一个共价连接。

[0107] 在常见实施方案中,载体蛋白质为CRM₁₉₇。在其他实施方案中,载体蛋白为SCP。

[0108] 在一些实施方案中,载体蛋白为CRM₁₉₇,且CRM₁₉₇与糖之间的共价连接在糖之每4、10、15或25个糖重复单元中出现至少一次。在其他实施方案中,载体蛋白为SCP,且SCP与糖之间的共价连接在糖之每4、10、15或25个糖重复单元中出现至少一次。

[0109] 本发明之糖缀合物及免疫原性组合物可含有并未与载体蛋白共价缀合但仍存在于糖缀合物组合物中之游离糖。游离糖可与糖缀合物非共价缔合(即,与糖缀合物非共价键结、吸附至糖缀合物、或者包覆于糖缀合物中或由糖缀合物包覆)。

[0110] 在一个优选实施方案中,与该糖之总量相比,糖缀合物包含少于约50%之游离糖。

在一个优选实施方案中,与该糖之总量相比,糖缀合物包含少于约40%之游离糖。在另一个优选实施方案中,与该糖之总量相比,糖缀合物包含少于约25%之游离糖。在一甚至更优选实施方案中,与该糖之总量相比,糖缀合物包含少于约20%之游离糖。在另一个优选实施方案中,与该糖之总量相比,糖缀合物包含少于约15%之游离糖。

[0111] 糖缀合物还可由其分子尺寸分布(K_d)表征。大小排阻层析介质(CL-4B)可用于测定缀合物之相对分子尺寸分布。在重力馈送柱(gravity fed column)中使用大小排阻层析(SEC)以得到缀合物之分子尺寸分布概况。自介质之孔中排阻之大分子比小分子洗脱得更快。使用级分收集器来收集柱洗脱液。通过糖分析级分进行比色测试。为了测定 K_d ,将柱进行校准,以确立分子被完全排阻之级分(V_0),($K_d=0$)及表示最大保留之级分(V_i),($K_d=1$)。达到指定样品属性之级分(V_e)通过表达式 $K_d = (V_e - V_0) / (V_i - V_0)$ 与 K_d 相关。

[0112] 在一个优选实施方案中,在CL-4B柱中,至少30%的本发明之糖缀合物具有小于或等于0.3之 K_d 。在一个优选实施方案中,在CL-4B柱中,至少40%的本发明之糖缀合物具有小于或等于0.3之 K_d 。在一个优选实施方案中,在CL-4B柱中,至少45%、50%、55%、60%、65%、70%、75%、80%或85%的本发明之糖缀合物具有小于或等于0.3之 K_d 。在一个优选实施方案中,在CL-4B柱中,至少60%的本发明之糖缀合物具有小于或等于0.3之 K_d 。在一个优选实施方案中,在CL-4B柱中,50%与80%之间的本发明之糖缀合物具有小于或等于0.3之 K_d 。在一个优选实施方案中,在CL-4B柱中,65%与80%之间的本发明之糖缀合物具有小于或等于0.3之 K_d 。

[0113] 1.3使用点击化学制备的本发明之荚膜糖糖缀合物

[0114] 本发明之糖缀合物系使用点击化学制备。本发明还涉及制备如本文所公开之糖缀合物的方法。

[0115] 根据本发明,点击化学包含三个步骤:(a)使经分离荚膜糖与碳酸衍生物及叠氮基接头在非质子性溶剂中反应以产生经活化叠氮基糖(糖活化),(b)使载体蛋白与带有N-羟基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应,其中NHS部分与氨基反应以形成酰胺键,从而获得经炔烃官能化之载体蛋白(载体蛋白活化),(c)通过 Cu^{+1} 介导之叠氮化物-炔烃环加成反应使步骤(a)之经活化叠氮基糖与步骤(b)之经活化炔烃-载体蛋白发生反应以形成糖缀合物。

[0116] 在步骤(a)之后,糖被称为经活化的,且在本文中被称作“经活化糖”或“经活化叠氮基糖”。

[0117] 在步骤(b)之后,载体被称为经活化的,且被称作“经活化载体”。

[0118] 如上文所提及,糖在活化(a)前,将尺寸设定为目标分子量(MW)范围。因此,在一个实施方案中,经分离糖在用碳酸衍生物及叠氮基接头活化之前经尺寸设定。在一个实施方案中,经分离糖经尺寸设定为上文所定义之目标分子量(MW)范围中之任一者。

[0119] 在一个实施方案中,该碳酸衍生物系选自由以下组成的组:1,1'-羰基二咪唑(CDI)、1,1'-羰基-二-(1,2,4-三唑)(CDT)、二琥珀酰亚胺碳酸酯(DSC)及N-羟基琥珀酰亚胺氯甲酸酯。

[0120] 在一个实施方案中,该碳酸衍生物为1,1'-羰基二咪唑(CDI)。在另一个实施方案中,该碳酸衍生物为1,1'-羰基-二-(1,2,4-三唑)(CDT)。在另一个实施方案中,该碳酸衍生物为二琥珀酰亚胺碳酸酯(DSC)。在又一个实施方案中,该碳酸衍生物为N-羟基琥珀酰亚

胺氯甲酸酯。

[0121] 在一个实施方案中,该碳酸衍生物为1,1'-羰基二咪唑(CDI)或1,1'-羰基-二-(1,2,4-三唑)(CDT)。优选地,该碳酸衍生物为1,1'-羰基二咪唑(CDI)。

[0122] 在一个实施方案中,该叠氮基接头为式(I)化合物,

[0123] $\text{H}_2\text{N}-\text{X}-\text{N}_3$ (I)

[0124] 其中X系选自由以下组成的组: $\text{CH}_2(\text{CH}_2)_n$ 、 $(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$ 、 $\text{NHC}(\text{O})(\text{CH}_2)_n$ 、 $\text{NHC}(\text{O})(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$ 、 $\text{OCH}_2(\text{CH}_2)_n$ 及 $\text{O}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$;其中n系选自1至10且m系选自1至4。

[0125] 在一个实施方案中,该叠氮基接头为式(I)化合物,其中X为 $\text{CH}_2(\text{CH}_2)_n$,且n系选自1至10。在一个实施方案中,n系选自1至5。在一个实施方案中,n系选自1至4。在一个实施方案中,n系选自1至3。在一个实施方案中,n系选自1至2。在一个特定实施方案中,n为1。在另一个实施方案中,n为2。在又一个实施方案中,n为3。在又一个实施方案中,n为4。在又一个实施方案中,n为5。在又一个实施方案中,n为6。在又一个实施方案中,n为7。在又一个实施方案中,n为8。在又一个实施方案中,n为9。在又一个实施方案中,n为10。

[0126] 在一个实施方案中,该叠氮基接头为式(I)化合物,其中X为 $(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$,其中m系选自1至4。在一个实施方案中,m系选自1至3。在一个实施方案中,m系选自1至2。在一个特定实施方案中,m为1。在另一个实施方案中,m为2。在又一个实施方案中,m为3。在又一个实施方案中,m为4。

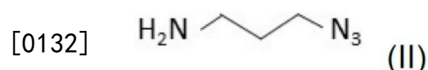
[0127] 在一个实施方案中,该叠氮基接头为式(I)化合物,其中X为 $\text{NHC}(\text{O})(\text{CH}_2)_n$,且n系选自1至10。在一个实施方案中,n系选自1至5。在一个实施方案中,n系选自1至4。在一个实施方案中,n系选自1至3。在一个实施方案中,n系选自1至2。在一个特定实施方案中,n为1。在另一个实施方案中,n为2。在又一个实施方案中,n为3。在又一个实施方案中,n为4。在又一个实施方案中,n为5。在又一个实施方案中,n为6。在又一个实施方案中,n为7。在又一个实施方案中,n为8。在又一个实施方案中,n为9。在又一个实施方案中,n为10。

[0128] 在一个实施方案中,该叠氮基接头为式(I)化合物,其中X为 $\text{NHC}(\text{O})(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$,其中m系选自1至4。在一个实施方案中,m系选自1至3。在一个实施方案中,m系选自1至2。在一个特定实施方案中,m为1。在另一个实施方案中,m为2。在又一个实施方案中,m为3。在又一个实施方案中,m为4。

[0129] 在一个实施方案中,该叠氮基接头为式(I)化合物,其中X为 $\text{OCH}_2(\text{CH}_2)_n$,且n系选自1至10。在一个实施方案中,n系选自1至5。在一个实施方案中,n系选自1至4。在一个实施方案中,n系选自1至3。在一个实施方案中,n系选自1至2。在一个特定实施方案中,n为1。在另一个实施方案中,n为2。在又一个实施方案中,n为3。在又一个实施方案中,n为4。在又一个实施方案中,n为5。在又一个实施方案中,n为6。在又一个实施方案中,n为7。在又一个实施方案中,n为8。在又一个实施方案中,n为9。在又一个实施方案中,n为10。

[0130] 在一个实施方案中,该叠氮基接头为式(I)化合物,其中X为 $\text{O}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$,其中m系选自1至4。在一个实施方案中,m系选自1至3。在一个实施方案中,m系选自1至2。在一个特定实施方案中,m为1。在另一个实施方案中,m为2。在又一个实施方案中,m为3。在又一个实施方案中,m为4。

[0131] 在一个实施方案中,该叠氮基接头为式(II)化合物,

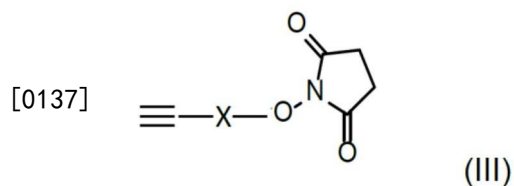


[0133] 在一个优选实施方案中,该叠氮基接头为3-叠氮基-丙胺。

[0134] 在一个实施方案中,该带有N-羟基琥珀酰亚胺(NHS)部分及炔烃基之试剂为带有N-羟基琥珀酰亚胺(NHS)部分及末端炔烃之试剂。

[0135] 在一个实施方案中,该带有N-羟基琥珀酰亚胺(NHS)部分及炔烃基之试剂为带有N-羟基琥珀酰亚胺(NHS)部分及环炔烃之试剂。

[0136] 在一个实施方案中,该带有N-羟基琥珀酰亚胺(NHS)部分及炔烃基之试剂为式(III)化合物,



[0138] 其中X系选自由以下组成的组: $\text{CH}_2\text{O}(\text{CH}_2)_n\text{CH}_2\text{C}=\text{O}$ 及 $\text{CH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_m(\text{CH}_2)_n\text{CH}_2\text{C}=\text{O}$,其中n系选自0至10且m系选自0至4。

[0139] 在一个实施方案中,该带有N-羟基琥珀酰亚胺(NHS)部分及炔烃基之试剂为式(III)化合物,其中X为 $\text{CH}_2\text{O}(\text{CH}_2)_n\text{CH}_2\text{C}=\text{O}$,其中n系选自0至10。在一个实施方案中,n系选自0至5。在一个实施方案中,n系选自0至4。在一个实施方案中,n系选自0至3。在一个实施方案中,n系选自0至2。在一个特定实施方案中,n为0。在一个特定实施方案中,n为1。在另一个实施方案中,n为2。在又一个实施方案中,n为3。在又一个实施方案中,n为4。在又一个实施方案中,n为5。在又一个实施方案中,n为6。在又一个实施方案中,n为7。在又一个实施方案中,n为8。在又一个实施方案中,n为9。在又一个实施方案中,n为10。

[0140] 在一个实施方案中,该带有N-羟基琥珀酰亚胺(NHS)部分及炔烃基之试剂为式(III)化合物,其中X为 $\text{CH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_m(\text{CH}_2)_n\text{CH}_2\text{C}=\text{O}$,其中n系选自0至10且m系选自0至4。在一个实施方案中,n系选自0至5。在一个实施方案中,n系选自0至4。在一个实施方案中,n系选自0至3。在一个实施方案中,n系选自0至2。在一个特定实施方案中,n为0。在一个特定实施方案中,n为1。在另一个实施方案中,n为2。在又一个实施方案中,n为3。在又一个实施方案中,n为4。在又一个实施方案中,n为5。在又一个实施方案中,n为6。在又一个实施方案中,n为7。在又一个实施方案中,n为8。在又一个实施方案中,n为9。在又一个实施方案中,n为10。在一个实施方案中,m系选自0至3。在一个实施方案中,m系选自0至2。在一个特定实施方案中,m为1。在一个特定实施方案中,m为1。在另一个实施方案中,m为2。在又一个实施方案中,m为3。在又一个实施方案中,m为4。

[0141] 在一个实施方案中,n系选自0至5且m系选自0至3。在一个实施方案中,n系选自0至5且m系选自0至2。

[0142] 在一个实施方案中,n系选自0至4且m系选自0至3。在一个实施方案中,n系选自0至4且m系选自0至2。

[0143] 在一个实施方案中,n系选自0至3且m系选自0至3。在一个实施方案中,n系选自0至3且m系选自0至2。

[0144] 在一个实施方案中,n系选自0至2且m系选自0至3。在一个实施方案中,n系选自0至

2且m系选自0至2。

[0145] 在一个实施方案中,n系选自0至1且m系选自0至3。在一个实施方案中,n系选自0至1且m系选自0至2。

[0146] 在一个实施方案中,n为0且m为0。在一个实施方案中,n为1且m为0。在一个实施方案中,n为2且m为0。在一个实施方案中,n为3且m为0。在一个实施方案中,n为4且m为0。在一个实施方案中,n为5且m为0。在一个实施方案中,n为6且m为0。在一个实施方案中,n为7且m为0。在一个实施方案中,n为8且m为0。在一个实施方案中,n为9且m为0。在一个实施方案中,n为10且m为0。

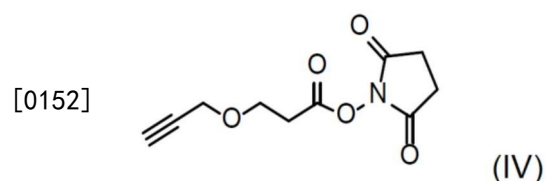
[0147] 在一个实施方案中,n为0且m为1。在一个实施方案中,n为1且m为1。在一个实施方案中,n为2且m为1。在一个实施方案中,n为3且m为1。在一个实施方案中,n为4且m为1。在一个实施方案中,n为5且m为1。在一个实施方案中,n为6且m为1。在一个实施方案中,n为7且m为1。在一个实施方案中,n为8且m为1。在一个实施方案中,n为9且m为1。在一个实施方案中,n为10且m为1。

[0148] 在一个实施方案中,n为0且m为2。在一个实施方案中,n为1且m为2。在一个实施方案中,n为2且m为2。在一个实施方案中,n为3且m为2。在一个实施方案中,n为4且m为2。在一个实施方案中,n为5且m为2。在一个实施方案中,n为6且m为2。在一个实施方案中,n为7且m为2。在一个实施方案中,n为8且m为2。在一个实施方案中,n为9且m为2。在一个实施方案中,n为10且m为2。

[0149] 在一个实施方案中,n为0且m为3。在一个实施方案中,n为1且m为3。在一个实施方案中,n为2且m为3。在一个实施方案中,n为3且m为3。在一个实施方案中,n为4且m为3。在一个实施方案中,n为5且m为3。在一个实施方案中,n为6且m为3。在一个实施方案中,n为7且m为3。在一个实施方案中,n为8且m为3。在一个实施方案中,n为9且m为3。在一个实施方案中,n为10且m为3。

[0150] 在一个实施方案中,n为0且m为4。在一个实施方案中,n为1且m为4。在一个实施方案中,n为2且m为4。在一个实施方案中,n为3且m为4。在一个实施方案中,n为4且m为4。在一个实施方案中,n为5且m为4。在一个实施方案中,n为6且m为4。在一个实施方案中,n为7且m为4。在一个实施方案中,n为8且m为4。在一个实施方案中,n为9且m为4。在一个实施方案中,n为10且m为4。

[0151] 在一个实施方案中,该带有N-羟基琥珀酰亚胺(NHS)部分及炔烃基之试剂为式(IV)化合物:



[0153] 在一个实施方案中,步骤a)包含使糖与碳酸衍生物反应,随后使经碳酸衍生物活化之糖与叠氮基接头在非质子性溶剂中反应以产生经活化叠氮基糖。

[0154] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.01至10摩尔当量之间的量的碳酸衍生物反应。

[0155] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在

0.05至10摩尔当量之间的量的碳酸衍生物反应。

[0156] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.1至10摩尔当量之间的量的碳酸衍生物反应。

[0157] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.2至10摩尔当量之间的量的碳酸衍生物反应。

[0158] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.3至10摩尔当量之间的量的碳酸衍生物反应。

[0159] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.4至10摩尔当量之间的量的碳酸衍生物反应。

[0160] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.5至10摩尔当量之间的量的碳酸衍生物反应。

[0161] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.8至10摩尔当量之间的量的碳酸衍生物反应。

[0162] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在1至10摩尔当量之间的量的碳酸衍生物反应。

[0163] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在2至10摩尔当量之间的量的碳酸衍生物反应。

[0164] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在3至10摩尔当量之间的量的碳酸衍生物反应。

[0165] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在5至10摩尔当量之间的量的碳酸衍生物反应。

[0166] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.01至5摩尔当量之间的量的碳酸衍生物反应。

[0167] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.05至5摩尔当量之间的量的碳酸衍生物反应。

[0168] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.1至5摩尔当量之间的量的碳酸衍生物反应。

[0169] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.2至5摩尔当量之间的量的碳酸衍生物反应。

[0170] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.3至5摩尔当量之间的量的碳酸衍生物反应。

[0171] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.4至5摩尔当量之间的量的碳酸衍生物反应。

[0172] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.5至5摩尔当量之间的量的碳酸衍生物反应。

[0173] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.8至5摩尔当量之间的量的碳酸衍生物反应。

[0174] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在1至5摩尔当量之间的量的碳酸衍生物反应。

[0175] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在2至5摩尔当量之间的量的碳酸衍生物反应。

[0176] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在3至5摩尔当量之间的量的碳酸衍生物反应。

[0177] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.01至3摩尔当量之间的量的碳酸衍生物反应。

[0178] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.05至3摩尔当量之间的量的碳酸衍生物反应。

[0179] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.1至3摩尔当量之间的量的碳酸衍生物反应。

[0180] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.2至3摩尔当量之间的量的碳酸衍生物反应。

[0181] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.3至3摩尔当量之间的量的碳酸衍生物反应。

[0182] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.4至3摩尔当量之间的量的碳酸衍生物反应。

[0183] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.5至3摩尔当量之间的量的碳酸衍生物反应。

[0184] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.8至3摩尔当量之间的量的碳酸衍生物反应。

[0185] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在1至3摩尔当量之间的量的碳酸衍生物反应。

[0186] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在2至3摩尔当量之间的量的碳酸衍生物反应。

[0187] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.01至2摩尔当量之间的量的碳酸衍生物反应。

[0188] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.05至2摩尔当量之间的量的碳酸衍生物反应。

[0189] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.1至2摩尔当量之间的量的碳酸衍生物反应。

[0190] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.2至2摩尔当量之间的量的碳酸衍生物反应。

[0191] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.3至2摩尔当量之间的量的碳酸衍生物反应。

[0192] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.4至2摩尔当量之间的量的碳酸衍生物反应。

[0193] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.5至2摩尔当量之间的量的碳酸衍生物反应。

[0194] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在

0.8至2摩尔当量之间的量的碳酸衍生物反应。

[0195] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在1至2摩尔当量之间的量的碳酸衍生物反应。

[0196] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.01至1摩尔当量之间的量的碳酸衍生物反应。

[0197] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.05至1摩尔当量之间的量的碳酸衍生物反应。

[0198] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.1至1摩尔当量之间的量的碳酸衍生物反应。

[0199] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.2至1摩尔当量之间的量的碳酸衍生物反应。

[0200] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.3至1摩尔当量之间的量的碳酸衍生物反应。

[0201] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.4至1摩尔当量之间的量的碳酸衍生物反应。

[0202] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.5至1摩尔当量之间的量的碳酸衍生物反应。

[0203] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.8至1摩尔当量之间的量的碳酸衍生物反应。

[0204] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.01至0.5摩尔当量之间的量的碳酸衍生物反应。

[0205] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.05至0.5摩尔当量之间的量的碳酸衍生物反应。

[0206] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.1至0.5摩尔当量之间的量的碳酸衍生物反应。

[0207] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.2至0.5摩尔当量之间的量的碳酸衍生物反应。

[0208] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.3至0.5摩尔当量之间的量的碳酸衍生物反应。

[0209] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.4至0.5摩尔当量之间的量的碳酸衍生物反应。

[0210] 在一个实施方案中,步骤a)包含使糖与一量之碳酸衍生物反应。

[0211] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.01至0.4摩尔当量之间的量的碳酸衍生物反应。

[0212] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.05至0.4摩尔当量之间的量的碳酸衍生物反应。

[0213] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.1至0.4摩尔当量之间的量的碳酸衍生物反应。

[0214] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在

0.2至0.4摩尔当量之间的量的碳酸衍生物反应。

[0215] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.3至0.4摩尔当量之间的量的碳酸衍生物反应。

[0216] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.01至0.3摩尔当量之间的量的碳酸衍生物反应。

[0217] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.05至0.3摩尔当量之间的量的碳酸衍生物反应。

[0218] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.1至0.3摩尔当量之间的量的碳酸衍生物反应。

[0219] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量在0.2至0.3摩尔当量之间的量的碳酸衍生物反应。

[0220] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量约0.01摩尔当量之量的碳酸衍生物反应。

[0221] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量约0.05摩尔当量之量的碳酸衍生物反应。

[0222] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量约0.08摩尔当量之量的碳酸衍生物反应。

[0223] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量约0.1摩尔当量之量的碳酸衍生物反应。

[0224] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量约0.2摩尔当量之量的碳酸衍生物反应。

[0225] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量约0.3摩尔当量之量的碳酸衍生物反应。

[0226] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量约0.4摩尔当量之量的碳酸衍生物反应。

[0227] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量约0.5摩尔当量之量的碳酸衍生物反应。

[0228] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量约1摩尔当量之量的碳酸衍生物反应。

[0229] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量约2摩尔当量之量的碳酸衍生物反应。

[0230] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量约3摩尔当量之量的碳酸衍生物反应。

[0231] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量约4摩尔当量之量的碳酸衍生物反应。

[0232] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量约5摩尔当量之量的碳酸衍生物反应。

[0233] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量约8摩尔当量之量的碳酸衍生物反应。

[0234] 在一个实施方案中,步骤a)包含使糖与相对于反应混合物中所存在之糖的量约10摩尔当量之量的碳酸衍生物反应。

[0235] 在一个实施方案中,在步骤a)处,经分离糖与碳酸衍生物在非质子性溶剂中反应。

[0236] 在一个实施方案中,经分离糖与碳酸衍生物在基本上由二甲亚砜(DMSO)或二甲基甲酰胺(DMF)组成之溶液中反应。在一个实施方案中,经分离糖与碳酸衍生物在基本上由二甲基甲酰胺(DMF)组成之溶液中反应。在一个实施方案中,经分离糖与碳酸衍生物在基本上由二甲亚砜(DMSO)组成之溶液中反应。

[0237] 在一个实施方案中,经分离糖与碳酸衍生物在基本上由二甲基乙酰胺组成之溶液中反应。在一个实施方案中,经分离糖与碳酸衍生物在基本上由N-甲基-2-吡咯烷酮组成之溶液中反应。在一个实施方案中,经分离糖与碳酸衍生物在基本上由六甲基磷酰胺(HMPA)组成之溶液中反应。

[0238] 在一个优选实施方案中,经分离糖与碳酸衍生物在基本上由二甲亚砜(DMSO)组成之溶液中反应。

[0239] 在一个实施方案中,经分离糖与碳酸衍生物在二甲亚砜(DMSO)或二甲基甲酰胺(DMF)中反应。在一个实施方案中,经分离糖与碳酸衍生物在二甲基甲酰胺(DMF)中反应。在一个实施方案中,经分离糖与碳酸衍生物在二甲亚砜(DMSO)中反应。

[0240] 在一个实施方案中,经分离糖与碳酸衍生物在二甲基乙酰胺中反应。在一个实施方案中,经分离糖与碳酸衍生物在N-甲基-2-吡咯烷酮中反应。在一个实施方案中,经分离糖与碳酸衍生物在六甲基磷酰胺(HMPA)中反应。

[0241] 在一个优选实施方案中,经分离糖与CDI在二甲亚砜(DMSO)中反应。在一个实施方案中,经分离糖与CDI在无水DMSO中反应。

[0242] 已出人意料地发现,使经分离糖与CDI在水分含量约0.1%至1%(v/v)之环境中反应能够避免副反应。因此,在一个实施方案中,经分离糖与CDI在包含0.1%至1%(v/v)水之非质子性溶剂中反应。在一个实施方案中,经分离糖与CDI在包含0.1%至0.8%(v/v)水之非质子性溶剂中反应。在一个实施方案中,经分离糖与CDI在包含0.1%至0.5%(v/v)水之非质子性溶剂中反应。在一个实施方案中,经分离糖与CDI在包含0.1%至0.4%(v/v)水之非质子性溶剂中反应。在一个实施方案中,经分离糖与CDI在包含0.1%至0.3%(v/v)水之非质子性溶剂中反应。在一个实施方案中,经分离糖与CDI在包含0.1%至0.2%(v/v)水之非质子性溶剂中反应。

[0243] 在一个实施方案中,经分离糖与CDI在包含0.2%至1%(v/v)水之非质子性溶剂中反应。在一个实施方案中,经分离糖与CDI在包含0.2%至0.8%(v/v)水之非质子性溶剂中反应。在一个实施方案中,经分离糖与CDI在包含0.2%至0.5%(v/v)水之非质子性溶剂中反应。在一个实施方案中,经分离糖与CDI在包含0.2%至0.4%(v/v)水之非质子性溶剂中反应。在一个实施方案中,经分离糖与CDI在包含0.2%至0.3%(v/v)水之非质子性溶剂中反应。

[0244] 在一个实施方案中,经分离糖与CDI在包含0.3%至0.8%(v/v)水之非质子性溶剂中反应。在一个实施方案中,经分离糖与CDI在包含0.3%至0.5%(v/v)水之非质子性溶剂中反应。在一个实施方案中,经分离糖与CDI在包含0.3%至0.4%(v/v)水之非质子性溶剂中反应。

[0245] 在一个实施方案中,经分离糖与CDI在包含约0.1% (v/v) 水之非质子性溶剂中反应。在一个实施方案中,经分离糖与CDI在包含约0.2% (v/v) 水之非质子性溶剂中反应。在一个实施方案中,经分离糖与CDI在包含约0.3% (v/v) 水之非质子性溶剂中反应。在一个实施方案中,经分离糖与CDI在包含约0.4% (v/v) 水之非质子性溶剂中反应。在一个实施方案中,经分离糖与CDI在包含约0.5% (v/v) 水之非质子性溶剂中反应。在一个实施方案中,经分离糖与CDI在包含约0.6% (v/v) 水之非质子性溶剂中反应。在一个实施方案中,经分离糖与CDI在包含约0.7% (v/v) 水之非质子性溶剂中反应。在一个实施方案中,经分离糖与CDI在包含约0.8% (v/v) 水之非质子性溶剂中反应。在一个实施方案中,经分离糖与CDI在包含约0.9% (v/v) 水之非质子性溶剂中反应。

[0246] 在一个实施方案中,经分离糖与CDI在包含0.1%至1% (v/v) 水之DMSO中反应。在一个实施方案中,经分离糖与CDI在包含0.1%至0.8% (v/v) 水之DMSO中反应。在一个实施方案中,经分离糖与CDI在包含0.1%至0.5% (v/v) 水之DMSO中反应。在一个实施方案中,经分离糖与CDI在包含0.1%至0.4% (v/v) 水之DMSO中反应。在一个实施方案中,经分离糖与CDI在包含0.1%至0.3% (v/v) 水之DMSO中反应。在一个实施方案中,经分离糖与CDI在包含0.1%至0.2% (v/v) 水之DMSO中反应。

[0247] 在一个实施方案中,经分离糖与CDI在包含0.2%至1% (v/v) 水之DMSO中反应。在一个实施方案中,经分离糖与CDI在包含0.2%至0.8% (v/v) 水之DMSO中反应。在一个实施方案中,经分离糖与CDI在包含0.2%至0.5% (v/v) 水之DMSO中反应。在一个实施方案中,经分离糖与CDI在包含0.2%至0.4% (v/v) 水之DMSO中反应。在一个实施方案中,经分离糖与CDI在包含0.2%至0.3% (v/v) 水之DMSO中反应。

[0248] 在一个实施方案中,经分离糖与CDI在包含0.3%至0.8% (v/v) 水之DMSO中反应。在一个实施方案中,经分离糖与CDI在包含0.3%至0.5% (v/v) 水之DMSO中反应。在一个实施方案中,经分离糖与CDI在包含0.3%至0.4% (v/v) 水之DMSO中反应。

[0249] 在一个实施方案中,经分离糖与CDI在包含约0.1% (v/v) 水之DMSO中反应。在一个实施方案中,经分离糖与CDI在包含约0.2% (v/v) 水之DMSO中反应。在一个实施方案中,经分离糖与CDI在包含约0.3% (v/v) 水之DMSO中反应。在一个实施方案中,经分离糖与CDI在包含约0.4% (v/v) 水之DMSO中反应。在一个实施方案中,经分离糖与CDI在包含约0.5% (v/v) 水之DMSO中反应。在一个实施方案中,经分离糖与CDI在包含约0.6% (v/v) 水之DMSO中反应。在一个实施方案中,经分离糖与CDI在包含约0.7% (v/v) 水之DMSO中反应。在一个实施方案中,经分离糖与CDI在包含约0.8% (v/v) 水之DMSO中反应。在一个实施方案中,经分离糖与CDI在包含约0.9% (v/v) 水之DMSO中反应。

[0250] 在一个实施方案中,在添加叠氮基接头之前通过添加水来淬灭游离碳酸衍生物。水可使游离CDI失活。因此,在一个实施方案中,在碳酸衍生物活化之后添加水。在一个实施方案中,添加水以使混合物中之总水含量达到约1%至约10% (v/v) 之间。在一个实施方案中,添加水以使混合物中之总水含量达到约1.2%至约8% (v/v) 之间。在一个实施方案中,添加水以使混合物中之总水含量达到约1.5%至约5% (v/v) 之间。在一个实施方案中,添加水以使混合物中之总水含量达到约1.5%至约3% (v/v) 之间。在一个实施方案中,添加水以使混合物中之总水含量达到约1.5%至约2.5% (v/v) 之间。在一个实施方案中,添加水以使混合物中之总水含量达到约1% (v/v)。在一个实施方案中,添加水以使混合物中之总水含

量达到约1.2% (v/v)。在一个实施方案中,添加水以使混合物中之总水含量达到约1.4% (v/v)。在一个实施方案中,添加水以使混合物中之总水含量达到约1.5% (v/v)。在一个实施方案中,添加水以使混合物中之总水含量达到约2% (v/v)。在一个实施方案中,添加水以使混合物中之总水含量达到约2.5% (v/v)。在一个实施方案中,添加水以使混合物中之总水含量达到约3% (v/v)。在一个实施方案中,添加水以使混合物中之总水含量达到约5% (v/v)。在一个实施方案中,添加水以使混合物中之总水含量达到约7% (v/v)。在一个实施方案中,添加水以使混合物中之总水含量达到约10% (v/v)。

[0251] 一旦糖已与碳酸衍生物反应且在最终用水淬灭碳酸衍生物之后,经碳酸衍生物活化之糖便与叠氮基接头反应。

[0252] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量(RU之摩尔当量)在0.01至10摩尔当量之间的量的叠氮基接头反应。

[0253] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量在0.01至8摩尔当量之间的量的叠氮基接头反应。

[0254] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量在0.01至5摩尔当量之间的量的叠氮基接头反应。

[0255] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量在0.01至4摩尔当量之间的量的叠氮基接头反应。

[0256] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量在0.01至3摩尔当量之间的量的叠氮基接头反应。

[0257] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量在0.01至2摩尔当量之间的量的叠氮基接头反应。

[0258] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量在0.01至1摩尔当量之间的量的叠氮基接头反应。

[0259] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量在0.01至0.5摩尔当量之间的量的叠氮基接头反应。

[0260] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量在0.01至0.1摩尔当量之间的量的叠氮基接头反应。

[0261] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量在0.05至10摩尔当量之间的量的叠氮基接头反应。

[0262] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量在0.05至8摩尔当量之间的量的叠氮基接头反应。

[0263] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量在0.05至5摩尔当量之间的量的叠氮基接头反应。

[0264] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量在0.05至4摩尔当量之间的量的叠氮基接头反应。

[0265] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量在0.05至3摩尔当量之间的量的叠氮基接头反应。

[0266] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量在0.05至2摩尔当量之间的量的叠氮基接头反应。

[0267] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量在0.05至1摩尔当量之间的量的叠氮基接头反应。

[0268] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量在0.05至0.5摩尔当量之间的量的叠氮基接头反应。

[0269] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量在0.05至0.1摩尔当量之间的量的叠氮基接头反应。

[0270] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量为0.1至10摩尔当量之间的量的叠氮基接头反应。

[0271] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量在0.1至8摩尔当量之间的量的叠氮基接头反应。

[0272] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量在0.1至5摩尔当量之间的量的叠氮基接头反应。

[0273] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量在0.1至4摩尔当量之间的量的叠氮基接头反应。

[0274] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量在0.1至3摩尔当量之间的量的叠氮基接头反应。

[0275] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量在0.1至2摩尔当量之间的量的叠氮基接头反应。

[0276] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量为0.1至1摩尔当量之间的量的叠氮基接头反应。

[0277] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量在0.1至0.5摩尔当量之间的量的叠氮基接头反应。

[0278] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量在0.5至10摩尔当量之间的量的叠氮基接头反应。

[0279] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量在0.5至8摩尔当量之间的量的叠氮基接头反应。

[0280] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量在0.5至5摩尔当量之间的量的叠氮基接头反应。

[0281] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量为0.5至4摩尔当量之间的量的叠氮基接头反应。

[0282] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量在0.5至3摩尔当量之间的量的叠氮基接头反应。

[0283] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量在0.5至2摩尔当量之间的量的叠氮基接头反应。

[0284] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量在0.5至1摩尔当量之间的量的叠氮基接头反应。

[0285] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量为1至10摩尔当量之间的量的叠氮基接头反应。

[0286] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化

糖之多糖重复单元的量在1至8摩尔当量之间的量的叠氮基接头反应。

[0287] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量为1至5摩尔当量之间的量的叠氮基接头反应。

[0288] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量为1至4摩尔当量之间的量的叠氮基接头反应。

[0289] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量为1至3摩尔当量之间的量的叠氮基接头反应。

[0290] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量在1至2摩尔当量之间的量的叠氮基接头反应。

[0291] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量为2至10摩尔当量之间的量的叠氮基接头反应。

[0292] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量在2至8毫尔当量之间的量的叠氮基接头反应。

[0293] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量在2至5摩尔当量之间的量的叠氮基接头反应。

[0294] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量在2至4摩尔当量之间的量的叠氮基接头反应。

[0295] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量在2至3摩尔当量之间的量的叠氮基接头反应。

[0296] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量在3至10摩尔当量之间的量的叠氮基接头反应。

[0297] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量为3至8摩尔当量之间的量的叠氮基接头反应。

[0298] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量在3至5摩尔当量之间的量的叠氮基接头反应。

[0299] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量为3至4摩尔当量之间的量的叠氮基接头反应。

[0300] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量在4至10摩尔当量之间的量的叠氮基接头反应。

[0301] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量在4至8摩尔当量之间的量的叠氮基接头反应。

[0302] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量在4至5摩尔当量之间的量的叠氮基接头反应。

[0303] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量在5至10摩尔当量之间的量的叠氮基接头反应。

[0304] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量为5至8摩尔当量之间的量的叠氮基接头反应。

[0305] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量为8至10摩尔当量之间的量的叠氮基接头反应。

[0306] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量约0.01摩尔当量之量的叠氮基接头反应。

[0307] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量约0.05摩尔当量之量的叠氮基接头反应。

[0308] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量约0.1摩尔当量之量的叠氮基接头反应。

[0309] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量约0.5摩尔当量之量的叠氮基接头反应。

[0310] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量约1摩尔当量之量的叠氮基接头反应。

[0311] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量约2摩尔当量之量的叠氮基接头反应。

[0312] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量约3摩尔当量之量的叠氮基接头反应。

[0313] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量约4摩尔当量之量的叠氮基接头反应。

[0314] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量约5摩尔当量之量的叠氮基接头反应。

[0315] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量约8摩尔当量之量的叠氮基接头反应。

[0316] 在一个实施方案中,步骤a)进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之多糖重复单元的量约10摩尔当量之量的叠氮基接头反应。

[0317] 在上述实施方案中,该碳酸衍生物优选为CDI。在另一个实施方案中,该碳酸衍生物为CDT。

[0318] 在一个实施方案中,步骤a)之后经活化糖之活化程度在0.5%至50%之间。叠氮基糖之活化程度定义为与叠氮基接头连接之重复单元的百分比。

[0319] 在一个实施方案中,步骤a)之后经活化糖之活化程度在1%至30%之间。在另一个实施方案中,步骤a)之后经活化糖之活化程度在2%至25%之间。在另一个实施方案中,步骤a)之后经活化糖之活化程度在3%至20%之间。

[0320] 在另一个实施方案中,步骤a)之后经活化糖之活化程度在3%至15%之间。在另一个实施方案中,步骤a)之后经活化糖之活化程度在4%至15%之间。在一个实施方案中,步骤a)之后经活化糖之活化程度在1%至6%之间。

[0321] 在一个实施方案中,步骤a)之后经活化糖之活化程度在3%至6%之间。在一个实施方案中,步骤a)之后经活化糖之活化程度在10%至15%之间。

[0322] 在一个实施方案中,步骤a)之后经活化糖之活化程度为约1%。在一个实施方案中,步骤a)之后经活化糖之活化程度为约2%。在一个实施方案中,步骤a)之后经活化糖之活化程度为约3%。在一个实施方案中,步骤a)之后经活化糖之活化程度为约4%。在一个实施方案中,步骤a)之后经活化糖之活化程度为约5%。在一个实施方案中,步骤a)之后经活化糖之活化程度为约6%。在一个实施方案中,步骤a)之后经活化糖之活化程度为约7%。在

一个实施方案中,步骤a)之后经活化糖之活化程度为约8%。在一个实施方案中,步骤a)之后经活化糖之活化程度为约9%。在一个实施方案中,步骤a)之后经活化糖之活化程度为约10%。在一个实施方案中,步骤a)之后经活化糖之活化程度为约11%。在一个实施方案中,步骤a)之后经活化糖之活化程度为约12%。在一个实施方案中,步骤a)之后经活化糖之活化程度为约13%。在一个实施方案中,步骤a)之后经活化糖之活化程度为约14%。在一个实施方案中,步骤a)之后经活化糖之活化程度为约15%。在一个实施方案中,步骤a)之后经活化糖之活化程度为约16%。在一个实施方案中,步骤a)之后经活化糖之活化程度为约17%。在一个实施方案中,步骤a)之后经活化糖之活化程度为约18%。在一个实施方案中,步骤a)之后经活化糖之活化程度为约19%。在一个实施方案中,步骤a)之后经活化糖之活化程度为约20%。

[0323] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸0.1至10摩尔当量之量的带有N-羟基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。

[0324] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸0.5至10摩尔当量之量的带有N-羟基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。

[0325] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸1至10摩尔当量之量的带有N-羟基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。

[0326] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸1.5至10摩尔当量之量的带有N-羟基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。

[0327] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸2至10摩尔当量之量的带有N-羟基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。

[0328] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸2.5至10摩尔当量之量的带有N-羟基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。

[0329] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸3至10摩尔当量之量的带有N-羟基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。

[0330] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸5至10摩尔当量之量的带有N-羟基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。

[0331] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸7.5至10摩尔当量之量的带有N-羟基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。

[0332] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸0.1至7.5摩尔当量之量的带有N-羟基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。

[0333] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸0.5至7.5摩尔当量之量的带有N-羟基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。

[0334] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸1至7.5摩尔当量之量的带有N-羟基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。

[0335] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸1.5至7.5摩尔当量之量的带有N-羟基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。

[0336] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸2至7.5摩尔当量之量的带有N-羟基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。

[0337] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸2.5至7.5摩

尔当量之量的带有N-羧基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。

[0338] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸3至7.5摩尔当量之量的带有N-羧基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。

[0339] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸5至7.5摩尔当量之量的带有N-羧基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。

[0340] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸0.1至5摩尔当量之量的带有N-羧基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。

[0341] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸0.5至5摩尔当量之量的带有N-羧基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。

[0342] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸1至5摩尔当量之量的带有N-羧基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。

[0343] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸1.5至5摩尔当量之量的带有N-羧基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。

[0344] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸2至5摩尔当量之量的带有N-羧基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。

[0345] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸2.5至5摩尔当量之量的带有N-羧基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。

[0346] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸3至5摩尔当量之量的带有N-羧基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。

[0347] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸0.1至3摩尔当量之量的带有N-羧基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。

[0348] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸0.5至3摩尔当量之量的带有N-羧基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。

[0349] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸1至3摩尔当量之量的带有N-羧基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。

[0350] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸1.5至3摩尔当量之量的带有N-羧基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。

[0351] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸2至3摩尔当量之量的带有N-羧基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。

[0352] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸2.5至3摩尔当量之量的带有N-羧基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。

[0353] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸0.1至2.5摩尔当量之量的带有N-羧基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。

[0354] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸0.5至2.5摩尔当量之量的带有N-羧基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。

[0355] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸1至2.5摩尔当量之量的带有N-羧基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。

[0356] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸1.5至2.5摩尔当量之量的带有N-羧基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。

- [0357] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸2至2.5摩尔当量之量的带有N-羧基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。
- [0358] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸0.1至2摩尔当量之量的带有N-羧基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。
- [0359] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸0.5至2摩尔当量之量的带有N-羧基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。
- [0360] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸1至2摩尔当量之量的带有N-羧基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。
- [0361] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸1.5至2摩尔当量之量的带有N-羧基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。
- [0362] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸0.1至1.5摩尔当量之量的带有N-羧基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。
- [0363] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸0.5至1.5摩尔当量之量的带有N-羧基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。
- [0364] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸1至1.5摩尔当量之量的带有N-羧基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。
- [0365] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸0.1至1摩尔当量之量的带有N-羧基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。
- [0366] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸0.5至1摩尔当量之量的带有N-羧基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。
- [0367] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸0.1至0.5摩尔当量之量的带有N-羧基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。
- [0368] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸约10摩尔当量之量的带有N-羧基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。
- [0369] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸约7.5摩尔当量之量的带有N-羧基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。
- [0370] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸约5摩尔当量之量的带有N-羧基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。
- [0371] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸约3摩尔当量之量的带有N-羧基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。
- [0372] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸约2.5摩尔当量之量的带有N-羧基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。
- [0373] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸约2摩尔当量之量的带有N-羧基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。
- [0374] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸约1.5摩尔当量之量的带有N-羧基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。
- [0375] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸约1摩尔当量之量的带有N-羧基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。
- [0376] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸约0.5摩尔

当量之量的带有N-羟基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。

[0377] 在一个实施方案中,步骤b)包含使载体蛋白与相对于载体上之赖氨酸约0.1摩尔当量之量的带有N-羟基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应。

[0378] 在一个实施方案中,步骤b)之后经活化载体之活化程度在1至50之间。经活化载体之活化程度定义为载体蛋白中变得与带有N-羟基琥珀酰亚胺(NHS)部分及炔烃基之试剂连接的赖氨酸残基之数目。

[0379] 在一个实施方案中,载体蛋白为CRM₁₉₇,其含有39个赖氨酸残基。在该实施方案中,步骤b)之后经活化载体之活化程度可在1至30之间。在另一个实施方案中,步骤b)之后经活化载体(CRM₁₉₇)之活化程度在5至20之间。在另一个实施方案中,步骤b)之后经活化载体(CRM₁₉₇)之活化程度在9至18之间。在另一个实施方案中,步骤b)之后经活化载体(CRM₁₉₇)之活化程度在8至11之间。在另一个实施方案中,步骤b)之后经活化载体(CRM₁₉₇)之活化程度在15至20之间。在另一个实施方案中,步骤b)之后经活化载体(CRM₁₉₇)之活化程度为约5。在另一个实施方案中,步骤b)之后经活化载体(CRM₁₉₇)之活化程度为约6。在另一个实施方案中,步骤b)之后经活化载体(CRM₁₉₇)之活化程度为约7。在另一个实施方案中,步骤b)之后经活化载体(CRM₁₉₇)之活化程度为约8。在另一个实施方案中,步骤b)之后经活化载体(CRM₁₉₇)之活化程度为约9。在另一个实施方案中,步骤b)之后经活化载体(CRM₁₉₇)之活化程度为约10。在另一个实施方案中,步骤b)之后经活化载体(CRM₁₉₇)之活化程度为约11。在另一个实施方案中,步骤b)之后经活化载体(CRM₁₉₇)之活化程度为约12。在另一个实施方案中,步骤b)之后经活化载体(CRM₁₉₇)之活化程度为约13。在另一个实施方案中,步骤b)之后经活化载体(CRM₁₉₇)之活化程度为约14。在另一个实施方案中,步骤b)之后经活化载体(CRM₁₉₇)之活化程度为约15。在另一个实施方案中,步骤b)之后经活化载体(CRM₁₉₇)之活化程度为约16。在另一个实施方案中,步骤b)之后经活化载体(CRM₁₉₇)之活化程度为约17。在另一个实施方案中,步骤b)之后经活化载体(CRM₁₉₇)之活化程度为约18。在另一个实施方案中,步骤b)之后经活化载体(CRM₁₉₇)之活化程度为约19。在另一个实施方案中,步骤b)之后经活化载体(CRM₁₉₇)之活化程度为约20。在另一个实施方案中,步骤b)之后经活化载体(CRM₁₉₇)之活化程度为约21。在另一个实施方案中,步骤b)之后经活化载体(CRM₁₉₇)之活化程度为约22。在另一个实施方案中,步骤b)之后经活化载体(CRM₁₉₇)之活化程度为约23。在另一个实施方案中,步骤b)之后经活化载体(CRM₁₉₇)之活化程度为约24。在另一个实施方案中,步骤b)之后经活化载体(CRM₁₉₇)之活化程度为约25。

[0380] 在一个实施方案中,载体蛋白为SCP或其片段。在该实施方案中,步骤b)之后经活化载体之活化程度可在1至50之间。在另一个实施方案中,步骤b)之后经活化载体(SCP)之活化程度在5至50之间。在另一个实施方案中,步骤b)之后经活化载体(SCP)之活化程度在7至45之间。在另一个实施方案中,步骤b)之后经活化载体(SCP)之活化程度在5至15之间。在另一个实施方案中,步骤b)之后经活化载体(SCP)之活化程度在20至30之间。在另一个实施方案中,步骤b)之后经活化载体(SCP)之活化程度在30至50之间。在另一个实施方案中,步骤b)之后经活化载体(SCP)之活化程度在30至40之间。在另一个实施方案中,步骤b)之后经活化载体(SCP)之活化程度在10至40之间。在另一个实施方案中,步骤b)之后经活化载体(SCP)之活化程度为约5。在另一个实施方案中,步骤b)之后经活化载体(SCP)之活化程度为约7。在另一个实施方案中,步骤b)之后经活化载体(SCP)之活化程度为约10。在另一个实施

方案中,步骤b)之后经活化载体(SCP)之活化程度为约13。在另一个实施方案中,步骤b)之后经活化载体(SCP)之活化程度为约15。在另一个实施方案中,步骤b)之后经活化载体(SCP)之活化程度为约20。在另一个实施方案中,步骤b)之后经活化载体(SCP)之活化程度为约26。在另一个实施方案中,步骤b)之后经活化载体(SCP)之活化程度为约30。在另一个实施方案中,步骤b)之后经活化载体(SCP)之活化程度为约35。在另一个实施方案中,步骤b)之后经活化载体(SCP)之活化程度为约37。在另一个实施方案中,步骤b)之后经活化载体(SCP)之活化程度为约40。在另一个实施方案中,步骤b)之后经活化载体(SCP)之活化程度为约45。在另一个实施方案中,步骤b)之后经活化载体(SCP)之活化程度为约50。

[0381] 在一个实施方案中,载体蛋白为TT或其片段。在该实施方案中,步骤b)之后经活化载体之活化程度可在1至30之间。在另一个实施方案中,步骤b)之后经活化载体(TT)之活化程度在5至25之间。在另一个实施方案中,步骤b)之后经活化载体(TT)之活化程度在7至25之间。在另一个实施方案中,步骤b)之后经活化载体(TT)之活化程度在10至20之间。在另一个实施方案中,步骤b)之后经活化载体(TT)之活化程度为约5。在另一个实施方案中,步骤b)之后经活化载体(TT)之活化程度为约7。在另一个实施方案中,步骤b)之后经活化载体(TT)之活化程度为约10。在另一个实施方案中,步骤b)之后经活化载体(TT)之活化程度为约12。在另一个实施方案中,步骤b)之后经活化载体(TT)之活化程度为约15。在另一个实施方案中,步骤b)之后经活化载体(TT)之活化程度为约20。在另一个实施方案中,步骤b)之后经活化载体(TT)之活化程度为约25。在另一个实施方案中,步骤b)之后经活化载体(TT)之活化程度为约30。

[0382] 在一个实施方案中,缀合反应c)系在水性缓冲液中进行。在一个实施方案中,缀合反应c)系在水性缓冲液中于作为催化剂之铜(I)存在下进行。在一个实施方案中,缀合反应c)系在水性缓冲液中在氧化剂及作为催化剂之铜(I)存在下进行。在一个优选实施方案中,缀合反应c)系在水性缓冲液中于作为催化剂之铜(I)及作为氧化剂之抗坏血酸盐存在下进行。在一个实施方案中,可进一步添加THPTA(三(3-羟丙基三唑基甲基)胺)及氨基胍以防止蛋白质发生副反应。因此,在一个优选实施方案中,缀合反应c)系在水性缓冲液中在作为催化剂之铜(I)及作为氧化剂之抗坏血酸盐存在下进行,其中反应混合物进一步包含THPTA(三(3-羟丙基三唑基甲基)胺)及氨基胍。

[0383] 在一个实施方案中,步骤c)处之经活化叠氮基糖与经活化炔烃-载体之初始输入比率(重量/重量)在0.1与3之间。在一个实施方案中,步骤c)处之经活化叠氮基糖与经活化炔烃-载体之初始输入比率(重量/重量)在0.5与2之间。在一个实施方案中,步骤c)处之经活化叠氮基糖与经活化炔烃-载体之初始输入比率(重量/重量)在0.6与1.5之间。在一个优选实施方案中,步骤c)处之经活化叠氮基糖与经活化炔烃-载体之初始输入比率(重量/重量)在0.8与1之间。在一个实施方案中,步骤c)处之经活化叠氮基糖与经活化炔烃-载体之初始输入比率(重量/重量)为约0.5。在一个实施方案中,步骤c)处之经活化叠氮基糖与经活化炔烃-载体之初始输入比率(重量/重量)为约0.6。在一个实施方案中,步骤c)处之经活化叠氮基糖与经活化炔烃-载体之初始输入比率(重量/重量)为约0.7。在一个实施方案中,步骤c)处之经活化叠氮基糖与经活化炔烃-载体之初始输入比率(重量/重量)为约0.8。在一个实施方案中,步骤c)处之经活化叠氮基糖与经活化炔烃-载体之初始输入比率(重量/重量)为约0.9。在一个实施方案中,步骤c)处之经活化叠氮基糖与经活化炔烃-载体之初始

输入比率(重量/重量)为约1。在一个实施方案中,步骤c)处之经活化叠氮基糖与经活化炔烃-载体之初始输入比率(重量/重量)为约1.1。在一个实施方案中,步骤c)处之经活化叠氮基糖与经活化炔烃-载体之初始输入比率(重量/重量)为约1.2。在一个实施方案中,步骤c)处之经活化叠氮基糖与经活化炔烃-载体之初始输入比率(重量/重量)为约1.3。在一个实施方案中,步骤c)处之经活化叠氮基糖与经活化炔烃-载体之初始输入比率(重量/重量)为约1.4。在一个实施方案中,步骤c)处之经活化叠氮基糖与经活化炔烃-载体之初始输入比率(重量/重量)为约1.5。在一个实施方案中,步骤c)处之经活化叠氮基糖与经活化炔烃-载体之初始输入比率(重量/重量)为约1.6。在一个实施方案中,步骤c)处之经活化叠氮基糖与经活化炔烃-载体之初始输入比率(重量/重量)为约1.7。在一个实施方案中,步骤c)处之经活化叠氮基糖与经活化炔烃-载体之初始输入比率(重量/重量)为约1.8。在一个实施方案中,步骤c)处之经活化叠氮基糖与经活化炔烃-载体之初始输入比率(重量/重量)为约1.9。在一个实施方案中,步骤c)处之经活化叠氮基糖与经活化炔烃-载体之初始输入比率(重量/重量)为约2。

[0384] 在点击缀合反应之后,缀合物中可能保留有未反应叠氮基,可使用适合的叠氮基封端剂对此等未反应叠氮基进行封端。因此,在一个实施方案中,在步骤c)之后,使用适合的叠氮基封端剂对缀合物中之未反应叠氮基进行封端。在一个实施方案中,此叠氮基封端剂为带有炔烃基之试剂。在一个实施方案中,此叠氮基封端剂为带有末端炔烃之试剂。在一个实施方案中,此叠氮基封端剂为带有环炔烃之试剂。

[0385] 在一个实施方案中,该叠氮基封端剂为式(V)化合物,

[0386] $\equiv\text{-X-OH}$ (V)

[0387] 其中X为 $(\text{CH}_2)_n$,其中n系选自1至15。

[0388] 在一个实施方案中,此叠氮基封端剂为炔丙醇。

[0389] 因此,在一个实施方案中,在步骤c)之后,该方法进一步包含用叠氮基封端剂对缀合物中保留之未反应叠氮基进行封端的步骤。

[0390] 在一个实施方案中,未反应叠氮基之封端系用相对于经活化糖之多糖重复单元的量为0.05至20摩尔当量之间的量的封端剂进行。

[0391] 在一个实施方案中,未反应叠氮基之封端系用相对于经活化糖之多糖重复单元的量为0.1至15摩尔当量之间的量的封端剂进行。

[0392] 在一个实施方案中,未反应叠氮基之封端系用相对于经活化糖之多糖重复单元的量为0.5至10摩尔当量之间的量的封端剂进行。

[0393] 在一个实施方案中,未反应叠氮基之封端系用相对于经活化糖之多糖重复单元的量为0.5至5摩尔当量之间的量的封端剂进行。

[0394] 在一个实施方案中,未反应叠氮基之封端系用相对于经活化糖之多糖重复单元的量为0.5至2摩尔当量之间的量的封端剂进行。

[0395] 在一个实施方案中,未反应叠氮基之封端系用相对于经活化糖之多糖重复单元的量为0.5至1摩尔当量之间的量的封端剂进行。

[0396] 在一个实施方案中,未反应叠氮基之封端系用相对于经活化糖之多糖重复单元的量为1至2摩尔当量之间的量的封端剂进行。

[0397] 在一个实施方案中,未反应叠氮基之封端系用相对于经活化糖之多糖重复单元的

量在0.75至1.5摩尔当量之间的量的封端剂进行。

[0398] 在一个实施方案中,未反应叠氮基之封端系用相对于经活化糖之多糖重复单元的量约1摩尔当量之量的封端剂进行。

[0399] 在一个实施方案中,未反应叠氮基之封端系用相对于经活化糖之多糖重复单元的量约1.5摩尔当量之量的封端剂进行。

[0400] 在一个实施方案中,未反应叠氮基之封端系用相对于经活化糖之多糖重复单元的量约0.5摩尔当量之量的封端剂进行。

[0401] 在一个实施方案中,未反应叠氮基之封端系用相对于经活化糖之多糖重复单元的量约2摩尔当量之量的封端剂进行。

[0402] 在点击缀合反应之后,未反应之炔烃基可仍存在于缀合物中,可使用适合的炔烃基封端剂对此等未反应之炔烃基进行封端。在一个实施方案中,此炔烃基封端剂为带有叠氮基之试剂。

[0403] 在一个实施方案中,该炔烃基封端剂为式(VI)化合物,

[0404] N_3-X-OH (VI)

[0405] 其中X为 $(CH_2)_n$,其中n系选自1至15。

[0406] 在一个实施方案中,此炔烃基封端剂为3-叠氮基-1-丙醇。

[0407] 因此,在一个实施方案中,在步骤c)之后,该方法进一步包含用炔烃基封端剂对缀合物中保留之未反应炔烃基进行封端的步骤。

[0408] 在一个实施方案中,未反应炔烃基之封端系用相对于经活化糖之多糖重复单元的量在0.05至20摩尔当量之间的量的封端剂进行。

[0409] 在一个实施方案中,未反应炔烃基之封端系用相对于经活化糖之多糖重复单元的量在0.1至15摩尔当量之间的量的封端剂进行。

[0410] 在一个实施方案中,未反应炔烃基之封端系用相对于经活化糖之多糖重复单元的量在0.5至10摩尔当量之间的量的封端剂进行。

[0411] 在一个实施方案中,未反应炔烃基之封端系用相对于经活化糖之多糖重复单元的量在0.5至5摩尔当量之间的量的封端剂进行。

[0412] 在一个实施方案中,未反应炔烃基之封端系用相对于经活化糖之多糖重复单元的量在0.5至2摩尔当量之间的量的封端剂进行。

[0413] 在一个实施方案中,未反应炔烃基之封端系用相对于经活化糖之多糖重复单元的量在0.5至1摩尔当量之间的量的封端剂进行。

[0414] 在一个实施方案中,未反应炔烃基之封端系用相对于经活化糖之多糖重复单元的量在1至5摩尔当量之间的量的封端剂进行。

[0415] 在一个实施方案中,未反应炔烃基之封端系用相对于经活化糖之多糖重复单元的量在1至2摩尔当量之间的量的封端剂进行。

[0416] 在一个实施方案中,未反应炔烃基之封端系用相对于经活化糖之多糖重复单元的量在1.5至2.5摩尔当量之间的量的封端剂进行。

[0417] 在一个实施方案中,未反应炔烃基之封端系用相对于经活化糖之多糖重复单元的量约0.5摩尔当量之量的封端剂进行。

[0418] 在一个实施方案中,未反应炔烃基之封端系用相对于经活化糖之多糖重复单元的

量约1摩尔当量之量的封端剂进行。

[0419] 在一个实施方案中,未反应炔烃基之封端系用相对于经活化糖之多糖重复单元的
量约1.5摩尔当量之量的封端剂进行。

[0420] 在一个实施方案中,未反应炔烃基之封端系用相对于经活化糖之多糖重复单元的
量约2摩尔当量之量的封端剂进行。

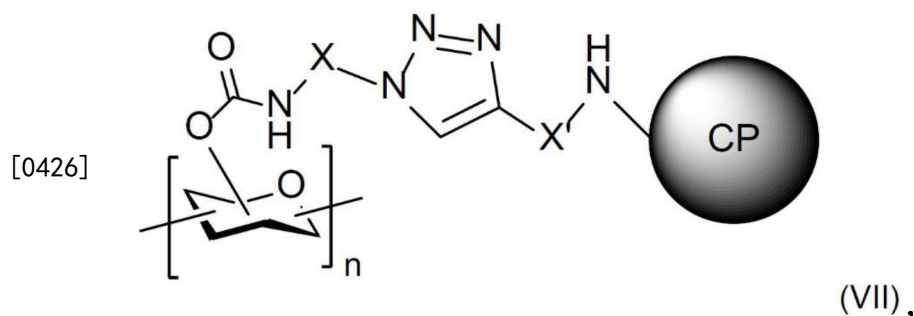
[0421] 在一个实施方案中,未反应炔烃基之封端系用相对于经活化糖之多糖重复单元的
量约2.5摩尔当量之量的封端剂进行。

[0422] 在一个实施方案中,未反应炔烃基之封端系用相对于经活化糖之多糖重复单元的
量约5摩尔当量之量的封端剂进行。

[0423] 在与载体蛋白缀合之后,可通过技术人员已知之多种技术来纯化(就糖-蛋白缀合物之量而言富集)糖缀合物。此等技术包括透析、浓缩/渗滤操作、切向流过滤沉淀/洗脱、柱层析(DEAE或疏水性相互作用层析)及深层过滤。因此,在一个实施方案中,用于产生本发明之糖缀合物的方法包含在产生糖缀合物之后对其进行纯化的步骤。

[0424] 在一方面中,本发明提供一种根据本文所公开之方法中之任一者产生的糖缀合物。

[0425] 在一方面中,本发明提供一种荚膜糖糖缀合物,其包含经由间隔子与载体蛋白(CP)共价缀合之荚膜糖且具有通式(VII):



[0427] 其中X系选自由以下组成的组: $\text{CH}_2(\text{CH}_2)_{n'}$ 、 $(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$ 、 $\text{NHCO}(\text{CH}_2)_{n'}$ 、 $\text{NHCO}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$ 、 $\text{OCH}_2(\text{CH}_2)_{n'}$ 及 $\text{O}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$;其中 n' 系选自1至10且 m 系选自1至4,

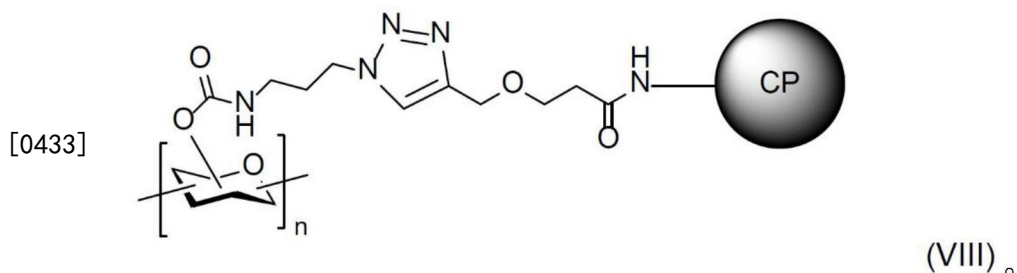
[0428] 且其中 X' 系选自由以下组成的组: $\text{CH}_2\text{O}(\text{CH}_2)_{n''}\text{CH}_2\text{C}=\text{O}$ 、 $\text{CH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_{m'}(\text{CH}_2)_{n''}\text{CH}_2\text{C}=\text{O}$,其中 n'' 系选自0至10且 m' 系选自0至4。其中方括号中之结构表示荚膜糖之重复单元。

[0429] 在一个实施方案中,荚膜糖不为来自肺炎链球菌血清型3之荚膜糖。

[0430] 式(VII)为本发明之糖缀合物之示意性表示。不应理解为糖之每一重复单元(方括号中之结构)处均存在连接。实情为,大部分糖重复单元保持未经修饰,且少数糖重复单元处存在载体蛋白与糖之共价连接。另外,个别载体蛋白(CP)分子可连接至多于一个糖分子,且个体糖分子可连接至多于一个个别载体蛋白(CP)分子。方括号中之结构表示荚膜糖之重复单元。

[0431] 在一个优选实施方案中,本发明提供一种糖缀合物,其包含经由间隔子与载体蛋白(CP)共价缀合之糖且具有通式(VII),其中X为 $\text{CH}_2(\text{CH}_2)_{n'}$,其中 n' 为2,且其中 X' 为 $\text{CH}_2\text{O}(\text{CH}_2)_{n''}\text{CH}_2\text{C}=\text{O}$,其中 n'' 为1。在一个实施方案中,糖不为来自肺炎链球菌血清型3之荚膜糖。

[0432] 因此,在一个优选实施方案中,本发明提供一种荚膜糖糖缀合物,其包含经由间隔子与载体蛋白(CP)共价缀合之荚膜糖且具有通式(VIII),



[0434] 其中方括号中之结构表示荚膜糖之重复单元。

[0435] 式(VIII)为本发明之优选糖缀合物之示意性表示。不应理解为糖之每一重复单元(方括号中之结构)处均存在连接。实情为,大部分糖重复单元保持未经修饰,且少数糖重复单元处存在载体蛋白与糖之共价连接。另外,个别载体蛋白(CP)分子可连接至多于一个糖分子,且个体糖分子可连接至多于一个个别载体蛋白(CP)分子。方括号中之结构为糖之重复单元的示意性表示。

[0436] 在一个实施方案中,糖不为来自肺炎链球菌血清型3之荚膜糖。

[0437] 在一个实施方案中,本发明提供一种糖缀合物,其包含经由间隔子与载体蛋白(CP)共价缀合之糖且具有通式(VII),其中X为 $\text{CH}_2(\text{CH}_2)_{n'}$,其中 n' 系选自1至10,且其中X'为 $\text{CH}_2\text{O}(\text{CH}_2)_{n''}\text{CH}_2\text{C}=\text{O}$,其中 n'' 系选自0至10。在一个实施方案中, n' 系选自1至5且 n'' 系选自0至10。在一个实施方案中, n' 系选自1至5且 n'' 系选自0至5。在一个实施方案中, n' 系选自1至3且 n'' 系选自0至3。在一个实施方案中, n' 系选自1至2且 n'' 系选自0至2。在一个特定实施方案中, n' 为1且 n'' 为0。在另一个实施方案中, n' 为2且 n'' 为0。在又一个实施方案中, n' 为3且 n'' 为0。在又一个实施方案中, n' 为4且 n'' 为0。在又一个实施方案中, n' 为5且 n'' 为0。在又一个实施方案中, n' 为6且 n'' 为0。在一个特定实施方案中, n' 为1且 n'' 为1。在另一个实施方案中, n' 为2且 n'' 为1。在又一个实施方案中, n' 为3且 n'' 为1。在又一个实施方案中, n' 为4且 n'' 为1。在又一个实施方案中, n' 为5且 n'' 为1。在又一个实施方案中, n' 为6且 n'' 为1。在一个特定实施方案中, n' 为1且 n'' 为2。在另一个实施方案中, n' 为2且 n'' 为2。在又一个实施方案中, n' 为3且 n'' 为2。在又一个实施方案中, n' 为4且 n'' 为2。在又一个实施方案中, n' 为5且 n'' 为2。在又一个实施方案中, n' 为6且 n'' 为2。在一个特定实施方案中, n' 为1且 n'' 为3。在另一个实施方案中, n' 为2且 n'' 为3。在又一个实施方案中, n' 为3且 n'' 为3。在又一个实施方案中, n' 为4且 n'' 为3。在又一个实施方案中, n' 为5且 n'' 为3。在又一个实施方案中, n' 为6且 n'' 为3。在一个特定实施方案中, n' 为1且 n'' 为4。在另一个实施方案中, n' 为2且 n'' 为4。在又一个实施方案中, n' 为3且 n'' 为4。在又一个实施方案中, n' 为4且 n'' 为4。在又一个实施方案中, n' 为5且 n'' 为4。在又一个实施方案中, n' 为6且 n'' 为4。在一个特定实施方案中, n' 为1且 n'' 为5。在另一个实施方案中, n' 为2且 n'' 为5。在又一个实施方案中, n' 为3且 n'' 为5。在又一个实施方案中, n' 为4且 n'' 为5。在又一个实施方案中, n' 为5且 n'' 为5。在又一个实施方案中, n' 为6且 n'' 为5。在一个特定实施方案中, n' 为1且 n'' 为6。在另一个实施方案中, n' 为2且 n'' 为6。在又一个实施方案中, n' 为3且 n'' 为6。在又一个实施方案中, n' 为4且 n'' 为6。在又一个实施方案中, n' 为5且 n'' 为6。在又一个实施方案中, n' 为6且 n'' 为6。优选地,糖不为来自肺炎链球菌血清型3之荚膜糖。

[0438] 在一个实施方案中,本发明提供一种糖缀合物,其包含经由间隔子与载体蛋白(CP)共价缀合之糖且具有通式(VII),其中X为 $\text{CH}_2(\text{CH}_2)_{n'}$,其中 n' 系选自1至10,且其中X'是

$\text{CH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_m(\text{CH}_2)_n\text{CH}_2\text{C}=\text{O}$, 其中 n' 系选自0至10且 m' 系选自0至4。

[0439] 在一个实施方案中, n' 系选自1至5, m' 系选自0至4,且 n'' 系选自0至10。在一个实施方案中, n' 系选自1至5, m' 系选自0至4,且 n'' 系选自0至5。在一个实施方案中, n' 系选自1至3, m' 系选自0至2,且 n'' 系选自0至3。在一个实施方案中, n' 系选自1至2, m' 系选自0至2,且 n'' 系选自0至1。

[0440] 在一个特定实施方案中, n' 为1, m' 为0,且 n'' 为0。在另一个实施方案中, n' 为1, m' 为1,且 n'' 为0。在另一个实施方案中, n' 为1, m' 为2,且 n'' 为0。在另一个实施方案中, n' 为1, m' 为3,且 n'' 为0。

[0441] 在另一个实施方案中, n' 为2, m' 为0,且 n'' 为0。在另一个实施方案中, n' 为2, m' 为1,且 n'' 为0。在另一个实施方案中, n' 为2, m' 为2,且 n'' 为0。在另一个实施方案中, n' 为2, m' 为3,且 n'' 为0。

[0442] 在又另一个实施方案中, n' 为3, m' 为0,且 n'' 为0。在又另一个实施方案中, n' 为3, m' 为1,且 n'' 为0。在又另一个实施方案中, n' 为3, m' 为2,且 n'' 为0。在又另一个实施方案中, n' 为3, m' 为3,且 n'' 为0。

[0443] 在又另一个实施方案中, n' 为4, m' 为0,且 n'' 为0。在又另一个实施方案中, n' 为4, m' 为1,且 n'' 为0。在又另一个实施方案中, n' 为4, m' 为2,且 n'' 为0。在又另一个实施方案中, n' 为4, m' 为3,且 n'' 为0。

[0444] 在又另一个实施方案中, n' 为5, m' 为0,且 n'' 为0。在又另一个实施方案中, n' 为5, m' 为1,且 n'' 为0。在又另一个实施方案中, n' 为5, m' 为2,且 n'' 为0。在又另一个实施方案中, n' 为5, m' 为3,且 n'' 为0。

[0445] 在一个特定实施方案中, n' 为1, m' 为0,且 n'' 为1。在一个特定实施方案中, n' 为1, m' 为1,且 n'' 为1。在一个特定实施方案中, n' 为1, m' 为2,且 n'' 为1。在一个特定实施方案中, n' 为1, m' 为3,且 n'' 为1。

[0446] 在另一个实施方案中, n' 为2, m' 为0,且 n'' 为1。在另一个实施方案中, n' 为2, m' 为1,且 n'' 为1。在另一个实施方案中, n' 为2, m' 为2,且 n'' 为1。在另一个实施方案中, n' 为2, m' 为3,且 n'' 为1。

[0447] 在又另一个实施方案中, n' 为3, m' 为0,且 n'' 为1。在又另一个实施方案中, n' 为3, m' 为1,且 n'' 为1。在又另一个实施方案中, n' 为3, m' 为2,且 n'' 为1。在又另一个实施方案中, n' 为3, m' 为3,且 n'' 为1。

[0448] 在又另一个实施方案中, n' 为4, m' 为0,且 n'' 为1。在又另一个实施方案中, n' 为4, m' 为1,且 n'' 为1。在又另一个实施方案中, n' 为4, m' 为2,且 n'' 为1。在又另一个实施方案中, n' 为4, m' 为3,且 n'' 为1。

[0449] 在又另一个实施方案中, n' 为5, m' 为0,且 n'' 为1。在又另一个实施方案中, n' 为5, m' 为1,且 n'' 为1。在又另一个实施方案中, n' 为5, m' 为2,且 n'' 为1。在又另一个实施方案中, n' 为5, m' 为3,且 n'' 为1。

[0450] 在一个特定实施方案中, n' 为1, m' 为0,且 n'' 为2。在一个特定实施方案中, n' 为1, m' 为1,且 n'' 为2。在一个特定实施方案中, n' 为1, m' 为2,且 n'' 为2。在一个特定实施方案中, n' 为1, m' 为3,且 n'' 为2。

[0451] 在另一个实施方案中, n' 为2, m' 为0,且 n'' 为2。在另一个实施方案中, n' 为2, m' 为

1,且n"为2。在另一个实施方案中,n'为2,m'为2,且n"为2。在另一个实施方案中,n'为2,m'为3,且n"为2。

[0452] 在又另一个实施方案中,n'为3,m'为0,且n"为2。在又另一个实施方案中,n'为3,m'为1,且n"为2。在又另一个实施方案中,n'为3,m'为2,且n"为2。在又另一个实施方案中,n'为3,m'为3,且n"为2。

[0453] 在又另一个实施方案中,n'为4,m'为0,且n"为2。在又另一个实施方案中,n'为4,m'为1,且n"为2。在又另一个实施方案中,n'为4,m'为2,且n"为2。在又另一个实施方案中,n'为4,m'为3,且n"为2。

[0454] 在又另一个实施方案中,n'为5,m'为0,且n"为2。在又另一个实施方案中,n'为5,m'为1,且n"为2。在又另一个实施方案中,n'为5,m'为2,且n"为2。在又另一个实施方案中,n'为5,m'为3,且n"为2。

[0455] 在一个特定实施方案中,n'为1,m'为0,且n"为3。在一个特定实施方案中,n'为1,m'为1,且n"为3。在一个特定实施方案中,n'为1,m'为2,且n"为3。在一个特定实施方案中,n'为1,m'为3,且n"为3。

[0456] 在另一个实施方案中,n'为2,m'为0,且n"为3。在另一个实施方案中,n'为2,m'为1,且n"为3。在另一个实施方案中,n'为2,m'为2,且n"为3。在另一个实施方案中,n'为2,m'为3,且n"为3。

[0457] 在又另一个实施方案中,n'为3,m'为0,且n"为3。在又另一个实施方案中,n'为3,m'为1,且n"为3。在又另一个实施方案中,n'为3,m'为2,且n"为3。在又另一个实施方案中,n'为3,m'为3,且n"为3。

[0458] 在又另一个实施方案中,n'为4,m'为0,且n"为3。在又另一个实施方案中,n'为4,m'为1,且n"为3。在又另一个实施方案中,n'为4,m'为2,且n"为3。在又另一个实施方案中,n'为4,m'为3,且n"为3。

[0459] 在又另一个实施方案中,n'为5,m'为0,且n"为3。在又另一个实施方案中,n'为5,m'为1,且n"为3。在又另一个实施方案中,n'为5,m'为2,且n"为3。在又另一个实施方案中,n'为5,m'为3,且n"为3。

[0460] 在一个特定实施方案中,n'为1,m'为0,且n"为4。在一个特定实施方案中,n'为1,m'为1,且n"为4。在一个特定实施方案中,n'为1,m'为2,且n"为4。在一个特定实施方案中,n'为1,m'为3,且n"为4。

[0461] 在另一个实施方案中,n'为2,m'为0,且n"为4。在另一个实施方案中,n'为2,m'为1,且n"为4。在另一个实施方案中,n'为2,m'为2,且n"为4。在另一个实施方案中,n'为2,m'为3,且n"为4。

[0462] 在又另一个实施方案中,n'为3,m'为0,且n"为4。在又另一个实施方案中,n'为3,m'为1,且n"为4。在又另一个实施方案中,n'为3,m'为2,且n"为4。在又另一个实施方案中,n'为3,m'为3,且n"为4。

[0463] 在又另一个实施方案中,n'为4,m'为0,且n"为4。在又另一个实施方案中,n'为4,m'为1,且n"为4。在又另一个实施方案中,n'为4,m'为2,且n"为4。在又另一个实施方案中,n'为4,m'为3,且n"为4。

[0464] 在又另一个实施方案中,n'为5,m'为0,且n"为4。在又另一个实施方案中,n'为5,

m'为1,且n"为4。在又一个实施方案中,n'为5,m'为2,且n"为4。在又一个实施方案中,n'为5,m'为3,且n"为4。

[0465] 在一个特定实施方案中,n'为1,m'为0,且n"为5。在一个特定实施方案中,n'为1,m'为1,且n"为5。在一个特定实施方案中,n'为1,m'为2,且n"为5。在一个特定实施方案中,n'为1,m'为3,且n"为5。

[0466] 在另一个实施方案中,n'为2,m'为0,且n"为5。在另一个实施方案中,n'为2,m'为1,且n"为5。在另一个实施方案中,n'为2,m'为2,且n"为5。在另一个实施方案中,n'为2,m'为3,且n"为5。

[0467] 在又一个实施方案中,n'为3,m'为0,且n"为5。在又一个实施方案中,n'为3,m'为1,且n"为5。在又一个实施方案中,n'为3,m'为2,且n"为5。在又一个实施方案中,n'为3,m'为3,且n"为5。

[0468] 在又一个实施方案中,n'为4,m'为0,且n"为5。在又一个实施方案中,n'为4,m'为1,且n"为5。在又一个实施方案中,n'为4,m'为2,且n"为5。在又一个实施方案中,n'为4,m'为3,且n"为5。

[0469] 在又一个实施方案中,n'为5,m'为0,且n"为5。在又一个实施方案中,n'为5,m'为1,且n"为5。在又一个实施方案中,n'为5,m'为2,且n"为5。在又一个实施方案中,n'为5,m'为3,且n"为5。

[0470] 优选地,糖不为来自肺炎链球菌血清型3之荚膜糖。

[0471] 在一个实施方案中,本发明提供一种糖缀合物,其包含经由间隔子与载体蛋白(CP)共价缀合之糖且具有通式(VII),其中X为 $(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$,其中m系选自1至4,且其中X'为 $\text{CH}_2\text{O}(\text{CH}_2)_n\text{CH}_2\text{C}=\text{O}$,其中n"系选自0至10。在一个实施方案中,m系选自1至3,且n"系选自0至10。在一个实施方案中,m系选自1至3,且n"系选自0至5。在一个实施方案中,m系选自1至2,且n"系选自0至3。在一个实施方案中,m系选自1至2,且n"系选自0至2。在一个特定实施方案中,m为1,且n"为0。在另一个实施方案中,m为2,且n"为0。在又一个实施方案中,m为3,且n"为0。在又一个实施方案中,m为4,且n"为0。在一个特定实施方案中,m为1,且n"为1。在另一个实施方案中,m为2,且n"为1。在又一个实施方案中,m为3,且n"为1。在又一个实施方案中,m为4,且n"为1。在一个特定实施方案中,m为1,且n"为2。在另一个实施方案中,m为2,且n"为2。在又一个实施方案中,m为3,且n"为2。在又一个实施方案中,m为4,且n"为2。在一个特定实施方案中,m为1,且n"为3。在另一个实施方案中,m为2,且n"为3。在又一个实施方案中,m为3,且n"为3。在又一个实施方案中,m为4,且n"为3。在一个特定实施方案中,m为1,且n"为4。在另一个实施方案中,m为2,且n"为4。在又一个实施方案中,m为3,且n"为4。在又一个实施方案中,m为4,且n"为4。在一个特定实施方案中,m为1,且n"为5。在另一个实施方案中,m为2,且n"为5。在又一个实施方案中,m为3,且n"为5。在又一个实施方案中,m为4,且n"为5。在一个特定实施方案中,m为1,且n"为6。在另一个实施方案中,m为2,且n"为6。在又一个实施方案中,m为3,且n"为6。在又一个实施方案中,m为4,且n"为6。优选地,糖不为来自肺炎链球菌血清型3之荚膜糖。

[0472] 在一个实施方案中,本发明提供一种糖缀合物,其包含经由间隔子与载体蛋白(CP)共价缀合之糖且具有通式(VII),其中X为 $(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$,其中m系选自1至4,且其中X'为 $\text{CH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_m(\text{CH}_2)_n\text{CH}_2\text{C}=\text{O}$,其中n"系选自0至10且m'系选自0至4。

[0473] 在一个实施方案中,m系选自1至3,m'系选自0至4,且n"系选自0至10。在一个实施方案中,m系选自1至2,m'系选自0至4,且n"系选自0至5。在一个实施方案中,m系选自1至2,m'系选自0至2,且n"系选自0至3。在一个实施方案中,m系选自1至2,m'系选自0至2,且n"系选自0至1。

[0474] 在一个特定实施方案中,m为1,m'为0,且n"为0。在另一个实施方案中,m为1,m'为1,且n"为0。在另一个实施方案中,m为1,m'为2,且n"为0。在另一个实施方案中,m为1,m'为3,且n"为0。

[0475] 在另一个实施方案中,m为2,m'为0,且n"为0。在另一个实施方案中,m为2,m'为1,且n"为0。在另一个实施方案中,m为2,m'为2,且n"为0。在另一个实施方案中,m为2,m'为3,且n"为0。

[0476] 在又另一个实施方案中,m为3,m'为0,且n"为0。在又另一个实施方案中,m为3,m'为1,且n"为0。在又另一个实施方案中,m为3,m'为2,且n"为0。在又另一个实施方案中,m为3,m'为3,且n"为0。

[0477] 在又另一个实施方案中,m为4,m'为0,且n"为0。在又另一个实施方案中,m为4,m'为1,且n"为0。在又另一个实施方案中,m为4,m'为2,且n"为0。在又另一个实施方案中,m为4,m'为3,且n"为0。

[0478] 在一个特定实施方案中,m为1,m'为0,且n"为1。在一个特定实施方案中,m为1,m'为1,且n"为1。在一个特定实施方案中,m为1,m'为2,且n"为1。在一个特定实施方案中,m为1,m'为3,且n"为1。

[0479] 在另一个实施方案中,m为2,m'为0,且n"为1。在另一个实施方案中,m为2,m'为1,且n"为1。在另一个实施方案中,m为2,m'为2,且n"为1。在另一个实施方案中,m为2,m'为3,且n"为1。

[0480] 在又另一个实施方案中,m为3,m'为0,且n"为1。在又另一个实施方案中,m为3,m'为1,且n"为1。在又另一个实施方案中,m为3,m'为2,且n"为1。在又另一个实施方案中,m为3,m'为3,且n"为1。

[0481] 在又另一个实施方案中,m为4,m'为0,且n"为1。在又另一个实施方案中,m为4,m'为1,且n"为1。在又另一个实施方案中,m为4,m'为2,且n"为1。在又另一个实施方案中,m为4,m'为3,且n"为1。

[0482] 在一个特定实施方案中,m为1,m'为0,且n"为2。在一个特定实施方案中,m为1,m'为1,且n"为2。在一个特定实施方案中,m为1,m'为2,且n"为2。在一个特定实施方案中,m为1,m'为3,且n"为2。

[0483] 在另一个实施方案中,m为2,m'为0,且n"为2。在另一个实施方案中,m为2,m'为1,且n"为2。在另一个实施方案中,m为2,m'为2,且n"为2。在另一个实施方案中,m为2,m'为3,且n"为2。

[0484] 在又另一个实施方案中,m为3,m'为0,且n"为2。在又另一个实施方案中,m为3,m'为1,且n"为2。在又另一个实施方案中,m为3,m'为2,且n"为2。在又另一个实施方案中,m为3,m'为3,且n"为2。

[0485] 在又另一个实施方案中,m为4,m'为0,且n"为2。在又另一个实施方案中,m为4,m'为1,且n"为2。在又另一个实施方案中,m为4,m'为2,且n"为2。在又另一个实施方案中,m为

4, m' 为 3, 且 n'' 为 2。

[0486] 在一个特定实施方案中, m 为 1, m' 为 0, 且 n'' 为 3。在一个特定实施方案中, m 为 1, m' 为 1, 且 n'' 为 3。在一个特定实施方案中, m 为 1, m' 为 2, 且 n'' 为 3。在一个特定实施方案中, m 为 1, m' 为 3, 且 n'' 为 3。

[0487] 在另一个实施方案中, m 为 2, m' 为 0, 且 n'' 为 3。在另一个实施方案中, m 为 2, m' 为 1, 且 n'' 为 3。在另一个实施方案中, m 为 2, m' 为 2, 且 n'' 为 3。在另一个实施方案中, m 为 2, m' 为 3, 且 n'' 为 3。

[0488] 在又一个实施方案中, m 为 3, m' 为 0, 且 n'' 为 3。在又一个实施方案中, m 为 3, m' 为 1, 且 n'' 为 3。在又一个实施方案中, m 为 3, m' 为 2, 且 n'' 为 3。在又一个实施方案中, m 为 3, m' 为 3, 且 n'' 为 3。

[0489] 在又一个实施方案中, m 为 4, m' 为 0, 且 n'' 为 3。在又一个实施方案中, m 为 4, m' 为 1, 且 n'' 为 3。在又一个实施方案中, m 为 4, m' 为 2, 且 n'' 为 3。在又一个实施方案中, m 为 4, m' 为 3, 且 n'' 为 3。

[0490] 在一个特定实施方案中, m 为 1, m' 为 0, 且 n'' 为 4。在一个特定实施方案中, m 为 1, m' 为 1, 且 n'' 为 4。在一个特定实施方案中, m 为 1, m' 为 2, 且 n'' 为 4。在一个特定实施方案中, m 为 1, m' 为 3, 且 n'' 为 4。

[0491] 在另一个实施方案中, m 为 2, m' 为 0, 且 n'' 为 4。在另一个实施方案中, m 为 2, m' 为 1, 且 n'' 为 4。在另一个实施方案中, m 为 2, m' 为 2, 且 n'' 为 4。在另一个实施方案中, m 为 2, m' 为 3, 且 n'' 为 4。

[0492] 在又一个实施方案中, m 为 3, m' 为 0, 且 n'' 为 4。在又一个实施方案中, m 为 3, m' 为 1, 且 n'' 为 4。在又一个实施方案中, m 为 3, m' 为 2, 且 n'' 为 4。在又一个实施方案中, m 为 3, m' 为 3, 且 n'' 为 4。

[0493] 在又一个实施方案中, m 为 4, m' 为 0, 且 n'' 为 4。在又一个实施方案中, m 为 4, m' 为 1, 且 n'' 为 4。在又一个实施方案中, m 为 4, m' 为 2, 且 n'' 为 4。在又一个实施方案中, m 为 4, m' 为 3, 且 n'' 为 4。

[0494] 在一个特定实施方案中, m 为 1, m' 为 0, 且 n'' 为 5。在一个特定实施方案中, m 为 1, m' 为 1, 且 n'' 为 5。在一个特定实施方案中, m 为 1, m' 为 2, 且 n'' 为 5。在一个特定实施方案中, m 为 1, m' 为 3, 且 n'' 为 5。

[0495] 在另一个实施方案中, m 为 2, m' 为 0, 且 n'' 为 5。在另一个实施方案中, m 为 2, m' 为 1, 且 n'' 为 5。在另一个实施方案中, m 为 2, m' 为 2, 且 n'' 为 5。在另一个实施方案中, m 为 2, m' 为 3, 且 n'' 为 5。

[0496] 在又一个实施方案中, m 为 3, m' 为 0, 且 n'' 为 5。在又一个实施方案中, m 为 3, m' 为 1, 且 n'' 为 5。在又一个实施方案中, m 为 3, m' 为 2, 且 n'' 为 5。在又一个实施方案中, m 为 3, m' 为 3, 且 n'' 为 5。

[0497] 在又一个实施方案中, m 为 4, m' 为 0, 且 n'' 为 5。在又一个实施方案中, m 为 4, m' 为 1, 且 n'' 为 5。在又一个实施方案中, m 为 4, m' 为 2, 且 n'' 为 5。在又一个实施方案中, m 为 4, m' 为 3, 且 n'' 为 5。

[0498] 优选地, 糖不为来自肺炎链球菌血清型 3 之荚膜糖。

[0499] 在一个实施方案中, 本发明提供一种糖缀合物, 其包含经由间隔子与载体蛋白

(CP) 共价缀合之糖且具有通式 (VII), 其中X为 $\text{NHC}(\text{CH}_2)_n$, 其中n'系选自1至10, 且其中X'为 $\text{CH}_2\text{O}(\text{CH}_2)_m\text{CH}_2\text{C}=\text{O}$, 其中n"系选自0至10。在一个实施方案中, n'系选自1至5, 且n"系选自0至10。在一个实施方案中, n'系选自1至5, 且n"系选自0至5。在一个实施方案中, n'系选自1至3, 且n"系选自0至3。在一个实施方案中, n'系选自1至2, 且n"系选自0至2。在一个特定实施方案中, n'为1, 且n"为0。在另一个实施方案中, n'为2, 且n"为0。在又一个实施方案中, n'为3, 且n"为0。在又一个实施方案中, n'为4, 且n"为0。在又一个实施方案中, n'为5, 且n"为0。在又一个实施方案中, n'为6, 且n"为0。在一个特定实施方案中, n'为1, 且n"为1。在另一个实施方案中, n'为2, 且n"为1。在又一个实施方案中, n'为3, 且n"为1。在又一个实施方案中, n'为4, 且n"为1。在又一个实施方案中, n'为5, 且n"为1。在又一个实施方案中, n'为6, 且n"为1。在一个特定实施方案中, n'为1, 且n"为2。在另一个实施方案中, n'为2, 且n"为2。在又一个实施方案中, n'为3, 且n"为2。在又一个实施方案中, n'为4, 且n"为2。在又一个实施方案中, n'为5, 且n"为2。在又一个实施方案中, n'为6, 且n"为2。在一个特定实施方案中, n'为1, 且n"为3。在另一个实施方案中, n'为2, 且n"为3。在又一个实施方案中, n'为3, 且n"为3。在又一个实施方案中, n'为4, 且n"为3。在又一个实施方案中, n'为5, 且n"为3。在又一个实施方案中, n'为6, 且n"为3。在一个特定实施方案中, n'为1, 且n"为4。在另一个实施方案中, n'为2, 且n"为4。在又一个实施方案中, n'为3, 且n"为4。在又一个实施方案中, n'为4, 且n"为4。在又一个实施方案中, n'为5, 且n"为4。在又一个实施方案中, n'为6, 且n"为4。在一个特定实施方案中, n'为1, 且n"为5。在另一个实施方案中, n'为2, 且n"为5。在又一个实施方案中, n'为3, 且n"为5。在又一个实施方案中, n'为4, 且n"为5。在又一个实施方案中, n'为5, 且n"为5。在又一个实施方案中, n'为6, 且n"为5。在一个特定实施方案中, n'为1, 且n"为6。在另一个实施方案中, n'为2, 且n"为6。在又一个实施方案中, n'为3, 且n"为6。在又一个实施方案中, n'为4, 且n"为6。在又一个实施方案中, n'为5, 且n"为6。在又一个实施方案中, n'为6, 且n"为6。优选地, 糖不为来自肺炎链球菌血清型3之荚膜糖。

[0500] 在一个实施方案中, 本发明提供一种糖缀合物, 其包含经由间隔子与载体蛋白 (CP) 共价缀合之糖且具有通式 (VII), 其中X为 $\text{NHC}(\text{CH}_2)_n$, 其中n'系选自1至10, 且其中X'为 $\text{CH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_m(\text{CH}_2)_n\text{CH}_2\text{C}=\text{O}$, 其中n"系选自0至10且m'系选自0至4。

[0501] 在一个实施方案中, n'系选自1至5, m'系选自0至4, 且n"系选自0至10。在一个实施方案中, n'系选自1至5, m'系选自0至4, 且n"系选自0至5。在一个实施方案中, n'系选自1至3, m'系选自0至2, 且n"系选自0至3。在一个实施方案中, n'系选自1至2, m'系选自0至2, 且n"系选自0至1。

[0502] 在一个特定实施方案中, n'为1, m'为0, 且n"为0。在另一个实施方案中, n'为1, m'为1, 且n"为0。在另一个实施方案中, n'为1, m'为2, 且n"为0。在另一个实施方案中, n'为1, m'为3, 且n"为0。

[0503] 在另一个实施方案中, n'为2, m'为0, 且n"为0。在另一个实施方案中, n'为2, m'为1, 且n"为0。在另一个实施方案中, n'为2, m'为2, 且n"为0。在另一个实施方案中, n'为2, m'为3, 且n"为0。

[0504] 在又一个实施方案中, n'为3, m'为0, 且n"为0。在又一个实施方案中, n'为3, m'为1, 且n"为0。在又一个实施方案中, n'为3, m'为2, 且n"为0。在又一个实施方案中,

n' 为 3, m' 为 3, 且 n'' 为 0。

[0505] 在又另一个实施方案中, n' 为 4, m' 为 0, 且 n'' 为 0。在又另一个实施方案中, n' 为 4, m' 为 1, 且 n'' 为 0。在又另一个实施方案中, n' 为 4, m' 为 2, 且 n'' 为 0。在又另一个实施方案中, n' 为 4, m' 为 3, 且 n'' 为 0。

[0506] 在又一个实施方案中, n' 为 5, m' 为 0, 且 n'' 为 0。在又一个实施方案中, n' 为 5, m' 为 1, 且 n'' 为 0。在又一个实施方案中, n' 为 5, m' 为 2, 且 n'' 为 0。在又一个实施方案中, n' 为 5, m' 为 3, 且 n'' 为 0。

[0507] 在一个特定实施方案中, n' 为1, m' 为0, 且 n'' 为1。在一个特定实施方案中, n' 为1, m' 为1, 且 n'' 为1。在一个特定实施方案中, n' 为1, m' 为2, 且 n'' 为1。在一个特定实施方案中, n' 为1, m' 为3, 且 n'' 为1。

[0508] 在另一个实施方案中,n'为2,m'为0,且n"为1。在另一个实施方案中,n'为2,m'为1,且n"为1。在另一个实施方案中,n'为2,m'为2,且n"为1。在另一个实施方案中,n'为2,m'为3,且n"为1。

[0509] 在又一个实施方案中, n' 为 3, m' 为 0, 且 n'' 为 1。在又一个实施方案中, n' 为 3, m' 为 1, 且 n'' 为 1。在又一个实施方案中, n' 为 3, m' 为 2, 且 n'' 为 1。在又一个实施方案中, n' 为 3, m' 为 3, 且 n'' 为 1。

[0510] 在又一个实施方案中,n'为4,m'为0,且n"为1。在又一个实施方案中,n'为4,m'为1,且n"为1。在又一个实施方案中,n'为4,m'为2,且n"为1。在又一个实施方案中,n'为4,m'为3,且n"为1。

[0511] 在又一个实施方案中, n' 为 5, m' 为 0, 且 n'' 为 1。在又一个实施方案中, n' 为 5, m' 为 1, 且 n'' 为 1。在又一个实施方案中, n' 为 5, m' 为 2, 且 n'' 为 1。在又一个实施方案中, n' 为 5, m' 为 3, 且 n'' 为 1。

[0512] 在一个特定实施方案中,n'为1,m'为0,且n"为2。在一个特定实施方案中,n'为1,m'为1,且n"为2。在一个特定实施方案中,n'为1,m'为2,且n"为2。在一个特定实施方案中,n'为1,m'为3,且n"为2。

[0513] 在另一个实施方案中, n' 为 2, m' 为 0, 且 n'' 为 2。在另一个实施方案中, n' 为 2, m' 为 1, 且 n'' 为 2。在另一个实施方案中, n' 为 2, m' 为 2, 且 n'' 为 2。在另一个实施方案中, n' 为 2, m' 为 3, 且 n'' 为 2。

[0514] 在又一个实施方案中, n' 为3, m' 为0, 且 n'' 为2。在又一个实施方案中, n' 为3, m' 为1, 且 n'' 为2。在又一个实施方案中, n' 为3, m' 为2, 且 n'' 为2。在又一个实施方案中, n' 为3, m' 为3, 且 n'' 为2。

[0515] 在又另一个实施方案中,n'为4,m'为0,且n"为2。在又另一个实施方案中,n'为4,m'为1,且n"为2。在又另一个实施方案中,n'为4,m'为2,且n"为2。在又另一个实施方案中,n'为4,m'为3,且n"为2。

[0516] 在又一个实施方案中,n'为5,m'为0,且n"为2。在又一个实施方案中,n'为5,m'为1,且n"为2。在又一个实施方案中,n'为5,m'为2,且n"为2。在又一个实施方案中,n'为5,m'为3,且n"为2。

[0517] 在一个特定实施方案中, n' 为 1, m' 为 0, 且 n'' 为 3。在一个特定实施方案中, n' 为 1, m' 为 1, 且 n'' 为 3。在一个特定实施方案中, n' 为 1, m' 为 2, 且 n'' 为 3。在一个特定实施方案中,

n' 为 1, m' 为 3, 且 n'' 为 3。

[0518] 在另一个实施方案中, n' 为 2, m' 为 0, 且 n'' 为 3。在另一个实施方案中, n' 为 2, m' 为 1, 且 n'' 为 3。在另一个实施方案中, n' 为 2, m' 为 2, 且 n'' 为 3。在另一个实施方案中, n' 为 2, m' 为 3, 且 n'' 为 3。

[0519] 在又一个实施方案中, n' 为3, m' 为0, 且 n'' 为3。在又一个实施方案中, n' 为3, m' 为1, 且 n'' 为3。在又一个实施方案中, n' 为3, m' 为2, 且 n'' 为3。在又一个实施方案中, n' 为3, m' 为3, 且 n'' 为3。

[0520] 在又一个实施方案中, n' 为4, m' 为0, 且 n'' 为3。在又一个实施方案中, n' 为4, m' 为1, 且 n'' 为3。在又一个实施方案中, n' 为4, m' 为2, 且 n'' 为3。在又一个实施方案中, n' 为4, m' 为3, 且 n'' 为3。

[0521] 在又一个实施方案中, n' 为 5, m' 为 0, 且 n'' 为 3。在又一个实施方案中, n' 为 5, m' 为 1, 且 n'' 为 3。在又一个实施方案中, n' 为 5, m' 为 2, 且 n'' 为 3。在又一个实施方案中, n' 为 5, m' 为 3, 且 n'' 为 3。

[0522] 在一个特定实施方案中,n'为1,m'为0,且n"为4。在一个特定实施方案中,n'为1,m'为1,且n"为4。在一个特定实施方案中,n'为1,m'为2,且n"为4。在一个特定实施方案中,n'为1,m'为3,且n"为4。

[0523] 在另一个实施方案中, n' 为 2, m' 为 0, 且 n'' 为 4。在另一个实施方案中, n' 为 2, m' 为 1, 且 n'' 为 4。在另一个实施方案中, n' 为 2, m' 为 2, 且 n'' 为 4。在另一个实施方案中, n' 为 2, m' 为 3, 且 n'' 为 4。

[0524] 在又一个实施方案中, n' 为3, m' 为0, 且 n'' 为4。在又一个实施方案中, n' 为3, m' 为1, 且 n'' 为4。在又一个实施方案中, n' 为3, m' 为2, 且 n'' 为4。在又一个实施方案中, n' 为3, m' 为3, 且 n'' 为4。

[0525] 在又一个实施方案中, n' 为4, m' 为0, 且 n'' 为4。在又一个实施方案中, n' 为4, m' 为1, 且 n'' 为4。在又一个实施方案中, n' 为4, m' 为2, 且 n'' 为4。在又一个实施方案中, n' 为4, m' 为3, 且 n'' 为4。

[0526] 在又另一个实施方案中,n'为5,m'为0,且n"为4。在又另一个实施方案中,n'为5,m'为1,且n"为4。在又另一个实施方案中,n'为5,m'为2,且n"为4。在又另一个实施方案中,n'为5,m'为3,且n"为4。

[0527] 在一个特定实施方案中,n'为1,m'为0,且n"为5。在一个特定实施方案中,n'为1,m'为1,且n"为5。在一个特定实施方案中,n'为1,m'为2,且n"为5。在一个特定实施方案中,n'为1,m'为3,且n"为5。

[0528] 在另一个实施方案中, n' 为 2, m' 为 0, 且 n'' 为 5。在另一个实施方案中, n' 为 2, m' 为 1, 且 n'' 为 5。在另一个实施方案中, n' 为 2, m' 为 2, 且 n'' 为 5。在另一个实施方案中, n' 为 2, m' 为 3, 且 n'' 为 5。

[0529] 在又一个实施方案中, n' 为3, m' 为0, 且 n'' 为5。在又一个实施方案中, n' 为3, m' 为1, 且 n'' 为5。在又一个实施方案中, n' 为3, m' 为2, 且 n'' 为5。在又一个实施方案中, n' 为3, m' 为3, 且 n'' 为5。

[0530] 在又一个实施方案中, n' 为4, m' 为0, 且 n'' 为5。在又一个实施方案中, n' 为4, m' 为1, 且 n'' 为5。在又一个实施方案中, n' 为4, m' 为2, 且 n'' 为5。在又一个实施方案中,

n'为4,m'为3,且n"为5。

[0531] 在又一个实施方案中,n'为5,m'为0,且n"为5。在又一个实施方案中,n'为5,m'为1,且n"为5。在又一个实施方案中,n'为5,m'为2,且n"为5。在又一个实施方案中,n'为5,m'为3,且n"为5。

[0532] 优选地,糖不为来自肺炎链球菌血清型3之荚膜糖。

[0533] 在一个实施方案中,本发明提供一种糖缀合物,其包含经由间隔子与载体蛋白(CP)共价缀合之糖且具有通式(VII),其中X为 $\text{NHCO}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$,其中m系选自1至4,且其中X'为 $\text{CH}_2\text{O}(\text{CH}_2)_n\text{CH}_2\text{C}=\text{O}$,其中n"系选自0至10。在一个实施方案中,m系选自1至3,且n"系选自0至10。在一个实施方案中,m系选自1至3,且n"系选自0至5。在一个实施方案中,m系选自1至2,且n"系选自0至3。在一个实施方案中,m系选自1至2,且n"系选自0至2。在一个特定实施方案中,m为1,且n"为0。在另一个实施方案中,m为2,且n"为0。在又一个实施方案中,m为3,且n"为0。在又一个实施方案中,m为4,且n"为0。在一个特定实施方案中,m为1,且n"为1。在另一个实施方案中,m为2,且n"为1。在又一个实施方案中,m为3,且n"为1。在又一个实施方案中,m为4,且n"为1。在一个特定实施方案中,m为1,且n"为2。在另一个实施方案中,m为2,且n"为2。在又一个实施方案中,m为3,且n"为2。在又一个实施方案中,m为4,且n"为2。在一个特定实施方案中,m为1,且n"为3。在另一个实施方案中,m为2,且n"为3。在又一个实施方案中,m为3,且n"为3。在又一个实施方案中,m为4,且n"为3。在一个特定实施方案中,m为1,且n"为4。在另一个实施方案中,m为2,且n"为4。在又一个实施方案中,m为3,且n"为4。在又一个实施方案中,m为4,且n"为4。在一个特定实施方案中,m为1,且n"为5。在另一个实施方案中,m为2,且n"为5。在又一个实施方案中,m为3,且n"为5。在又一个实施方案中,m为4,且n"为5。在一个特定实施方案中,m为1,且n"为6。在另一个实施方案中,m为2,且n"为6。在又一个实施方案中,m为3,且n"为6。在又一个实施方案中,m为4,且n"为6。优选地,糖不为来自肺炎链球菌血清型3之荚膜糖。

[0534] 在一个实施方案中,本发明提供一种糖缀合物,其包含经由间隔子与载体蛋白(CP)共价缀合之糖且具有通式(VII),其中X为 $\text{NHCO}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$,其中m系选自1至4,且其中X'为 $\text{CH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_{m'}(\text{CH}_2)_n\text{CH}_2\text{C}=\text{O}$,其中n"系选自0至10且m'系选自0至4。

[0535] 在一个实施方案中,m系选自1至3,m'系选自0至4,且n"系选自0至10。在一个实施方案中,m系选自1至2,m'系选自0至4,且n"系选自0至5。在一个实施方案中,m系选自1至2,m'系选自0至2,且n"系选自0至3。在一个实施方案中,m系选自1至2,m'系选自0至2,且n"系选自0至1。

[0536] 在一个特定实施方案中,m为1,m'为0,且n"为0。在另一个实施方案中,m为1,m'为1,且n"为0。在另一个实施方案中,m为1,m'为2,且n"为0。在另一个实施方案中,m为1,m'为3,且n"为0。

[0537] 在另一个实施方案中,m为2,m'为0,且n"为0。在另一个实施方案中,m为2,m'为1,且n"为0。在另一个实施方案中,m为2,m'为2,且n"为0。在另一个实施方案中,m为2,m'为3,且n"为0。

[0538] 在又一个实施方案中,m为3,m'为0,且n"为0。在又一个实施方案中,m为3,m'为1,且n"为0。在又一个实施方案中,m为3,m'为2,且n"为0。在又一个实施方案中,m为3,m'为3,且n"为0。

[0539] 在又一个实施方案中,m为4,m'为0,且n"为0。在又一个实施方案中,m为4,m'为1,且n"为0。在又一个实施方案中,m为4,m'为2,且n"为0。在又一个实施方案中,m为4,m'为3,且n"为0。

[0540] 在一个特定实施方案中,m为1,m'为0,且n"为1。在一个特定实施方案中,m为1,m'为1,且n"为1。在一个特定实施方案中,m为1,m'为2,且n"为1。在一个特定实施方案中,m为1,m'为3,且n"为1。

[0541] 在另一个实施方案中,m为2,m'为0,且n"为1。在另一个实施方案中,m为2,m'为1,且n"为1。在另一个实施方案中,m为2,m'为2,且n"为1。在另一个实施方案中,m为2,m'为3,且n"为1。

[0542] 在又一个实施方案中,m为3,m'为0,且n"为1。在又一个实施方案中,m为3,m'为1,且n"为1。在又一个实施方案中,m为3,m'为2,且n"为1。在又一个实施方案中,m为3,m'为3,且n"为1。

[0543] 在又一个实施方案中,m为4,m'为0,且n"为1。在又一个实施方案中,m为4,m'为1,且n"为1。在又一个实施方案中,m为4,m'为2,且n"为1。在又一个实施方案中,m为4,m'为3,且n"为1。

[0544] 在一个特定实施方案中,m为1,m'为0,且n"为2。在一个特定实施方案中,m为1,m'为1,且n"为2。在一个特定实施方案中,m为1,m'为2,且n"为2。在一个特定实施方案中,m为1,m'为3,且n"为2。

[0545] 在另一个实施方案中,m为2,m'为0,且n"为2。在另一个实施方案中,m为2,m'为1,且n"为2。在另一个实施方案中,m为2,m'为2,且n"为2。在另一个实施方案中,m为2,m'为3,且n"为2。

[0546] 在又一个实施方案中,m为3,m'为0,且n"为2。在又一个实施方案中,m为3,m'为1,且n"为2。在又一个实施方案中,m为3,m'为2,且n"为2。在又一个实施方案中,m为3,m'为3,且n"为2。

[0547] 在又一个实施方案中,m为4,m'为0,且n"为2。在又一个实施方案中,m为4,m'为1,且n"为2。在又一个实施方案中,m为4,m'为2,且n"为2。在又一个实施方案中,m为4,m'为3,且n"为2。

[0548] 在一个特定实施方案中,m为1,m'为0,且n"为3。在一个特定实施方案中,m为1,m'为1,且n"为3。在一个特定实施方案中,m为1,m'为2,且n"为3。在一个特定实施方案中,m为1,m'为3,且n"为3。

[0549] 在另一个实施方案中,m为2,m'为0,且n"为3。在另一个实施方案中,m为2,m'为1,且n"为3。在另一个实施方案中,m为2,m'为2,且n"为3。在另一个实施方案中,m为2,m'为3,且n"为3。

[0550] 在又一个实施方案中,m为3,m'为0,且n"为3。在又一个实施方案中,m为3,m'为1,且n"为3。在又一个实施方案中,m为3,m'为2,且n"为3。在又一个实施方案中,m为3,m'为3,且n"为3。

[0551] 在又一个实施方案中,m为4,m'为0,且n"为3。在又一个实施方案中,m为4,m'为1,且n"为3。在又一个实施方案中,m为4,m'为2,且n"为3。在又一个实施方案中,m为4,m'为3,且n"为3。

[0552] 在一个特定实施方案中,m为1,m'为0,且n"为4。在一个特定实施方案中,m为1,m'为1,且n"为4。在一个特定实施方案中,m为1,m'为2,且n"为4。在一个特定实施方案中,m为1,m'为3,且n"为4。

[0553] 在另一个实施方案中,m为2,m'为0,且n"为4。在另一个实施方案中,m为2,m'为1,且n"为4。在另一个实施方案中,m为2,m'为2,且n"为4。在另一个实施方案中,m为2,m'为3,且n"为4。

[0554] 在又另一个实施方案中,m为3,m'为0,且n"为4。在又另一个实施方案中,m为3,m'为1,且n"为4。在又另一个实施方案中,m为3,m'为2,且n"为4。在又另一个实施方案中,m为3,m'为3,且n"为4。

[0555] 在又另一个实施方案中,m为4,m'为0,且n"为4。在又另一个实施方案中,m为4,m'为1,且n"为4。在又另一个实施方案中,m为4,m'为2,且n"为4。在又另一个实施方案中,m为4,m'为3,且n"为4。

[0556] 在一个特定实施方案中,m为1,m'为0,且n"为5。在一个特定实施方案中,m为1,m'为1,且n"为5。在一个特定实施方案中,m为1,m'为2,且n"为5。在一个特定实施方案中,m为1,m'为3,且n"为5。

[0557] 在另一个实施方案中,m为2,m'为0,且n"为5。在另一个实施方案中,m为2,m'为1,且n"为5。在另一个实施方案中,m为2,m'为2,且n"为5。在另一个实施方案中,m为2,m'为3,且n"为5。

[0558] 在又另一个实施方案中,m为3,m'为0,且n"为5。在又另一个实施方案中,m为3,m'为1,且n"为5。在又另一个实施方案中,m为3,m'为2,且n"为5。在又另一个实施方案中,m为3,m'为3,且n"为5。

[0559] 在又另一个实施方案中,m为4,m'为0,且n"为5。在又另一个实施方案中,m为4,m'为1,且n"为5。在又另一个实施方案中,m为4,m'为2,且n"为5。在又另一个实施方案中,m为4,m'为3,且n"为5。优选地,糖不为来自肺炎链球菌血清型3之荚膜糖。

[0560] 在一个实施方案中,本发明提供一种糖缀合物,其包含经由间隔子与载体蛋白(CP)共价缀合之糖且具有通式(VII),其中X为 $\text{OCH}_2(\text{CH}_2)_n$,其中n'系选自1至10,且其中X'为 $\text{CH}_2\text{O}(\text{CH}_2)_n\text{CH}_2\text{C}=\text{O}$,其中n"系选自0至10。

[0561] 在一个实施方案中,n'系选自1至5,且n"系选自0至10。在一个实施方案中,n'系选自1至5,且n"系选自0至5。在一个实施方案中,n'系选自1至3,且n"系选自0至3。在一个实施方案中,n'系选自1至2,且n"系选自0至2。在一个特定实施方案中,n'为1,且n"为0。在另一个实施方案中,n'为2,且n"为0。在又另一个实施方案中,n'为3,且n"为0。在又另一个实施方案中,n'为4,且n"为0。在又另一个实施方案中,n'为5,且n"为0。在又另一个实施方案中,n'为6,且n"为0。在一个特定实施方案中,n'为1,且n"为1。在另一个实施方案中,n'为2,且n"为1。在又另一个实施方案中,n'为3,且n"为1。在又另一个实施方案中,n'为4,且n"为1。在又另一个实施方案中,n'为5,且n"为1。在又另一个实施方案中,n'为6,且n"为1。在一个特定实施方案中,n'为1,且n"为2。在另一个实施方案中,n'为2,且n"为2。在又另一个实施方案中,n'为3,且n"为2。在又另一个实施方案中,n'为4,且n"为2。在又另一个实施方案中,n'为5,且n"为2。在又另一个实施方案中,n'为6,且n"为2。在一个特定实施方案中,n'为1,且n"为3。在另一个实施方案中,n'为2,且n"为3。在又另一个实施方案中,n'为3,且n"为3。

在又一个实施方案中, n' 为4, 且 n'' 为3。在又一个实施方案中, n' 为5, 且 n'' 为3。在又一个实施方案中, n' 为6, 且 n'' 为3。在一个特定实施方案中, n' 为1, 且 n'' 为4。在另一个实施方案中, n' 为2, 且 n'' 为4。在又一个实施方案中, n' 为3, 且 n'' 为4。在又一个实施方案中, n' 为4, 且 n'' 为4。在又一个实施方案中, n' 为5, 且 n'' 为4。在又一个实施方案中, n' 为6, 且 n'' 为4。在一个特定实施方案中, n' 为1, 且 n'' 为5。在另一个实施方案中, n' 为2, 且 n'' 为5。在又一个实施方案中, n' 为3, 且 n'' 为5。在又一个实施方案中, n' 为4, 且 n'' 为5。在又一个实施方案中, n' 为5, 且 n'' 为5。在又一个实施方案中, n' 为6, 且 n'' 为5。在一个特定实施方案中, n' 为1, 且 n'' 为6。在另一个实施方案中, n' 为2, 且 n'' 为6。在又一个实施方案中, n' 为3, 且 n'' 为6。在又一个实施方案中, n' 为4, 且 n'' 为6。在又一个实施方案中, n' 为5, 且 n'' 为6。在又一个实施方案中, n' 为6, 且 n'' 为6。优选地, 糖不为来自肺炎链球菌血清型3之荚膜糖。

[0562] 在一个实施方案中, 本发明提供一种糖缀合物, 其包含经由间隔子与载体蛋白(CP) 共价缀合之糖且具有通式(VII), 其中X为 $\text{OCH}_2(\text{CH}_2)_n$, 其中 n' 系选自1至10, 且其中X' 为 $\text{CH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_m(\text{CH}_2)_n\text{CH}_2\text{C}=\text{O}$, 其中 n'' 系选自0至10且 m' 系选自0至4。

[0563] 在一个实施方案中, n' 系选自1至5, m' 系选自0至4, 且 n'' 系选自0至10。在一个实施方案中, n' 系选自1至5, m' 系选自0至4, 且 n'' 系选自0至5。在一个实施方案中, n' 系选自1至3, m' 系选自0至2, 且 n'' 系选自0至3。在一个实施方案中, n' 系选自1至2, m' 系选自0至2, 且 n'' 系选自0至1。

[0564] 在一个特定实施方案中, n' 为1, m' 为0, 且 n'' 为0。在另一个实施方案中, n' 为1, m' 为1, 且 n'' 为0。在另一个实施方案中, n' 为1, m' 为2, 且 n'' 为0。在另一个实施方案中, n' 为1, m' 为3, 且 n'' 为0。

[0565] 在另一个实施方案中, n' 为2, m' 为0, 且 n'' 为0。在另一个实施方案中, n' 为2, m' 为1, 且 n'' 为0。在另一个实施方案中, n' 为2, m' 为2, 且 n'' 为0。在另一个实施方案中, n' 为2, m' 为3, 且 n'' 为0。

[0566] 在又一个实施方案中, n' 为3, m' 为0, 且 n'' 为0。在又一个实施方案中, n' 为3, m' 为1, 且 n'' 为0。在又一个实施方案中, n' 为3, m' 为2, 且 n'' 为0。在又一个实施方案中, n' 为3, m' 为3, 且 n'' 为0。

[0567] 在又一个实施方案中, n' 为4, m' 为0, 且 n'' 为0。在又一个实施方案中, n' 为4, m' 为1, 且 n'' 为0。在又一个实施方案中, n' 为4, m' 为2, 且 n'' 为0。在又一个实施方案中, n' 为4, m' 为3, 且 n'' 为0。

[0568] 在又一个实施方案中, n' 为5, m' 为0, 且 n'' 为0。在又一个实施方案中, n' 为5, m' 为1, 且 n'' 为0。在又一个实施方案中, n' 为5, m' 为2, 且 n'' 为0。在又一个实施方案中, n' 为5, m' 为3, 且 n'' 为0。

[0569] 在一个特定实施方案中, n' 为1, m' 为0, 且 n'' 为1。在一个特定实施方案中, n' 为1, m' 为1, 且 n'' 为1。在一个特定实施方案中, n' 为1, m' 为2, 且 n'' 为1。在一个特定实施方案中, n' 为1, m' 为3, 且 n'' 为1。

[0570] 在另一个实施方案中, n' 为2, m' 为0, 且 n'' 为1。在另一个实施方案中, n' 为2, m' 为1, 且 n'' 为1。在另一个实施方案中, n' 为2, m' 为2, 且 n'' 为1。在另一个实施方案中, n' 为2, m' 为3, 且 n'' 为1。

[0571] 在又一个实施方案中, n' 为3, m' 为0, 且 n'' 为1。在又一个实施方案中, n' 为3, m' 为1, 且 n'' 为1。在又一个实施方案中, n' 为3, m' 为2, 且 n'' 为1。在又一个实施方案中, n' 为3, m' 为3, 且 n'' 为1。

[0572] 在又另一个实施方案中, n' 为4, m' 为0, 且 n'' 为1。在又另一个实施方案中, n' 为4, m' 为1, 且 n'' 为1。在又另一个实施方案中, n' 为4, m' 为2, 且 n'' 为1。在又另一个实施方案中, n' 为4, m' 为3, 且 n'' 为1。

[0573] 在又一个实施方案中, n' 为 5, m' 为 0, 且 n'' 为 1。在又一个实施方案中, n' 为 5, m' 为 1, 且 n'' 为 1。在又一个实施方案中, n' 为 5, m' 为 2, 且 n'' 为 1。在又一个实施方案中, n' 为 5, m' 为 3, 且 n'' 为 1。

[0574] 在一个特定实施方案中,n'为1,m'为0,且n"为2。在一个特定实施方案中,n'为1,m'为1,且n"为2。在一个特定实施方案中,n'为1,m'为2,且n"为2。在一个特定实施方案中,n'为1,m'为3,且n"为2。

[0575] 在另一个实施方案中, n' 为 2, m' 为 0, 且 n'' 为 2。在另一个实施方案中, n' 为 2, m' 为 1, 且 n'' 为 2。在另一个实施方案中, n' 为 2, m' 为 2, 且 n'' 为 2。在另一个实施方案中, n' 为 2, m' 为 3, 且 n'' 为 2。

[0576] 在又一个实施方案中, n' 为3, m' 为0, 且 n'' 为2。在又一个实施方案中, n' 为3, m' 为1, 且 n'' 为2。在又一个实施方案中, n' 为3, m' 为2, 且 n'' 为2。在又一个实施方案中, n' 为3, m' 为3, 且 n'' 为2。

[0577] 在又另一个实施方案中,n'为4,m'为0,且n"为2。在又另一个实施方案中,n'为4,m'为1,且n"为2。在又另一个实施方案中,n'为4,m'为2,且n"为2。在又另一个实施方案中,n'为4,m'为3,且n"为2。

[0578] 在又一个实施方案中, n' 为 5, m' 为 0, 且 n'' 为 2。在又一个实施方案中, n' 为 5, m' 为 1, 且 n'' 为 2。在又一个实施方案中, n' 为 5, m' 为 2, 且 n'' 为 2。在又一个实施方案中, n' 为 5, m' 为 3, 且 n'' 为 2。

[0579] 在一个特定实施方案中,n'为1,m'为0,且n"为3。在一个特定实施方案中,n'为1,m'为1,且n"为3。在一个特定实施方案中,n'为1,m'为2,且n"为3。在一个特定实施方案中,n'为1,m'为3,且n"为3。

[0580] 在另一个实施方案中,n'为2,m'为0,且n"为3。在另一个实施方案中,n'为2,m'为1,且n"为3。在另一个实施方案中,n'为2,m'为2,且n"为3。在另一个实施方案中,n'为2,m'为3,且n"为3。

[0581] 在又一个实施方案中, n' 为 3, m' 为 0, 且 n'' 为 3。在又一个实施方案中, n' 为 3, m' 为 1, 且 n'' 为 3。在又一个实施方案中, n' 为 3, m' 为 2, 且 n'' 为 3。在又一个实施方案中, n' 为 3, m' 为 3, 且 n'' 为 3。

[0582] 在又另一个实施方案中,n'为4,m'为0,且n"为3。在又另一个实施方案中,n'为4,m'为1,且n"为3。在又另一个实施方案中,n'为4,m'为2,且n"为3。在又另一个实施方案中,n'为4,m'为3,且n"为3。

[0583] 在又一个实施方案中, n' 为 5, m' 为 0, 且 n'' 为 3。在又一个实施方案中, n' 为 5, m' 为 1, 且 n'' 为 3。在又一个实施方案中, n' 为 5, m' 为 2, 且 n'' 为 3。在又一个实施方案中, n' 为 5, m' 为 3, 且 n'' 为 3。

[0584] 在一个特定实施方案中, n' 为1, m' 为0, 且 n'' 为4。在一个特定实施方案中, n' 为1, m' 为1, 且 n'' 为4。在一个特定实施方案中, n' 为1, m' 为2, 且 n'' 为4。在一个特定实施方案中, n' 为1, m' 为3, 且 n'' 为4。

[0585] 在另一个实施方案中, n' 为2, m' 为0, 且 n'' 为4。在另一个实施方案中, n' 为2, m' 为1, 且 n'' 为4。在另一个实施方案中, n' 为2, m' 为2, 且 n'' 为4。在另一个实施方案中, n' 为2, m' 为3, 且 n'' 为4。

[0586] 在又一个实施方案中, n' 为3, m' 为0, 且 n'' 为4。在又一个实施方案中, n' 为3, m' 为1, 且 n'' 为4。在又一个实施方案中, n' 为3, m' 为2, 且 n'' 为4。在又一个实施方案中, n' 为3, m' 为3, 且 n'' 为4。

[0587] 在又一个实施方案中, n' 为4, m' 为0, 且 n'' 为4。在又一个实施方案中, n' 为4, m' 为1, 且 n'' 为4。在又一个实施方案中, n' 为4, m' 为2, 且 n'' 为4。在又一个实施方案中, n' 为4, m' 为3, 且 n'' 为4。

[0588] 在又一个实施方案中, n' 为5, m' 为0, 且 n'' 为4。在又一个实施方案中, n' 为5, m' 为1, 且 n'' 为4。在又一个实施方案中, n' 为5, m' 为2, 且 n'' 为4。在又一个实施方案中, n' 为5, m' 为3, 且 n'' 为4。

[0589] 在一个特定实施方案中, n' 为1, m' 为0, 且 n'' 为5。在一个特定实施方案中, n' 为1, m' 为1, 且 n'' 为5。在一个特定实施方案中, n' 为1, m' 为2, 且 n'' 为5。在一个特定实施方案中, n' 为1, m' 为3, 且 n'' 为5。

[0590] 在另一个实施方案中, n' 为2, m' 为0, 且 n'' 为5。在另一个实施方案中, n' 为2, m' 为1, 且 n'' 为5。在另一个实施方案中, n' 为2, m' 为2, 且 n'' 为5。在另一个实施方案中, n' 为2, m' 为3, 且 n'' 为5。

[0591] 在又一个实施方案中, n' 为3, m' 为0, 且 n'' 为5。在又一个实施方案中, n' 为3, m' 为1, 且 n'' 为5。在又一个实施方案中, n' 为3, m' 为2, 且 n'' 为5。在又一个实施方案中, n' 为3, m' 为3, 且 n'' 为5。

[0592] 在又一个实施方案中, n' 为4, m' 为0, 且 n'' 为5。在又一个实施方案中, n' 为4, m' 为1, 且 n'' 为5。在又一个实施方案中, n' 为4, m' 为2, 且 n'' 为5。在又一个实施方案中, n' 为4, m' 为3, 且 n'' 为5。

[0593] 在又一个实施方案中, n' 为5, m' 为0, 且 n'' 为5。在又一个实施方案中, n' 为5, m' 为1, 且 n'' 为5。在又一个实施方案中, n' 为5, m' 为2, 且 n'' 为5。在又一个实施方案中, n' 为5, m' 为3, 且 n'' 为5。

[0594] 优选地, 糖不为来自肺炎链球菌血清型3之荚膜糖。

[0595] 在一个实施方案中, 本发明提供一种糖缀合物, 其包含经由间隔子与载体蛋白(CP) 共价缀合之糖且具有通式(VII), 其中X为 $O(CH_2CH_2O)_mCH_2CH_2$, 其中m系选自1至4, 且其中X' 为 $CH_2O(CH_2)_nCH_2C=O$, 其中 n'' 系选自0至10。

[0596] 在一个实施方案中, m系选自1至3, 且 n'' 系选自0至10。在一个实施方案中, m系选自1至3, 且 n'' 系选自0至5。在一个实施方案中, m系选自1至2, 且 n'' 系选自0至3。在一个实施方案中, m系选自1至2, 且 n'' 系选自0至2。在一个特定实施方案中, m为1, 且 n'' 为0。在另一个实施方案中, m为2, 且 n'' 为0。在又一个实施方案中, m为3, 且 n'' 为0。在又一个实施方案中, m为4, 且 n'' 为0。在一个特定实施方案中, m为1, 且 n'' 为1。在另一个实施方案中, m为2, 且 n'' 为

1.在又一个实施方案中,m为3,且n”为1。在又一个实施方案中,m为4,且n”为1。在一个特定实施方案中,m为1,且n”为2。在另一个实施方案中,m为2,且n”为2。在又一个实施方案中,m为3,且n”为2。在又一个实施方案中,m为4,且n”为2。在一个特定实施方案中,m为1,且n”为3。在另一个实施方案中,m为2,且n”为3。在又一个实施方案中,m为3,且n”为3。在又一个实施方案中,m为4,且n”为3。在一个特定实施方案中,m为1,且n”为4。在另一个实施方案中,m为2,且n”为4。在又一个实施方案中,m为3,且n”为4。在又一个实施方案中,m为4,且n”为4。在一个特定实施方案中,m为1,且n”为5。在另一个实施方案中,m为2,且n”为5。在又一个实施方案中,m为3,且n”为5。在又一个实施方案中,m为4,且n”为5。在一个特定实施方案中,m为1,且n”为6。在另一个实施方案中,m为2,且n”为6。在又一个实施方案中,m为3,且n”为6。在又一个实施方案中,m为4,且n”为6。优选地,糖不为来自肺炎链球菌血清型3之荚膜糖。

[0597] 在一个实施方案中,本发明提供一种糖缀合物,其包含经由间隔子与载体蛋白(CP)共价缀合之糖且具有通式(VII),其中X为 $O(CH_2CH_2)_mCH_2CH_2$,其中m系选自1至4,且其中X’为 $CH_2O(CH_2CH_2)_m(CH_2)_{n'}CH_2C=O$,其中n”系选自0至10且m’系选自0至4。

[0598] 在一个实施方案中,m系选自1至3,m’系选自0至4,且n”系选自0至10。在一个实施方案中,m系选自1至2,m’系选自0至4,且n”系选自0至5。在一个实施方案中,m系选自1至2,m’系选自0至2,且n”系选自0至3。在一个实施方案中,m系选自1至2,m’系选自0至2,且n”系选自0至1。

[0599] 在一个特定实施方案中,m为1,m’为0,且n”为0。在另一个实施方案中,m为1,m’为1,且n”为0。在另一个实施方案中,m为1,m’为2,且n”为0。在另一个实施方案中,m为1,m’为3,且n”为0。

[0600] 在另一个实施方案中,m为2,m’为0,且n”为0。在另一个实施方案中,m为2,m’为1,且n”为0。在另一个实施方案中,m为2,m’为2,且n”为0。在另一个实施方案中,m为2,m’为3,且n”为0。

[0601] 在又一个实施方案中,m为3,m’为0,且n”为0。在又一个实施方案中,m为3,m’为1,且n”为0。在又一个实施方案中,m为3,m’为2,且n”为0。在又一个实施方案中,m为3,m’为3,且n”为0。

[0602] 在又一个实施方案中,m为4,m’为0,且n”为0。在又一个实施方案中,m为4,m’为1,且n”为0。在又一个实施方案中,m为4,m’为2,且n”为0。在又一个实施方案中,m为4,m’为3,且n”为0。

[0603] 在一个特定实施方案中,m为1,m’为0,且n”为1。在一个特定实施方案中,m为1,m’为1,且n”为1。在一个特定实施方案中,m为1,m’为2,且n”为1。在一个特定实施方案中,m为1,m’为3,且n”为1。

[0604] 在另一个实施方案中,m为2,m’为0,且n”为1。在另一个实施方案中,m为2,m’为1,且n”为1。在另一个实施方案中,m为2,m’为2,且n”为1。在另一个实施方案中,m为2,m’为3,且n”为1。

[0605] 在又一个实施方案中,m为3,m’为0,且n”为1。在又一个实施方案中,m为3,m’为1,且n”为1。在又一个实施方案中,m为3,m’为2,且n”为1。在又一个实施方案中,m为3,m’为3,且n”为1。

[0606] 在又一个实施方案中,m为4,m'为0,且n"为1。在又一个实施方案中,m为4,m'为1,且n"为1。在又一个实施方案中,m为4,m'为2,且n"为1。在又一个实施方案中,m为4,m'为3,且n"为1。

[0607] 在一个特定实施方案中,m为1,m'为0,且n"为2。在一个特定实施方案中,m为1,m'为1,且n"为2。在一个特定实施方案中,m为1,m'为2,且n"为2。在一个特定实施方案中,m为1,m'为3,且n"为2。

[0608] 在另一个实施方案中,m为2,m'为0,且n"为2。在另一个实施方案中,m为2,m'为1,且n"为2。在另一个实施方案中,m为2,m'为2,且n"为2。在另一个实施方案中,m为2,m'为3,且n"为2。

[0609] 在又一个实施方案中,m为3,m'为0,且n"为2。在又一个实施方案中,m为3,m'为1,且n"为2。在又一个实施方案中,m为3,m'为2,且n"为2。在又一个实施方案中,m为3,m'为3,且n"为2。

[0610] 在又一个实施方案中,m为4,m'为0,且n"为2。在又一个实施方案中,m为4,m'为1,且n"为2。在又一个实施方案中,m为4,m'为2,且n"为2。在又一个实施方案中,m为4,m'为3,且n"为2。

[0611] 在一个特定实施方案中,m为1,m'为0,且n"为3。在一个特定实施方案中,m为1,m'为1,且n"为3。在一个特定实施方案中,m为1,m'为2,且n"为3。在一个特定实施方案中,m为1,m'为3,且n"为3。

[0612] 在另一个实施方案中,m为2,m'为0,且n"为3。在另一个实施方案中,m为2,m'为1,且n"为3。在另一个实施方案中,m为2,m'为2,且n"为3。在另一个实施方案中,m为2,m'为3,且n"为3。

[0613] 在又一个实施方案中,m为3,m'为0,且n"为3。在又一个实施方案中,m为3,m'为1,且n"为3。在又一个实施方案中,m为3,m'为2,且n"为3。在又一个实施方案中,m为3,m'为3,且n"为3。

[0614] 在又一个实施方案中,m为4,m'为0,且n"为3。在又一个实施方案中,m为4,m'为1,且n"为3。在又一个实施方案中,m为4,m'为2,且n"为3。在又一个实施方案中,m为4,m'为3,且n"为3。

[0615] 在一个特定实施方案中,m为1,m'为0,且n"为4。在一个特定实施方案中,m为1,m'为1,且n"为4。在一个特定实施方案中,m为1,m'为2,且n"为4。在一个特定实施方案中,m为1,m'为3,且n"为4。

[0616] 在另一个实施方案中,m为2,m'为0,且n"为4。在另一个实施方案中,m为2,m'为1,且n"为4。在另一个实施方案中,m为2,m'为2,且n"为4。在另一个实施方案中,m为2,m'为3,且n"为4。

[0617] 在又一个实施方案中,m为3,m'为0,且n"为4。在又一个实施方案中,m为3,m'为1,且n"为4。在又一个实施方案中,m为3,m'为2,且n"为4。在又一个实施方案中,m为3,m'为3,且n"为4。

[0618] 在又一个实施方案中,m为4,m'为0,且n"为4。在又一个实施方案中,m为4,m'为1,且n"为4。在又一个实施方案中,m为4,m'为2,且n"为4。在又一个实施方案中,m为4,m'为3,且n"为4。

[0619] 在一个特定实施方案中,m为1,m'为0,且n"为5。在一个特定实施方案中,m为1,m'为1,且n"为5。在一个特定实施方案中,m为1,m'为2,且n"为5。在一个特定实施方案中,m为1,m'为3,且n"为5。

[0620] 在另一个实施方案中,m为2,m'为0,且n"为5。在另一个实施方案中,m为2,m'为1,且n"为5。在另一个实施方案中,m为2,m'为2,且n"为5。在另一个实施方案中,m为2,m'为3,且n"为5。

[0621] 在又一个实施方案中,m为3,m'为0,且n"为5。在又一个实施方案中,m为3,m'为1,且n"为5。在又一个实施方案中,m为3,m'为2,且n"为5。在又一个实施方案中,m为3,m'为3,且n"为5。

[0622] 在又一个实施方案中,m为4,m'为0,且n"为5。在又一个实施方案中,m为4,m'为1,且n"为5。在又一个实施方案中,m为4,m'为2,且n"为5。在又一个实施方案中,m为4,m'为3,且n"为5。

[0623] 优选地,糖不为来自肺炎链球菌血清型3之荚膜糖。

[0624] 优选地,本发明之糖缀合物之糖不为来自肺炎链球菌血清型3之荚膜糖。

[0625] 1.5 本发明之糖缀合物之载体蛋白

[0626] 糖缀合物之组分为与糖缀合之载体蛋白。术语“蛋白载体”或“载体蛋白”或“载体”可在本文中互换使用。载体蛋白应适于标准缀合程序。

[0627] 在一个优选实施方案中,本发明之糖缀合物之载体蛋白系在由以下组成的组中选择:DT(白喉类毒素)、TT(破伤风类毒素)或TT之片段C、CRM₁₉₇(白喉毒素的无毒但抗原性相同的变体)、其他DT突变体(诸如CRM₁₇₆、CRM₂₂₈、CRM₄₅(Uchida等人(1973) J. Biol. Chem. 218: 3838-3844)、CRM₉、CRM₁₀₂、CRM₁₀₃或CRM₁₀₇;及Nicholls及Youle在Genetically Engineered Toxins,编:Frankel, Maecel Dekker Inc. (1992)中描述之其他突变;Glu-148缺失或突变为Asp、Gln或Ser,及/或Ala 158缺失或突变为Gly,及美国专利第4,709,017号及第4,950,740号中所公开之其他突变;至少一或多个残基Lys 516、Lys 526、Phe 530及/或Lys 534之突变,及美国专利第5,917,017号及第6,455,673号中所公开之其他突变;或美国专利第5,843,711号中所公开之片段,肺炎链球菌性肺炎链球菌溶血素(ply)(Kuo等人(1995) Infect Immun 63:2706-2713)包括以某种方式解毒之ply,例如dPLY-GMBS(WO 2004/081515、WO 2006/032499)或dPLY-formol、PhtX(包括PhtA、PhtB、PhtD、PhtE(PhtA、PhtB、PhtD或PhtE之序列公开于WO 00/37105及WO 00/39299中)及Pht蛋白之融合物,例如PhtDE融合物、PhtBE融合物、Pht A-E(WO 01/98334、WO 03/054007、WO 2009/000826)、OMPC(脑膜炎球菌性外膜蛋白)(其通常提取自脑膜炎奈瑟氏菌血清群B(EP0372501))、PorB(来自脑膜炎奈瑟氏菌)、PD(流感嗜血杆菌蛋白D;参见例如EP0594610 B)或其免疫功能等效物、合成肽(EP0378881、EP0427347)、热休克蛋白(WO 93/17712、WO 94/03208)、百日咳蛋白(WO 98/58668、EP0471177)、细胞因子、淋巴因子、生长因子或激素(WO 91/01146)、人工蛋白质,包含来自各种病原体衍生抗原之多种人类CD4+T细胞表位(Falugi等人(2001) Eur J Immunol 31: 3816-3824),诸如N19蛋白(Baraldoi等人(2004) Infect Immun 72:4884-4887)、肺炎链球菌性表面蛋白PspA(WO 02/091998)、铁摄取蛋白(WO 01/72337)、艰难梭菌之毒素A或B(WO 00/61761)、转铁蛋白结合蛋白、肺炎链球菌性黏附蛋白(PsaA)、重组铜绿假单胞菌(Pseudomonas aeruginosa)外毒素A(尤其其无毒突变体(诸如具有谷氨酸553处之取代的

外毒素A(Douglas等人(1987)J.Bacteriol.169(11):4967-4971))。其他蛋白,诸如卵白蛋白、匙孔血蓝蛋白(KLH)、牛血清白蛋白(BSA)或结核菌素之纯化蛋白衍生物(PPD)还可用作载体蛋白。其他适合的载体蛋白包括失活的细菌毒素,诸如霍乱类毒素(例如如WO 2004/083251中所描述);大肠杆菌LT;大肠杆菌ST;及来自铜绿假单胞菌之外毒素A。另一适合的载体蛋白为来自链球菌(SCP)之C5a肽酶。

[0628] 在一个优选实施方案中,本发明之糖缀合物之载体蛋白为TT、DT、DT突变体(诸如CRM₁₉₇)或来自链球菌之C5a肽酶(SCP)。

[0629] 在一个实施方案中,本发明之糖缀合物之载体蛋白为DT(白喉类毒素)。在另一个实施方案中,本发明之糖缀合物之载体蛋白为TT(破伤风类毒素)。

[0630] 在另一个实施方案中,本发明之糖缀合物之载体蛋白为PD(流感嗜血杆菌蛋白D;参见例如EP0594610 B)。

[0631] 在一个优选实施方案中,本发明之糖缀合物之载体蛋白为CRM₁₉₇或来自链球菌之C5a肽酶(SCP)。

[0632] 在一个优选实施方案中,本发明之糖缀合物之载体蛋白为CRM₁₉₇。CRM₁₉₇蛋白为一种无毒形式之白喉毒素,但在免疫学上与白喉毒素无法区分。CRM₁₉₇系由受无毒噬菌体 β 197^{tox-}感染之白喉棒状杆菌(*Corynebacterium diphtheriae*)产生,该无毒噬菌体由产毒棒状杆菌噬菌体 β 之亚硝基胍诱变产生(Uchida等人(1971)Nature New Biology 233:8-11)。CRM₁₉₇蛋白具有与白喉毒素相同的分子量,但因结构基因中之单个碱基变化(鸟嘌呤变为腺嘌呤)而与白喉毒素不同。此单个碱基变化引起成熟蛋白中之氨基酸取代(谷氨酸取代甘氨酸),且消除白喉毒素之毒性特性。CRM₁₉₇蛋白为糖之安全且有效的T细胞依赖性载体。关于CRM₁₉₇及其产生之其他细节可例如见于美国专利第5,614,382号中。

[0633] 在一个实施方案中,本发明之糖缀合物之载体蛋白为CRM₁₉₇之A链(参见CN103495161)。在一个实施方案中,本发明之糖缀合物之载体蛋白为经由通过基因重组之大肠杆菌进行表达而获得的CRM₁₉₇之A链(参见CN103495161)。

[0634] 在其他优选实施方案中,本发明之糖缀合物之载体蛋白为SCP(链球菌C5a肽酶)。 β 溶血性链球菌之两种重要菌种酿脓链球菌(A组链球菌,GAS)及无乳链球菌(B组链球菌,GBS)引起多种严重人类感染,自轻症咽炎及脓疱病至严重侵袭性疾病(诸如坏死性筋膜炎(GAS)及新生儿败血症(GBS))都有,已发展出击败这种免疫反应的方法。 β 溶血性链球菌(包括GAS及GBS)之所有人类分离株均产生特异性失活C5a的高度保守的细胞壁蛋白SCP(链球菌C5a肽酶)。来自GAS及GBS之scp基因编码含有1,134至1,181个氨基酸之多肽(Brown等人,PNAS,2005,第102卷,第51期,第18391-18396页)。前31个残基为输出信号前序列且在穿过细胞质膜时被移除。随后68个残基充当原序列且必须经移除以产生活性SCP。随后10个残基可经移除而不损失蛋白酶活性。在另一端,以Lys-1034开始为四个连续的17-残基基序,接着为细胞分选及细胞壁附着信号。此组合信号由含有LPTTND序列之20个残基亲水性序列、17个残基疏水性序列及短碱性羧基端构成。

[0635] SCP可分成结构域(参见Brown等人,PNAS,2005,第102卷,第51期第18391-18396页之图1B)。此等结构域为前结构域/后结构域(其包含输出信号前序列(通常为前31个残基)及原序列(通常为随后68个残基))、蛋白酶结构域(其分解为两部分(蛋白酶部分1,通常为残基89至333/334;及蛋白酶结构域部分2,且通常为残基467/468至583/584)、蛋白酶相关

结构域(PA域)(通常为残基333/334至467/468)、三个纤连蛋白III型(Fn)结构域(Fn1,通常为残基583/584至712/713;Fn2,通常为残基712/713至928/929/930;通常Fn3,残基929/930至1029/1030/1031)及细胞壁锚定结构域(通常为残基1029/1030/1031至C端)。

[0636] 在一个实施方案中,本发明之糖缀合物之载体蛋白为来自GBS之SCP(SCPB)。SCPB之实例提供于W097/26008之SEQ ID.N0:3处。还参见W000/34487之SEQ ID N0:3。

[0637] 在另一个优选实施方案中,本发明之糖缀合物之载体蛋白为来自GAS之SCP(SCPA)。SCPA之实例可见于W097/26008之SEQ ID.No.1及SEQ ID.No.2。还参见W000/34487之SEQ ID N0:1、2及23。

[0638] 在一个优选实施方案中,本发明之糖缀合物之载体蛋白为无酶促活性的SCP。

[0639] 在其他优选实施方案中,本发明之糖缀合物之载体蛋白为无酶促活性的来自GBS之SCP(SCPB)。

[0640] 在另一个优选实施方案中,本发明之糖缀合物之载体蛋白为无酶促活性的来自GAS之SCP(SCPA)。

[0641] 在一个实施方案中,本发明之糖缀合物之载体蛋白为SCP之片段。在一个实施方案中,本发明之糖缀合物之载体蛋白为SCPA之片段。优选地,本发明之糖缀合物之载体蛋白为SCPB之片段。

[0642] 在一个实施方案中,本发明之糖缀合物之载体蛋白为SCP之片段,其包含蛋白酶结构域、蛋白酶相关结构域(PA域)及三个纤连蛋白III型(Fn)结构域,但不包含输出信号前序列、原序列及细胞壁锚定域。

[0643] 在一个实施方案中,本发明之糖缀合物之载体蛋白为SCP之片段,其包含蛋白酶结构域、蛋白酶相关结构域(PA域)及三个纤连蛋白III型(Fn)结构域,但不包含输出信号前序列、原序列及细胞壁锚定域。

[0644] 在一个实施方案中,本发明之糖缀合物之载体蛋白为无酶促活性的SCP之片段,其包含蛋白酶结构域、蛋白酶相关结构域(PA域)以及三个纤连蛋白III型(Fn)结构域中之两者,但不包含输出信号前序列、原序列及细胞壁锚定域。

[0645] 在一个实施方案中,本发明之糖缀合物之载体蛋白为无酶促活性的SCP之片段。在一个实施方案中,该无酶促活性的SCP之片段包含蛋白酶结构域、蛋白酶相关结构域(PA域)及三个纤连蛋白III型(Fn)结构域,但不包含输出信号前序列、原序列及细胞壁锚定域。

[0646] 在一个实施方案中,本发明之糖缀合物之载体蛋白为无酶促活性的SCPA之片段。在一个实施方案中,该无酶促活性的SCPA之片段包含蛋白酶结构域、蛋白酶相关结构域(PA域)及三个纤连蛋白III型(Fn)结构域,但不包含输出信号前序列、原序列及细胞壁锚定域。

[0647] 在一个优选实施方案中,本发明之糖缀合物之载体蛋白为无酶促活性的SCPB之片段。优选地,该无酶促活性的SCPB之片段包含蛋白酶结构域、蛋白酶相关结构域(PA域)及三个纤连蛋白III型(Fn)结构域,但不包含输出信号前序列、原序列及细胞壁锚定域。

[0648] 在一个实施方案中,SCP之酶促活性系通过置换野生型序列之至少一个氨基酸而失活。在一个实施方案中,该置换系选自由D130A、H193A、N295A及S512A组成的组。数目指示根据W000/34487之SEQ ID N0:1之编号的肽酶中之氨基酸残基位置。

[0649] 因此,在一个实施方案中,本发明之糖缀合物之载体蛋白为无酶促活性的SCP,其中该失活系通过置换野生型序列之至少一个氨基酸来实现。优选地,该至少一个氨基酸置

换系在蛋白酶结构域中。在一个实施方案中,该至少一个氨基酸置换系在蛋白酶结构域之部分1中。在一个实施方案中,该至少一个氨基酸置换系在蛋白酶结构域之部分2中。在一个实施方案中,该置换系选自由D130A、H193A、N295A及S512A组成的组。在一个实施方案中,该置换为D130A。在另一个实施方案中,该置换为H193A。在另一个实施方案中,该置换为N295A。在又另一个实施方案中,该置换为S512A。

[0650] 在一个实施方案中,本发明之糖缀合物之载体蛋白为无酶促活性的SCPA,其中该失活系通过置换野生型序列之至少一个氨基酸来实现。优选地,该至少一个氨基酸置换系在蛋白酶结构域中。在一个实施方案中,该至少一个氨基酸置换系在蛋白酶结构域之部分1中。在一个实施方案中,该至少一个氨基酸置换系在蛋白酶结构域之部分2中。在一个实施方案中,该置换系选自由D130A、H193A、N295A及S512A组成的组。在一个实施方案中,该置换为D130A。在另一个实施方案中,该置换为H193A。在另一个实施方案中,该置换为N295A。在又另一个实施方案中,该置换为S512A。

[0651] 在一个实施方案中,本发明之糖缀合物之载体蛋白为无酶促活性的SCPB,其中该失活系通过置换野生型序列之至少一个氨基酸来实现。优选地,该至少一个氨基酸置换系在蛋白酶结构域中。在一个实施方案中,该至少一个氨基酸置换系在蛋白酶结构域之部分1中。在一个实施方案中,该至少一个氨基酸置换系在蛋白酶结构域之部分2中。在一个实施方案中,该置换系选自由D130A、H193A、N295A及S512A组成的组。在一个实施方案中,该置换为D130A。在另一个实施方案中,该置换为H193A。在另一个实施方案中,该置换为N295A。在又另一个实施方案中,该置换为S512A。

[0652] 在一个实施方案中,本发明之糖缀合物之载体蛋白为无酶促活性的SCP之片段,其中该失活系通过置换野生型序列之至少一个氨基酸来实现。优选地,该至少一个氨基酸置换系在蛋白酶结构域中。在一个实施方案中,该至少一个氨基酸置换系在蛋白酶结构域之部分1中。在一个实施方案中,该至少一个氨基酸置换系在蛋白酶结构域之部分2中。在一个实施方案中,该置换系选自由D130A、H193A、N295A及S512A组成的组。在一个实施方案中,该置换为D130A。在另一个实施方案中,该置换为H193A。在另一个实施方案中,该置换为N295A。在又另一个实施方案中,该置换为S512A。

[0653] 在一个实施方案中,本发明之糖缀合物之载体蛋白为无酶促活性的SCP之片段,其包含蛋白酶结构域、蛋白酶相关结构域(PA域)及三个纤连蛋白III型(Fn)结构域但不包含输出信号前序列、原序列及细胞壁锚定域,其中该失活系通过置换野生型序列之至少一个氨基酸来实现。优选地,该至少一个氨基酸置换系在蛋白酶结构域中。在一个实施方案中,该至少一个氨基酸置换系在蛋白酶结构域之部分1中。在一个实施方案中,该至少一个氨基酸置换系在蛋白酶结构域之部分2中。在一个实施方案中,该置换系选自由D130A、H193A、N295A及S512A组成的组。在一个实施方案中,该置换为D130A。在另一个实施方案中,该置换为H193A。在另一个实施方案中,该置换为N295A。在又另一个实施方案中,该置换为S512A。

[0654] 在一个实施方案中,本发明之糖缀合物之载体蛋白为无酶促活性的SCPA之片段,其包含蛋白酶结构域、蛋白酶相关结构域(PA域)及三个纤连蛋白III型(Fn)结构域但不包含输出信号前序列、原序列及细胞壁锚定域,其中该不活化系通过置换野生型序列之至少一个氨基酸来实现。优选地,该至少一个氨基酸置换系在蛋白酶结构域中。在一个实施方案中,该至少一个氨基酸置换系在蛋白酶结构域之部分1中。在一个实施方案中,该至少一个

氨基酸置换系在蛋白酶结构域之部分2中。在一个实施方案中,该置换系选自由D130A、H193A、N295A及S512A组成的组。在一个实施方案中,该置换为D130A。在另一个实施方案中,该置换为H193A。在另一个实施方案中,该置换为N295A。在又另一个实施方案中,该置换为S512A。

[0655] 在一个实施方案中,本发明之糖缀合物之载体蛋白为无酶促活性的SCPB之片段,其包含蛋白酶结构域、蛋白酶相关结构域(PA域)及三个纤连蛋白III型(Fn)结构域但不包含输出信号前序列、原序列及细胞壁锚定域,其中该失活系通过置换野生型序列之至少一个氨基酸来实现。优选地,该至少一个氨基酸置换系在蛋白酶结构域中。在一个实施方案中,该至少一个氨基酸置换系在蛋白酶结构域之部分1中。在一个实施方案中,该至少一个氨基酸置换系在蛋白酶结构域之部分2中。在一个实施方案中,该置换系选自由D130A、H193A、N295A及S512A组成的组。在一个实施方案中,该置换为D130A。在另一个实施方案中,该置换为H193A。在另一个实施方案中,该置换为N295A。在又另一个实施方案中,该置换为S512A。

[0656] 在一个实施方案中,SCP之酶促活性系通过置换野生型序列之至少两个氨基酸而失活。在一个实施方案中,该至少两个氨基酸置换系选自由D130A、H193A、N295A及S512A组成的组。在一个实施方案中,该至少两个氨基酸置换为D130A及H193A。在一个实施方案中,该至少两个氨基酸置换为D130A及N295A。在一个实施方案中,该至少两个氨基酸置换为D130A及S512A。在一个实施方案中,该至少两个氨基酸置换为H193A及N295A。在一个实施方案中,该至少两个氨基酸置换为H193A及S512A。在一个实施方案中,该至少两个氨基酸置换为N295A及S512A。

[0657] 因此,在一个实施方案中,本发明之糖缀合物之载体蛋白为无酶促活性的SCP,其中该失活系通过置换野生型序列之至少两个氨基酸来实现。优选地,该至少两个氨基酸置换系在蛋白酶结构域中。在一个实施方案中,该至少两个氨基酸置换系在蛋白酶结构域之部分1中。在一个实施方案中,该至少两个氨基酸置换系在蛋白酶结构域之部分2中。在一个实施方案中,该至少两个氨基酸置换系选自由D130A、H193A、N295A及S512A组成的组。在一个实施方案中,该至少两个氨基酸置换为D130A及H193A。在一个实施方案中,该至少两个氨基酸置换为D130A及N295A。优选地,该至少两个氨基酸置换为D130A及S512A。在一个实施方案中,该至少两个氨基酸置换为H193A及N295A。在一个实施方案中,该至少两个氨基酸置换为H193A及S512A。在一个实施方案中,该至少两个氨基酸置换为N295A及S512A。

[0658] 在一个实施方案中,本发明之糖缀合物之载体蛋白为无酶促活性的SCPA,其中该失活系通过置换野生型序列之至少两个氨基酸来实现。优选地,该至少两个氨基酸置换系在蛋白酶结构域中。在一个实施方案中,该至少两个氨基酸置换系在蛋白酶结构域之部分1中。在一个实施方案中,该至少两个氨基酸置换系在蛋白酶结构域之部分2中。在一个实施方案中,该至少两个氨基酸置换系选自由D130A、H193A、N295A及S512A组成的组。在一个实施方案中,该至少两个氨基酸置换为D130A及H193A。在一个实施方案中,该至少两个氨基酸置换为D130A及N295A。优选地,该至少两个氨基酸置换为D130A及S512A。在一个实施方案中,该至少两个氨基酸置换为H193A及N295A。在一个实施方案中,该至少两个氨基酸置换为H193A及S512A。在一个实施方案中,该至少两个氨基酸置换为N295A及S512A。

[0659] 在一个实施方案中,本发明之糖缀合物之载体蛋白为无酶促活性的SCPB,其中该

失活系通过置换野生型序列之至少两个氨基酸来实现。优选地,该至少两个氨基酸置换系在蛋白酶结构域中。在一个实施方案中,该至少两个氨基酸置换系在蛋白酶结构域之部分1中。在一个实施方案中,该至少两个氨基酸置换系在蛋白酶结构域之部分2中。在一个实施方案中,该至少两个氨基酸置换系选自由D130A、H193A、N295A及S512A组成的组。在一个实施方案中,该至少两个氨基酸置换为D130A及H193A。在一个实施方案中,该至少两个氨基酸置换为D130A及N295A。优选地,该至少两个氨基酸置换为D130A及S512A。在一个实施方案中,该至少两个氨基酸置换为H193A及N295A。在一个实施方案中,该至少两个氨基酸置换为H193A及S512A。在一个实施方案中,该至少两个氨基酸置换为N295A及S512A。

[0660] 在一个实施方案中,本发明之糖缀合物之载体蛋白为无酶促活性的SCP之片段,其中该失活系通过置换野生型序列之至少两个氨基酸来实现。优选地,该至少两个氨基酸置换系在蛋白酶结构域中。在一个实施方案中,该至少两个氨基酸置换系在蛋白酶结构域之部分1中。在一个实施方案中,该至少两个氨基酸置换系在蛋白酶结构域之部分2中。在一个实施方案中,该至少两个氨基酸置换系选自由D130A、H193A、N295A及S512A组成的组。在一个实施方案中,该至少两个氨基酸置换为D130A及H193A。在一个实施方案中,该至少两个氨基酸置换为D130A及N295A。优选地,该至少两个氨基酸置换为D130A及S512A。在一个实施方案中,该至少两个氨基酸置换为H193A及N295A。在一个实施方案中,该至少两个氨基酸置换为H193A及S512A。在一个实施方案中,该至少两个氨基酸置换为N295A及S512A。

[0661] 在一个实施方案中,本发明之糖缀合物之载体蛋白为无酶促活性的SCP之片段,其包含蛋白酶结构域、蛋白酶相关结构域(PA域)及三个纤连蛋白III型(Fn)结构域但不包含输出信号前序列、原序列及细胞壁锚定域,其中该失活系通过置换野生型序列之至少两个氨基酸来实现。优选地,该至少两个氨基酸置换系在蛋白酶结构域中。在一个实施方案中,该至少两个氨基酸置换系在蛋白酶结构域之部分1中。在一个实施方案中,该至少两个氨基酸置换系在蛋白酶结构域之部分2中。在一个实施方案中,该至少两个氨基酸置换系选自由D130A、H193A、N295A及S512A组成的组。在一个实施方案中,该至少两个氨基酸置换为D130A及H193A。在一个实施方案中,该至少两个氨基酸置换为D130A及N295A。优选地,该至少两个氨基酸置换为D130A及S512A。在一个实施方案中,该至少两个氨基酸置换为H193A及N295A。在一个实施方案中,该至少两个氨基酸置换为H193A及S512A。在一个实施方案中,该至少两个氨基酸置换为N295A及S512A。

[0662] 在一个实施方案中,本发明之糖缀合物之载体蛋白为无酶促活性的SCPA之片段,其包含蛋白酶结构域、蛋白酶相关结构域(PA域)及三个纤连蛋白III型(Fn)结构域但不包含输出信号前序列、原序列及细胞壁锚定域,其中该失活系通过置换野生型序列之至少两个氨基酸来实现。优选地,该至少两个氨基酸置换系在蛋白酶结构域中。在一个实施方案中,该至少两个氨基酸置换系在蛋白酶结构域之部分1中。在一个实施方案中,该至少一个氨基酸置换系在蛋白酶结构域之部分2中。在一个实施方案中,该至少两个氨基酸置换系选自由D130A、H193A、N295A及S512A组成的组。在一个实施方案中,该至少两个氨基酸置换为D130A及H193A。在一个实施方案中,该至少两个氨基酸置换为D130A及N295A。优选地,该至少两个氨基酸置换为D130A及S512A。在一个实施方案中,该至少两个氨基酸置换为H193A及N295A。在一个实施方案中,该至少两个氨基酸置换为H193A及S512A。在一个实施方案中,该至少两个氨基酸置换为N295A及S512A。

[0663] 在一个实施方案中,本发明之糖缀合物之载体蛋白为无酶促活性的SCPB之片段,其包含蛋白酶结构域、蛋白酶相关结构域(PA域)及三个纤连蛋白III型(Fn)结构域但不包含输出信号前序列、原序列及细胞壁锚定域,其中该失活系通过置换野生型序列之至少两个氨基酸来实现。优选地,该至少两个氨基酸置换系在蛋白酶结构域中。在一个实施方案中,该至少两个氨基酸置换系在蛋白酶结构域之部分1中。在一个实施方案中,该至少两个氨基酸置换系在蛋白酶结构域之部分2中。在一个实施方案中,该至少两个氨基酸置换系选自由D130A、H193A、N295A及S512A组成的组。在一个实施方案中,该至少两个氨基酸置换为D130A及H193A。在一个实施方案中,该至少两个氨基酸置换为D130A及N295A。优选地,该至少两个氨基酸置换为D130A及S512A。在一个实施方案中,该至少两个氨基酸置换为H193A及N295A。在一个实施方案中,该至少两个氨基酸置换为H193A及S512A。在一个实施方案中,该至少两个氨基酸置换为N295A及S512A。

[0664] 在一个实施方案中,SCP之酶促活性系通过置换野生型序列之至少三个氨基酸而失活。在一个实施方案中,该至少三个氨基酸置换系选自由D130A、H193A、N295A及S512A组成的组。在一个实施方案中,该至少三个氨基酸置换为D130A、H193A及N295A。在一个实施方案中,该至少三个氨基酸置换为D130A、H193A及S512A。在一个实施方案中,该至少三个氨基酸置换为D130A、N295A及S512A。在一个实施方案中,该至少三个氨基酸置换为H193A、N295A及S512A。

[0665] 因此,在一个实施方案中,本发明之糖缀合物之载体蛋白为无酶促活性的SCP,其中该失活系通过置换野生型序列之至少三个氨基酸来实现。优选地,该至少三个氨基酸置换系在蛋白酶结构域中。在一个实施方案中,该至少三个氨基酸置换系在蛋白酶结构域之部分1中。在一个实施方案中,该至少三个氨基酸置换系在蛋白酶结构域之部分2中。在一个实施方案中,该至少三个氨基酸置换系选自由D130A、H193A、N295A及S512A组成的组。在一个实施方案中,该至少三个氨基酸置换为D130A、H193A及N295A。在一个实施方案中,该至少三个氨基酸置换为D130A、H193A及S512A。在一个实施方案中,该至少三个氨基酸置换为D130A、N295A及S512A。在一个实施方案中,该至少三个氨基酸置换为H193A、N295A及S512A。

[0666] 在一个实施方案中,本发明之糖缀合物之载体蛋白为无酶促活性的SCPA,其中该失活系通过置换野生型序列之至少三个氨基酸来实现。优选地,该至少三个氨基酸置换系在蛋白酶结构域中。在一个实施方案中,该至少三个氨基酸置换系在蛋白酶结构域之部分1中。在一个实施方案中,该至少三个氨基酸置换系在蛋白酶结构域之部分2中。在一个实施方案中,该至少三个氨基酸置换系选自由D130A、H193A、N295A及S512A组成的组。在一个实施方案中,该至少三个氨基酸置换为D130A、H193A及N295A。在一个实施方案中,该至少三个氨基酸置换为D130A、H193A及S512A。在一个实施方案中,该至少三个氨基酸置换为D130A、N295A及S512A。在一个实施方案中,该至少三个氨基酸置换为H193A、N295A及S512A。

[0667] 在一个实施方案中,本发明之糖缀合物之载体蛋白为无酶促活性的SCPB,其中该失活系通过置换野生型序列之至少三个氨基酸来实现。优选地,该至少三个氨基酸置换系在蛋白酶结构域中。在一个实施方案中,该至少三个氨基酸置换系在蛋白酶结构域之部分1中。在一个实施方案中,该至少三个氨基酸置换系在蛋白酶结构域之部分2中。在一个实施方案中,该至少三个氨基酸置换系选自由D130A、H193A、N295A及S512A组成的组。在一个实施方案中,该至少三个氨基酸置换为D130A、H193A及N295A。在一个实施方案中,该至少三个

氨基酸置换为D130A、H193A及S512A。在一个实施方案中,该至少三个氨基酸置换为D130A、N295A及S512A。在一个实施方案中,该至少三个氨基酸置换为H193A、N295A及S512A。

[0668] 在一个实施方案中,本发明之糖缀合物之载体蛋白为无酶促活性的SCP之片段,其中该失活系通过置换野生型序列之至少三个氨基酸来实现。优选地,该至少三个氨基酸置换系在蛋白酶结构域中。在一个实施方案中,该至少三个氨基酸置换系在蛋白酶结构域之部分1中。在一个实施方案中,该至少三个氨基酸置换系在蛋白酶结构域之部分2中。在一个实施方案中,该至少三个氨基酸置换系选自由D130A、H193A、N295A及S512A组成的组。在一个实施方案中,该至少三个氨基酸置换为D130A、H193A及N295A。在一个实施方案中,该至少三个氨基酸置换为D130A、H193A及S512A。在一个实施方案中,该至少三个氨基酸置换为D130A、N295A及S512A。在一个实施方案中,该至少三个氨基酸置换为H193A、N295A及S512A。

[0669] 在一个实施方案中,本发明之糖缀合物之载体蛋白为无酶促活性的SCP之片段,其包含蛋白酶结构域、蛋白酶相关结构域(PA域)及三个纤连蛋白III型(Fn)结构域但不包含输出信号前序列、原序列及细胞壁锚定域,其中该失活系通过置换野生型序列之至少三个氨基酸来实现。优选地,该至少三个氨基酸置换系在蛋白酶结构域中。在一个实施方案中,该至少三个氨基酸置换系在蛋白酶结构域之部分1中。在一个实施方案中,该至少三个氨基酸置换系在蛋白酶结构域之部分2中。在一个实施方案中,该至少三个氨基酸置换系选自由D130A、H193A、N295A及S512A组成的组。在一个实施方案中,该至少三个氨基酸置换为D130A、H193A及N295A。在一个实施方案中,该至少三个氨基酸置换为D130A、H193A及S512A。在一个实施方案中,该至少三个氨基酸置换为D130A、N295A及S512A。在一个实施方案中,该至少三个氨基酸置换为H193A、N295A及S512A。

[0670] 在一个实施方案中,本发明之糖缀合物之载体蛋白为无酶促活性的SCPA之片段,其包含蛋白酶结构域、蛋白酶相关结构域(PA域)及三个纤连蛋白III型(Fn)结构域但不包含输出信号前序列、原序列及细胞壁锚定域,其中该失活系通过置换野生型序列之至少三个氨基酸来实现。优选地,该至少三个氨基酸置换系在蛋白酶结构域中。在一个实施方案中,该至少三个氨基酸置换系在蛋白酶结构域之部分1中。在一个实施方案中,该至少三个氨基酸置换系在蛋白酶结构域之部分2中。在一个实施方案中,该至少三个氨基酸置换系选自由D130A、H193A、N295A及S512A组成的组。在一个实施方案中,该至少三个氨基酸置换为D130A、H193A及N295A。在一个实施方案中,该至少三个氨基酸置换为D130A、H193A及S512A。在一个实施方案中,该至少三个氨基酸置换为D130A、N295A及S512A。在一个实施方案中,该至少三个氨基酸置换为H193A、N295A及S512A。

[0671] 在一个实施方案中,本发明之糖缀合物之载体蛋白为无酶促活性的SCPB之片段,其包含蛋白酶结构域、蛋白酶相关结构域(PA域)及三个纤连蛋白III型(Fn)结构域但不包含输出信号前序列、原序列及细胞壁锚定域,其中该失活系通过置换野生型序列之至少三个氨基酸来实现。优选地,该至少三个氨基酸置换系在蛋白酶结构域中。在一个实施方案中,该至少三个氨基酸置换系在蛋白酶结构域之部分1中。在一个实施方案中,该至少三个氨基酸置换系在蛋白酶结构域之部分2中。在一个实施方案中,该至少三个氨基酸置换系选自由D130A、H193A、N295A及S512A组成的组。在一个实施方案中,该至少三个氨基酸置换为D130A、H193A及N295A。在一个实施方案中,该至少三个氨基酸置换为D130A、H193A及S512A。在一个实施方案中,该至少三个氨基酸置换为D130A、N295A及S512A。在一个实施方案中,该

至少三个氨基酸置换为H193A、N295A及S512A。

[0672] 在一个实施方案中,SCP之酶促活性系通过置换野生型序列之至少四个氨基酸而失活。在一个实施方案中,该至少四个氨基酸置换为D130A、H193A、N295A及S512A。

[0673] 因此,在一个实施方案中,本发明之糖缀合物之载体蛋白为无酶促活性的SCP,其中该失活系通过置换野生型序列之至少四个氨基酸来实现。优选地,该至少四个氨基酸置换系在蛋白酶结构域中。在一个实施方案中,该至少四个氨基酸置换系在蛋白酶结构域之部分1中。在一个实施方案中,该至少四个氨基酸置换系在蛋白酶结构域之部分2中。在一个实施方案中,该至少四个氨基酸置换为D130A、H193A、N295A及S512A。

[0674] 在一个实施方案中,本发明之糖缀合物之载体蛋白为无酶促活性的SCPA,其中该失活系通过置换野生型序列之至少四个氨基酸来实现。优选地,该至少四个氨基酸置换系在蛋白酶结构域中。在一个实施方案中,该至少四个氨基酸置换系在蛋白酶结构域之部分1中。在一个实施方案中,该至少四个氨基酸置换系在蛋白酶结构域之部分2中。在一个实施方案中,该至少四个氨基酸置换为D130A、H193A、N295A及S512A。

[0675] 在一个实施方案中,本发明之糖缀合物之载体蛋白为无酶促活性的SCPB,其中该失活系通过置换野生型序列之至少四个氨基酸来实现。优选地,该至少四个氨基酸置换系在蛋白酶结构域中。在一个实施方案中,该至少四个氨基酸置换系在蛋白酶结构域之部分1中。在一个实施方案中,该至少四个氨基酸置换系在蛋白酶结构域之部分2中。在一个实施方案中,该至少四个氨基酸置换为D130A、H193A、N295A及S512A。

[0676] 在一个实施方案中,本发明之糖缀合物之载体蛋白为无酶促活性的SCP之片段,其中该失活系通过置换野生型序列之至少四个氨基酸来实现。优选地,该至少四个氨基酸置换系在蛋白酶结构域中。在一个实施方案中,该至少四个氨基酸置换系在蛋白酶结构域之部分1中。在一个实施方案中,该至少四个氨基酸置换系在蛋白酶结构域之部分2中。在一个实施方案中,该至少四个氨基酸置换为D130A、H193A、N295A及S512A。

[0677] 在一个实施方案中,本发明之糖缀合物之载体蛋白为无酶促活性的SCP之片段,其包含蛋白酶结构域、蛋白酶相关结构域(PA域)及三个纤连蛋白III型(Fn)结构域但不包含输出信号前序列、原序列及细胞壁锚定域,其中该失活系通过置换野生型序列之至少四个氨基酸来实现。优选地,该至少四个氨基酸置换系在蛋白酶结构域中。在一个实施方案中,该至少四个氨基酸置换系在蛋白酶结构域之部分1中。在一个实施方案中,该至少四个氨基酸置换系在蛋白酶结构域之部分2中。在一个实施方案中,该至少四个氨基酸置换为D130A、H193A、N295A及S512A。

[0678] 在一个实施方案中,本发明之糖缀合物之载体蛋白为无酶促活性的SCPA之片段,其包含蛋白酶结构域、蛋白酶相关结构域(PA域)及三个纤连蛋白III型(Fn)结构域但不包含输出信号前序列、原序列及细胞壁锚定域,其中该失活系通过置换野生型序列之至少四个氨基酸来实现。优选地,该至少四个氨基酸置换系在蛋白酶结构域中。在一个实施方案中,该至少四个氨基酸置换系在蛋白酶结构域之部分1中。在一个实施方案中,该至少一个氨基酸置换系在蛋白酶结构域之部分2中。在一个实施方案中,该至少四个氨基酸置换为D130A、H193A、N295A及S512A。

[0679] 在一个实施方案中,本发明之糖缀合物之载体蛋白为无酶促活性的SCPB之片段,其包含蛋白酶结构域、蛋白酶相关结构域(PA域)及三个纤连蛋白III型(Fn)结构域但不包

含输出信号前序列、原序列及细胞壁锚定域,其中该失活系通过置换野生型序列之至少四个氨基酸来实现。优选地,该至少四个氨基酸置换系在蛋白酶结构域中。在一个实施方案中,该至少四个氨基酸置换系在蛋白酶结构域之部分1中。在一个实施方案中,该至少四个氨基酸置换系在蛋白酶结构域之部分2中。在一个实施方案中,该至少四个氨基酸置换为D130A、H193A、N295A及S512A。

[0680] 在一个特定实施方案中,本发明之糖缀合物之载体蛋白为由SEQ ID NO:41组成的无酶促活性的SCP之片段。

[0681] 在一个特定实施方案中,本发明之糖缀合物之载体蛋白为由SEQ ID NO:42组成的无酶促活性的SCP之片段。

[0682] SEQ ID NO:41:

MAKTADTPATSKATIRDLNDPSQVKTLQEKGAGTVVAVIAAGFDKNH
EAWRLTDKAKARYQSKEDLEKAKKEHGITYGEWVNDKVAYYHDYSKDGKT
AVDQEHGTHVSGILSGNAPSETKEPYRLEGAMPEAQLLLMRVEIVNGLAD
YARNYAQAIRDAINLGAKVINMSFGNAALAYANLPDETKKAFDYAKSKGV
SIVTSAGNDSSFSGGKTRLPLADHPDYGVVGTPAAADSTLTVASYSPDKQL
TETVTVKTADQQDKEMPVLSTNRFEPNKAYDYAYANRGTKEDDFKDVKGK
IALIERGDIDFKDKIAKAKKAGAVGVLIYDNQDKGFPIELPNVDQMPAAF
ISRKDGLLLKDNPPQKTITFNATPKVLPTASGTKLSRFSSWGLTADGNIKP
DIAAPGQDILSSVANNKYAKLSGTAMSAPLVAGIMGLLQEYETQYPDMT
PSERLDLAKKVLMSATALYDEDEKAYFSPRQQGAGAVDAKKASAATMYV
TDKDNTSSKVHLNNVSDKFEVTVTVHNKSDKPQELYYQATVQTDKVDGKH
FALAPKALYETSWQKITIPANSSKQVTVPIDASRFSKDLLAQMKNGYFLE
GFVRFKQDPKKEELMSIPYIGFRGDFGNLSALEKPIYDSKDGSSYYHEAN
SDAKDQLDGDGLQFYALKNNFTALTTESNPWTIIKAVKEGVENIEDIESS
EITETIFAGTFAKQDDDSHYIHRHANGKPYAAISPNGDGNRDYVQFQGT
FLRNAKNLVAEVLDEKGNVVTSEVTEQVVKNNNDLASTLGSTRFEKTR
WDGKDKDGKVVANGTYTYRVRYTPISSGAKEQHTDFDVVDNTTPEVATS
ATFSTEDRRLTLASKPKTSQPVYRERIAITYMDEDLPTTEYISPNEGDTF
TLPEEAETMEGATVPLKMSDFTYVVEDMAGNITYTPVTKLLEGHSNKPEQ

[0684] SEQ ID NO:41之长度为950个氨基酸。

[0685] SEQ ID NO:42:

[0686] AKTADTPATSKATIRDLNDPSQVKTLQEKGAGTVVAVIAAGFDKNH

EAWRLTDKAKARYQSKEDLEKAKKEHGITYGEWVNDKVAYYHDYSKDGKT
AVDQEHGTHVSGILSGNAPSETKEPYRLEGAMPEAQLLLMRVEIVNGLAD
YARNYAQAIRDAINLGAKVINMSFGNAALAYANLPDETKKAFDYAKSKGV
SIVTSAGNDSSFSGGKTRLPLADHPDYGVVGTAAADSTLTVASYSPDKQL
TETVTVKTADQQDKEMPVLSTNRFEPNKAYDYAYANRGTKEDDFKDVKGK
IALIERGDIDFKDKIAKAKKAGAVGVLIYDNQDKGFPIELPNVDQMPAAF
ISRKDGLLLKDNPPQKTITFNATPKVLPTASGTLKSRFSSWGLTADGNIKP
DIAAPGQDILSSVANNKYAKLSGTAMSAPLVAGIMGLLQEQYETQYPDMT
[0687] PSERLDLAKKVLMSATALYDEDEKAYFSPRQQGAGAVDAKKASAATMYV
TDKDNTSSKVHLNNVSDKFEVTVTVHNKSDKPQELYYQATVQTDKVDGKH
FALAPKALYETSWQKITIPANSSKQVTVPIDASRFSDLLAQMKNGYFLE
GFVRFKQDPKKEELMSIPYIGFRGDFGNLSALEKPIYDSKDGSSYYHEAN
SDAKDQLDGDGLQFYALKNNFTALTTESNPWTIIKAVKEGVENIEDIESS
EITETIFAGTFAKQDDDSHYIHRHANGKPYAAISPNGDGNRDYVQFQGT
FLRNAKNLVAEVL DKEGNVVTSEVTEQVVKNNNDLASTLGSTRFEKTR
WDGKDKDGKVVANGTYTYRVRYTPISSGAKEQHTDFDVVDNTTPEVATS
ATFSTEDRRLTLASKPKTSQPVYRERIAITYMDEDLPTTEYISPNEGTF
TLPEEAETMEGATVPLKMSDFTYVVEDMAGNITYTPVTKLLEGHSNKPEQ

[0688] SEQ ID NO:42之长度为949个氨基酸。

[0689] 在一个特定实施方案中,本发明之糖缀合物之载体蛋白为由与SEQ ID NO:41具有至少90%同一性之多肽组成的无酶促活性的SCP之片段。

[0690] 在一个特定实施方案中,本发明之糖缀合物之载体蛋白为由与SEQ ID NO:41具有至少95%同一性之多肽组成的无酶促活性的SCP之片段。

[0691] 在一个特定实施方案中,本发明之糖缀合物之载体蛋白为由与SEQ ID NO:41具有至少99%同一性之多肽组成的无酶促活性的SCP之片段。

[0692] 在一个特定实施方案中,本发明之糖缀合物之载体蛋白为由与SEQ ID NO:41具有至少99.5%同一性之多肽组成的无酶促活性的SCP之片段。

[0693] 在一个特定实施方案中,本发明之糖缀合物之载体蛋白为由与SEQ ID NO:41具有至少99.8%同一性之多肽组成的无酶促活性的SCP之片段。

[0694] 在一个特定实施方案中,本发明之糖缀合物之载体蛋白为由与SEQ ID NO:41具有至少99.85%同一性之多肽组成的无酶促活性的SCP之片段。

[0695] 在一个特定实施方案中,本发明之糖缀合物之载体蛋白为由与SEQ ID NO:42具有至少90%同一性之多肽组成的无酶促活性的SCP之片段。

[0696] 在一个特定实施方案中,本发明之糖缀合物之载体蛋白为由与SEQ ID NO:42具有至少95%同一性之多肽组成的无酶促活性的SCP之片段。

[0697] 在一个特定实施方案中,本发明之糖缀合物之载体蛋白为由与SEQ ID NO:42具有

至少99%同一性之多肽组成的无酶促活性的SCP之片段。

[0698] 在一个特定实施方案中,本发明之糖缀合物之载体蛋白为由与SEQ ID NO:42具有至少99.5%同一性之多肽组成的无酶促活性的SCP之片段。

[0699] 在一个特定实施方案中,本发明之糖缀合物之载体蛋白为由与SEQ ID NO:42具有至少99.8%同一性之多肽组成的无酶促活性的SCP之片段。

[0700] 在一个特定实施方案中,本发明之糖缀合物之载体蛋白为由与SEQ ID NO:42具有至少99.85%同一性之多肽组成的无酶促活性的SCP之片段。

[0701] 2免疫原性组合物

[0702] 2.1本发明之糖缀合物之组合

[0703] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物(如上文部分1处所公开)。

[0704] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含1至25种不同糖缀合物。

[0705] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含1至25种来自不同肺炎链球菌血清型之糖缀合物(1至25种肺炎链球菌缀合物)。在一个实施方案中,本发明涉及一种免疫原性组合物,其包含来自7、8、9、10、11、12、13、14、15、16、17、18、19、20、21、22、23、24或25种不同肺炎链球菌血清型之糖缀合物。在一个实施方案中,免疫原性组合物包含来自16或20种不同肺炎链球菌血清型之糖缀合物。在一个实施方案中,免疫原性组合物为7、8、9、10、11、12、13、14、15、16、17、18、19或20价肺炎链球菌缀合物组合物。在一个实施方案中,免疫原性组合物为14、15、16、17、18或19价肺炎链球菌缀合物组合物。在一个实施方案中,免疫原性组合物为16价肺炎链球菌缀合物组合物。在一个实施方案中,免疫原性组合物为19价肺炎链球菌缀合物组合物。在一个实施方案中,免疫原性组合物为20价肺炎链球菌缀合物组合物。

[0706] 在一个实施方案中,免疫原性组合物为21、22、23、24或25价肺炎链球菌缀合物组合物。在一个实施方案中,免疫原性组合物为21价肺炎链球菌缀合物组合物。在一个实施方案中,免疫原性组合物为22价肺炎链球菌缀合物组合物。在一个实施方案中,免疫原性组合物为23价肺炎链球菌缀合物组合物。在一个实施方案中,免疫原性组合物为24价肺炎链球菌缀合物组合物。在一个实施方案中,免疫原性组合物为25价肺炎链球菌缀合物组合物。

[0707] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含来自肺炎链球菌血清型1、3、4、5、6B、7F、9V、14、18C、19F及23F之糖缀合物。在一个实施方案中,免疫原性组合物为11价肺炎链球菌缀合物组合物。

[0708] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含来自肺炎链球菌血清型1、3、4、5、6A、6B、7F、9V、14、18C、19A、19F及23F之糖缀合物。在一个实施方案中,免疫原性组合物为13价肺炎链球菌缀合物组合物。

[0709] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且进一步包含来自肺炎链球菌血清型1、3、4、5、6A、6B、7F、9V、14、18C、19A、19F、22F、23F及33F之糖缀合物。在一个实施方案中,免疫原性组合物为15价肺炎链球菌缀合物组合物。

[0710] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含来自肺炎链球菌血清型1、3、4、5、6A、6B、7F、8、9V、10A、11A、12F、14、15B、18C、19A、

19F、22F、23F及33F之糖缀合物。在一个实施方案中,免疫原性组合物为20价肺炎链球菌缀合物组合物。

[0711] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且进一步包含来自肺炎链球菌血清型1、2、3、4、5、6A、6B、7F、8、9V、10A、11A、12F、14、15B、18C、19A、19F、22F、23F及33F之糖缀合物。在一个实施方案中,免疫原性组合物为21价肺炎链球菌缀合物组合物。

[0712] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含来自肺炎链球菌血清型1、3、4、5、6A、6B、7F、8、9V、9N、10A、11A、12F、14、15B、18C、19A、19F、22F、23F及33F之糖缀合物。在一个实施方案中,免疫原性组合物为21价肺炎链球菌缀合物组合物。

[0713] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含来自肺炎链球菌血清型1、3、2、4、5、6A、6B、7F、8、9V、9N、10A、11A、12F、14、15B、18C、19A、19F、22F、23F及33F之糖缀合物。在一个实施方案中,免疫原性组合物为22价肺炎链球菌缀合物组合物。

[0714] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含来自肺炎链球菌血清型1、3、2、4、5、6A、6B、7F、8、9V、9N、10A、11A、12F、14、15B、17F、18C、19A、19F、22F、23F及33F之糖缀合物。在一个实施方案中,免疫原性组合物为23价肺炎链球菌缀合物组合物。

[0715] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含来自肺炎链球菌血清型1、2、3、4、5、6A、6B、7F、8、9V、9N、10A、11A、12F、14、15B、17F、18C、19A、19F、20、22F、23F及33F之糖缀合物。在一个实施方案中,免疫原性组合物为24价肺炎链球菌缀合物组合物。

[0716] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含来自肺炎链球菌血清型1、3、4、5、6A、6B、7F、8、9V、10A、11A、12F、14、15A、15B、18C、19A、19F、22F、23F及33F之糖缀合物。在一个实施方案中,免疫原性组合物为21价肺炎链球菌缀合物组合物。

[0717] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含来自肺炎链球菌血清型1、3、4、5、6A、6B、7F、8、9V、10A、11A、12F、14、15B、18C、19A、19F、22F、23A、23F及33F之糖缀合物。在一个实施方案中,免疫原性组合物为21价肺炎链球菌缀合物组合物。

[0718] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含来自肺炎链球菌血清型1、3、4、5、6A、6B、7F、8、9V、10A、11A、12F、14、15B、18C、19A、19F、22F、23B、23F及33F之糖缀合物。在一个实施方案中,免疫原性组合物为21价肺炎链球菌缀合物组合物。

[0719] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含来自肺炎链球菌血清型1、3、4、5、6A、6B、7F、8、9V、10A、11A、12F、14、15B、18C、19A、19F、22F、23F、24F及33F之糖缀合物。在一个实施方案中,免疫原性组合物为21价肺炎链球菌缀合物组合物。

[0720] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物

且包含来自肺炎链球菌血清型1、3、4、5、6A、6B、7F、8、9V、10A、11A、12F、14、15B、18C、19A、19F、22F、23F、33F及35B之糖缀合物。在一个实施方案中,免疫原性组合物为21价肺炎链球菌缀合物组合物。

[0721] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含来自肺炎链球菌血清型1、3、4、5、6A、6B、7F、8、9V、10A、11A、12F、14、15A、15B、18C、19A、19F、22F、23A、23F及33F之糖缀合物。在一个实施方案中,免疫原性组合物为22价肺炎链球菌缀合物组合物。

[0722] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且进一步包含来自肺炎链球菌血清型1、3、4、5、6A、6B、7F、8、9V、10A、11A、12F、14、15A、15B、18C、19A、19F、22F、23B、23F及33F之糖缀合物。在一个实施方案中,免疫原性组合物为22价肺炎链球菌缀合物组合物。

[0723] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含来自肺炎链球菌血清型1、3、4、5、6A、6B、7F、8、9V、10A、11A、12F、14、15A、15B、18C、19A、19F、22F、23F、24F及33F之糖缀合物。在一个实施方案中,免疫原性组合物为22价肺炎链球菌缀合物组合物。

[0724] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含来自肺炎链球菌血清型1、3、4、5、6A、6B、7F、8、9V、10A、11A、12F、14、15A、15B、18C、19A、19F、22F、23F、33F及35B之糖缀合物。在一个实施方案中,免疫原性组合物为22价肺炎链球菌缀合物组合物。

[0725] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含来自肺炎链球菌血清型1、3、4、5、6A、6B、7F、8、9V、10A、11A、12F、14、15B、18C、19A、19F、22F、23A、23B、23F及33F之糖缀合物。在一个实施方案中,免疫原性组合物为22价肺炎链球菌缀合物组合物。

[0726] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含来自肺炎链球菌血清型1、3、4、5、6A、6B、7F、8、9V、10A、11A、12F、14、15B、18C、19A、19F、22F、23A、23F、24F及33F之糖缀合物。在一个实施方案中,免疫原性组合物为22价肺炎链球菌缀合物组合物。

[0727] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含来自肺炎链球菌血清型1、3、4、5、6A、6B、7F、8、9V、10A、11A、12F、14、15B、18C、19A、19F、22F、23A、23F、33F及35B之糖缀合物。在一个实施方案中,免疫原性组合物为22价肺炎链球菌缀合物组合物。

[0728] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含来自肺炎链球菌血清型1、3、4、5、6A、6B、7F、8、9V、10A、11A、12F、14、15B、18C、19A、19F、22F、23B、23F、24F及33F之糖缀合物。在一个实施方案中,免疫原性组合物为22价肺炎链球菌缀合物组合物。

[0729] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含来自肺炎链球菌血清型1、3、4、5、6A、6B、7F、8、9V、10A、11A、12F、14、15B、18C、19A、19F、22F、23B、23F、33F及35B之糖缀合物。在一个实施方案中,免疫原性组合物为22价肺炎链球菌缀合物组合物。

[0730] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含来自肺炎链球菌血清型1、3、4、5、6A、6B、7F、8、9V、10A、11A、12F、14、15B、18C、19A、19F、22F、23F、24F、33F及35B之糖缀合物。在一个实施方案中,免疫原性组合物为22价肺炎链球菌缀合物组合物。

[0731] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含来自肺炎链球菌血清型1、3、4、5、6A、6B、7F、8、9V、10A、11A、12F、14、15A、15B、18C、19A、19F、22F、23A、23B、23F及33F之糖缀合物。在一个实施方案中,免疫原性组合物为23价肺炎链球菌缀合物组合物。

[0732] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含来自肺炎链球菌血清型1、3、4、5、6A、6B、7F、8、9V、10A、11A、12F、14、15A、15B、18C、19A、19F、22F、23A、23F、24F及33F之糖缀合物。在一个实施方案中,免疫原性组合物为23价肺炎链球菌缀合物组合物。

[0733] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含来自肺炎链球菌血清型1、3、4、5、6A、6B、7F、8、9V、10A、11A、12F、14、15A、15B、18C、19A、19F、22F、23A、23F、33F及35B之糖缀合物。在一个实施方案中,免疫原性组合物为23价肺炎链球菌缀合物组合物。

[0734] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含来自肺炎链球菌血清型1、3、4、5、6A、6B、7F、8、9V、10A、11A、12F、14、15A、15B、18C、19A、19F、22F、23B、23F、24F及33F之糖缀合物。在一个实施方案中,免疫原性组合物为23价肺炎链球菌缀合物组合物。

[0735] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含来自肺炎链球菌血清型1、3、4、5、6A、6B、7F、8、9V、10A、11A、12F、14、15A、15B、18C、19A、19F、22F、23B、23F、33F及35B之糖缀合物。在一个实施方案中,免疫原性组合物为23价肺炎链球菌缀合物组合物。

[0736] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含来自肺炎链球菌血清型1、3、4、5、6A、6B、7F、8、9V、10A、11A、12F、14、15A、15B、18C、19A、19F、22F、23F、24F、33F及35B之糖缀合物。在一个实施方案中,免疫原性组合物为23价肺炎链球菌缀合物组合物。

[0737] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含来自肺炎链球菌血清型1、3、4、5、6A、6B、7F、8、9V、10A、11A、12F、14、15B、18C、19A、19F、22F、23A、23B、23F、33F及35B之糖缀合物。在一个实施方案中,免疫原性组合物为23价肺炎链球菌缀合物组合物。

[0738] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含来自肺炎链球菌血清型1、3、4、5、6A、6B、7F、8、9V、10A、11A、12F、14、15B、18C、19A、19F、22F、23A、23B、23F、24F及33F之糖缀合物。在一个实施方案中,免疫原性组合物为23价肺炎链球菌缀合物组合物。

[0739] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含来自肺炎链球菌血清型1、3、4、5、6A、6B、7F、8、9V、10A、11A、12F、14、15B、18C、19A、19F、22F、23A、23F、24F、33F及35B之糖缀合物。在一个实施方案中,免疫原性组合物为23价

肺炎链球菌缀合物组合物。

[0740] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含来自肺炎链球菌血清型1、3、4、5、6A、6B、7F、8、9V、10A、11A、12F、14、15B、18C、19A、19F、22F、23B、23F、24F、33F及35B之糖缀合物。在一个实施方案中,免疫原性组合物为23价肺炎链球菌缀合物组合物。

[0741] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含来自肺炎链球菌血清型1、3、4、5、6A、6B、7F、8、9V、10A、11A、12F、14、15A、15B、18C、19A、19F、22F、23A、23B、23F、24F及33F之糖缀合物。在一个实施方案中,免疫原性组合物为24价肺炎链球菌缀合物组合物。

[0742] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含来自肺炎链球菌血清型1、3、4、5、6A、6B、7F、8、9V、10A、11A、12F、14、15A、15B、18C、19A、19F、22F、23A、23B、23F、33F及35B之糖缀合物。在一个实施方案中,免疫原性组合物为24价肺炎链球菌缀合物组合物。

[0743] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含来自肺炎链球菌血清型1、3、4、5、6A、6B、7F、8、9V、10A、11A、12F、14、15A、15B、18C、19A、19F、22F、23A、23F、24F、33F及35B之糖缀合物。在一个实施方案中,免疫原性组合物为24价肺炎链球菌缀合物组合物。

[0744] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含来自肺炎链球菌血清型1、3、4、5、6A、6B、7F、8、9V、10A、11A、12F、14、15A、15B、18C、19A、19F、22F、23B、23F、24F、33F及35B之糖缀合物。在一个实施方案中,免疫原性组合物为24价肺炎链球菌缀合物组合物。

[0745] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含来自肺炎链球菌血清型1、3、4、5、6A、6B、7F、8、9V、10A、11A、12F、14、15B、18C、19A、19F、22F、23A、23B、23F、24F、33F及35B之糖缀合物。在一个实施方案中,免疫原性组合物为24价肺炎链球菌缀合物组合物。

[0746] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含来自肺炎链球菌血清型1、3、4、5、6A、6B、7F、8、9V、10A、11A、12F、14、15A、15B、18C、19A、19F、22F、23A、23B、23F、24F、33F及35B之糖缀合物。在一个实施方案中,免疫原性组合物为25价肺炎链球菌缀合物组合物。

[0747] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含选自由以下组成的组的本发明之糖缀合物:来自肺炎链球菌血清型2、7C、9N、10B、15A、16F、17F、20、21、22A、23A、23B、24B、24F、27、29、31、33B、34、35B、35F及38之糖缀合物。

[0748] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含选自由以下组成的组的本发明之糖缀合物:来自肺炎链球菌血清型2、7C、9N、10B、15A、16F、17F、20、21、22A、23A、23B、24B、24F、27、29、31、33B、34、35B、35F及38之糖缀合物。在一个实施方案中,免疫原性组合物为21价肺炎链球菌缀合物组合物。

[0749] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含二十一种选自由以下组成的组之糖缀合物:来自肺炎链球菌血清型2、7C、9N、10B、15A、16F、17F、20、21、22A、23A、23B、24B、24F、27、29、31、33B、34、35B、35F及38之糖缀合物。

在一个实施方案中,免疫原性组合物为22价肺炎链球菌缀合物组合物。

[0750] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含至少一种选自由以下组成的组之糖缀合物:来自肺炎链球菌血清型2、7C、9N、10B、15A、16F、17F、19A、19F、20、21、22A、23A、23B、24B、24F、27、29、31、33B、34、35B、35F及38之糖缀合物。

[0751] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含二十二种选自由以下组成的组之糖缀合物:来自肺炎链球菌血清型2、7C、9N、10B、15A、16F、17F、19A、19F、20、21、22A、23A、23B、24B、24F、27、29、31、33B、34、35B、35F及38之糖缀合物。在一个实施方案中,免疫原性组合物为23价肺炎链球菌缀合物组合物。

[0752] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含二十三种选自由以下组成的组之糖缀合物:来自肺炎链球菌血清型2、7C、9N、10B、15A、16F、17F、19A、19F、20、21、22A、23A、23B、24B、24F、27、29、31、33B、34、35B、35F及38之糖缀合物。在一个实施方案中,免疫原性组合物为24价肺炎链球菌缀合物组合物。

[0753] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含来自肺炎链球菌血清型2、9N、15A、17F、20、23A、23B、24F及35B之糖缀合物。在一个实施方案中,免疫原性组合物为10价肺炎链球菌缀合物组合物。

[0754] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含来自肺炎链球菌血清型2、9N、15A、17F、19A、19F、20、23A、23B、24F及35B之糖缀合物。在一个实施方案中,免疫原性组合物为12价肺炎链球菌缀合物组合物。

[0755] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含来自肺炎链球菌血清型2、7C、9N、10B、15A、16F、17F、20、21、22A、23A、23B、24B、24F、27、29、31、33B、34、35B、35F及38之糖缀合物。在一个实施方案中,免疫原性组合物为23价肺炎链球菌缀合物组合物。

[0756] 在一个实施方案中,本发明涉及一种免疫原性组合物,其包含本发明之糖缀合物且包含来自肺炎链球菌血清型2、7C、9N、10B、15A、16F、17F、19A、19F、20、21、22A、23A、23B、24B、24F、27、29、31、33B、34、35B、35F及38之糖缀合物。在一个实施方案中,免疫原性组合物为25价肺炎链球菌缀合物组合物。

[0757] 在一个优选实施方案中,糖各自个别地与蛋白载体之不同分子缀合(蛋白载体之各分子仅具有与其缀合之一类型之糖)。在该实施方案中,荚膜糖被称为个别地与载体蛋白缀合。优选地,上述免疫原性组合物之所有糖缀合物均个别地与载体蛋白缀合。

[0758] 在上述免疫原性组合物中之任一者之一实施方案中,本发明之糖缀合物与CRM₁₉₇缀合。在上述免疫原性组合物中之任一者之一实施方案中,本发明之糖缀合物与SCP缀合。

[0759] 在上述免疫原性组合物中之任一者之一实施方案中,来自肺炎链球菌血清型22F之糖缀合物与CRM₁₉₇缀合。在上述免疫原性组合物中之任一者之一实施方案中,来自肺炎链球菌血清型33F之糖缀合物与CRM₁₉₇缀合。在上述免疫原性组合物中之任一者之一实施方案中,来自肺炎链球菌血清型15B之糖缀合物与CRM₁₉₇缀合。在上述免疫原性组合物中之任一者之一实施方案中,来自肺炎链球菌血清型12F之糖缀合物与CRM₁₉₇缀合。在上述免疫原性组合物中之任一者之一实施方案中,来自肺炎链球菌血清型10A之糖缀合物

与CRM₁₉₇缀合。在上述免疫原性组合物中之任一者之一个实施方案中,来自肺炎链球菌血清型11A之糖缀合物与CRM₁₉₇缀合。在上述免疫原性组合物中之任一者之一个实施方案中,来自肺炎链球菌血清型8之糖缀合物与CRM₁₉₇缀合。在上述免疫原性组合物中之任一者之一个实施方案中,来自肺炎链球菌血清型4、6B、9V、14、18C、19F及23F之糖缀合物与CRM₁₉₇缀合。在上述免疫原性组合物中之任一者之一个实施方案中,来自肺炎链球菌血清型1、5及7F之糖缀合物与CRM₁₉₇缀合。在上述免疫原性组合物中之任一者之一个实施方案中,来自肺炎链球菌血清型6A及19A之糖缀合物与CRM₁₉₇缀合。

[0760] 在上述免疫原性组合物中之任一者之一个实施方案中,上述免疫原性组合物中之任一者之糖缀合物均个别地与CRM₁₉₇缀合。

[0761] 在上述免疫原性组合物中之任一者之一个实施方案中,本发明之糖缀合物与SCP缀合且其他糖缀合物均个别地与CRM₁₉₇缀合。

[0762] 在一个实施方案中,上述免疫原性组合物中之任一者之来自肺炎链球菌血清型1、4、5、6B、7F、9V、14及/或23F之糖缀合物个别地与PD缀合。

[0763] 在一个实施方案中,上述免疫原性组合物中之任一者之来自肺炎链球菌血清型18C之糖缀合物与TT缀合。

[0764] 在一个实施方案中,上述免疫原性组合物中之任一者之来自肺炎链球菌血清型19F之糖缀合物与DT缀合。

[0765] 在一个实施方案中,上述免疫原性组合物中之任一者之来自肺炎链球菌血清型1、4、5、6B、7F、9V、14及/或23F之糖缀合物个别地与PD缀合,来自肺炎链球菌血清型18C之糖缀合物与TT缀合,且来自肺炎链球菌血清型19F之糖缀合物与DT缀合。

[0766] 在一个实施方案中,上述免疫原性组合物包含8至25种不同肺炎链球菌血清型。

[0767] 本发明之组合物可包括少量游离载体。当给定载体蛋白以游离及缀合形式存在于本发明之组合物中时,未缀合形式优选总体上不超过组合物中之载体蛋白之总量的5%,且更优选以少于2重量%存在。

[0768] 2.2 本发明之免疫原性组合物之剂量

[0769] 各剂量中之糖缀合物之量经选择为诱导免疫保护性反应而无典型疫苗接种者中之明显不良副作用之量。此种量将视采用何种特定免疫原及其呈递方式而变化。

[0770] 免疫原性组合物中之特定糖缀合物之量可基于该缀合物之总糖(缀合糖及未缀合糖)来计算。举例而言,在100μg糖剂量中,具有20%游离糖之糖缀合物将具有约80μg之缀合糖及约20μg之未缀合糖。糖缀合物之量可视细菌及细菌血清型而变化。可通过糖醛酸分析来测定糖浓度。

[0771] 免疫原性组合物中之不同糖组分之“免疫原性量”可不同,且各自可包含约0.5μg、约0.75μg、约1μg、约2μg、约3μg、约4μg、约5μg、约6μg、约7μg、约8μg、约9μg、约10μg、约15μg、约20μg、约30μg、约40μg、约50μg、约60μg、约70μg、约80μg、约90μg或约100μg之任何特定糖抗原。

[0772] 一般而言,各剂量将包含0.1μg至100μg之糖。在一个实施方案中,各剂量将包含0.1μg至100μg之糖。在一个优选实施方案中,各剂量将包含0.5μg至20μg。在一个优选实施方案中,各剂量将包含1.0μg至10μg。在一甚至更优选实施方案中,各剂量将包含2.0μg至5.0μg之血清型3多糖。考虑以上范围中之任一者内之任何全数整数作为本发明之一个实施

方案。

[0773] 在一个实施方案中,各剂量将包含约0.5 μ g之糖。在一个实施方案中,各剂量将包含约0.55 μ g之糖。在一个实施方案中,各剂量将包含约0.75 μ g之糖。在一个实施方案中,各剂量将包含约1.0 μ g之糖。在一个实施方案中,各剂量将包含约1.1 μ g之糖。在一个实施方案中,各剂量将包含约1.5 μ g之糖。在一个实施方案中,各剂量将包含约2.0 μ g之糖。在一个实施方案中,各剂量将包含约2.2 μ g之糖。在一个实施方案中,各剂量将包含约2.5 μ g之糖。在一个实施方案中,各剂量将包含约3.0 μ g之糖。在一个实施方案中,各剂量将包含约3.5 μ g之糖。在一个实施方案中,各剂量将包含约4.0 μ g糖。在一个实施方案中,各剂量将包含约4.4 μ g之糖。在一个实施方案中,各剂量将包含约5.0 μ g之糖。在一个实施方案中,各剂量将包含约5.5 μ g之糖。在一个实施方案中,各剂量将包含约6.0 μ g之糖。

[0774] 一般而言,各剂量将包含0.1 μ g至100 μ g之针对给定细菌或血清型之糖。在一个实施方案中,各剂量将包含0.1 μ g至100 μ g之针对给定细菌或血清型之糖。在一个优选实施方案中,各剂量将包含0.5 μ g至20 μ g。在一个优选实施方案中,各剂量将包含1.0 μ g至10 μ g。在一甚至更优选实施方案中,各剂量将包含2.0 μ g至5.0 μ g之针对给定细菌或血清型之糖。考虑以上范围中之任一者内之任何全数整数作为本发明之一实施方案。

[0775] 在一个实施方案中,各剂量将包含约0.5 μ g之用于各特定糖缀合物之糖。在一个实施方案中,各剂量将包含约0.55 μ g之用于各特定糖缀合物之糖。在一个实施方案中,各剂量将包含约0.75 μ g之用于各特定糖缀合物之糖。在一个实施方案中,各剂量将包含约1.0 μ g之用于各特定糖缀合物之糖。在一个实施方案中,各剂量将包含约1.1 μ g之用于各特定糖缀合物之糖。在一个实施方案中,各剂量将包含约1.5 μ g之用于各特定糖缀合物之糖。在一个实施方案中,各剂量将包含约2.0 μ g之用于各特定糖缀合物之糖。在一个实施方案中,各剂量将包含约2.2 μ g之用于各特定糖缀合物之糖。在一个实施方案中,各剂量将包含约2.5 μ g之用于各特定糖缀合物之糖。在一个实施方案中,各剂量将包含约3.0 μ g之用于各特定糖缀合物之糖。在一个实施方案中,各剂量将包含约3.5 μ g之用于各特定糖缀合物之糖。在一个实施方案中,各剂量将包含约4.0 μ g之用于各特定糖缀合物之糖。在一个实施方案中,各剂量将包含约4.4 μ g之用于各特定糖缀合物之糖。在一个实施方案中,各剂量将包含约5.0 μ g之用于各特定糖缀合物之糖。在一个实施方案中,各剂量将包含约5.5 μ g之用于各特定糖缀合物之糖。在一个实施方案中,各剂量将包含约6.0 μ g之用于各特定糖缀合物之糖。

[0776] 2.3载体量

[0777] 一般而言,各剂量将包含10 μ g至150 μ g之载体蛋白,尤其15 μ g至100 μ g之载体蛋白,更尤其25 μ g至75 μ g之载体蛋白,且甚至更尤其40 μ g至60 μ g之载体蛋白。在一个实施方案中,该载体蛋白为CRM₁₉₇。在一个实施方案中,该载体蛋白为SCP。

[0778] 在一个实施方案中,各剂量将包含约10 μ g、约15 μ g、约20 μ g、约25 μ g、约26 μ g、约27 μ g、约28 μ g、约29 μ g、约30 μ g、约31 μ g、约32 μ g、约33 μ g、约34 μ g、约35 μ g、约36 μ g、约37 μ g、约38 μ g、约39 μ g、约40 μ g、约41 μ g、约42 μ g、约43 μ g、约44 μ g、约45 μ g、约46 μ g、约47 μ g、约48 μ g、约49 μ g、约50 μ g、约51 μ g、约52 μ g、约53 μ g、约54 μ g、约55 μ g、约56 μ g、约57 μ g、约58 μ g、约59 μ g、约60 μ g、约61 μ g、约62 μ g、约63 μ g、约64 μ g、约65 μ g、约66 μ g、约67 μ g、约68 μ g、约69 μ g、约70 μ g、约71 μ g、约72 μ g、约73 μ g、约74 μ g或约75 μ g之载体蛋白。

[0779] 在一个实施方案中,各剂量将包含约25 μ g、约26 μ g、约27 μ g、约28 μ g、约29 μ g、约30 μ

g、约31 μ g、约32 μ g、约33 μ g、约34 μ g、约35 μ g、约36 μ g、约37 μ g、约38 μ g、约39 μ g、约40 μ g、约41 μ g、约42 μ g、约43 μ g、约44 μ g、约45 μ g、约46 μ g、约47 μ g、约48 μ g、约49 μ g、约50 μ g、约51 μ g、约52 μ g、约53 μ g、约54 μ g、约55 μ g、约56 μ g、约57 μ g、约58 μ g、约59 μ g、约60 μ g、约61 μ g、约62 μ g、约63 μ g、约64 μ g、约65 μ g、约66 μ g、约67 μ g、约68 μ g、约69 μ g、约70 μ g、约71 μ g、约72 μ g、约73 μ g、约74 μ g或约75 μ g之载体蛋白。

[0780] 在一个实施方案中,各剂量将包含约30 μ g之载体蛋白。在一个实施方案中,各剂量将包含约31 μ g之载体蛋白。在一个实施方案中,各剂量将包含约32 μ g之载体蛋白。在一个实施方案中,各剂量将包含约33 μ g之载体蛋白。在一个实施方案中,各剂量将包含约34 μ g之载体蛋白。在一个实施方案中,各剂量将包含约45 μ g之载体蛋白。

[0781] 在一个实施方案中,各剂量将包含约40 μ g之载体蛋白。在一个实施方案中,各剂量将包含约41 μ g之载体蛋白。在一个实施方案中,各剂量将包含约42 μ g之载体蛋白。在一个实施方案中,各剂量将包含约43 μ g之载体蛋白。在一个实施方案中,各剂量将包含约44 μ g之载体蛋白。在一个实施方案中,各剂量将包含约45 μ g之载体蛋白。

[0782] 在一个实施方案中,各剂量将包含约48 μ g之载体蛋白。在一个实施方案中,各剂量将包含约49 μ g之载体蛋白。在一个实施方案中,各剂量将包含约50 μ g之载体蛋白。在一个实施方案中,各剂量将包含约51 μ g之载体蛋白。在一个实施方案中,各剂量将包含约52 μ g之载体蛋白。在一个实施方案中,各剂量将包含约53 μ g之载体蛋白。

[0783] 在一个实施方案中,该载体蛋白为CRM₁₉₇。

[0784] 在一个实施方案中,该载体蛋白为SCP。

[0785] 2.4其他抗原

[0786] 本发明之免疫原性组合物包含缀合之糖抗原(糖缀合物)。其还可进一步包含来自其他病原体,尤其来自细菌及/或病毒之抗原。优选的其他抗原系选自:白喉类毒素(D)、破伤风类毒素(T)、百日咳抗原(P)(其通常为非细胞型(Pa))、乙型肝炎病毒(HBV)表面抗原(HBsAg)、甲型肝炎病毒(HAV)抗原、缀合之流感嗜血杆菌b型荚膜糖(Hib)、失活的脊髓灰质炎病毒疫苗(IPV)。

[0787] 在一个实施方案中,本发明之免疫原性组合物包含D-T-Pa。在一个实施方案中,本发明之免疫原性组合物包含D-T-Pa-Hib、D-T-Pa-IPV或D-T-Pa-HBsAg。在一个实施方案中,本发明之免疫原性组合物包含D-T-Pa-HBsAg-IPV或D-T-Pa-HBsAg-Hib。在一个实施方案中,本发明之免疫原性组合物包含D-T-Pa-HBsAg-IPV-Hib。

[0788] 百日咳抗原:百日咳博德特氏菌(*Bordetella pertussis*)引起百日咳。疫苗中之百日咳抗原为细胞型(全细胞,呈失活的百日咳博德特氏菌细胞形式)或非细胞型的。细胞型百日咳抗原之制备已有完备记载(例如其可通过使百日咳博德特氏菌之I期培养物热灭活获得)。然而,优选地,本发明使用非细胞型抗原。在使用非细胞型抗原之情况下,优选使用以下抗原中之一者、两者或(优选地)三者:(1)脱毒之百日咳毒素(百日咳类毒素或PT);(2)丝状血凝素(FHA);(3)百日咳杆菌黏附素(pertactin)(也称为69千道尔顿外膜蛋白)。FHA及百日咳杆菌黏附素在根据本发明使用之前可用甲醛处理。PT优选通过用甲醛及/或戊二醛处理来解毒。非细胞型百日咳抗原优选吸附至一或多种铝盐佐剂上。作为替代方案,其可以未吸附状态添加。在添加百日咳杆菌黏附素之情况下,其优选已吸附至氢氧化铝佐剂上。PT及FHA可吸附至氢氧化铝佐剂或磷酸铝上。最优选地,PT、FHA及百日咳杆菌黏附素皆

吸附至氢氧化铝。

[0789] 失活的脊髓灰质炎病毒疫苗：脊髓灰质炎病毒引起脊髓灰质炎。本发明之优选实施方案使用IPV,而非使用口服脊髓灰质炎病毒疫苗。在向患者施用之前,脊髓灰质炎病毒必须为失活的,且此可通过用甲醛处理来实现。脊髓灰质炎可由三种类型之脊髓灰质炎病毒中之一者引起。三种类型类似且引起相同症状,但其在抗原上不同且一种类型之感染不会避免其他类型之感染。因此,优选使用本发明中之三种脊髓灰质炎病毒抗原:1型脊髓灰质炎病毒(例如Mahoney病毒株)、2型脊髓灰质炎病毒(例如MEF-1病毒株)及3型脊髓灰质炎病毒(例如Saukett病毒株)。病毒优选分别生长、纯化及灭活,且接着合并,得到供本发明使用之主体三价混合物。

[0790] 白喉类毒素:白喉棒状杆菌引起白喉。可对白喉毒素进行处理(例如使用福尔马林或甲醛)以去除毒性,同时保留在注射之后诱导特异性抗毒素抗体之能力。此等白喉类毒素用于白喉疫苗中。优选的白喉类毒素为通过甲醛处理制备的那些。白喉类毒素可通过使白喉棒状杆菌在生长培养基中生长,随后甲醛处理、超滤及沉淀来获得。接着可通过包含无菌过滤及/或透析之方法处理类毒素化物质。白喉类毒素优选吸附至氢氧化铝佐剂上。

[0791] 破伤风类毒素:破伤风梭菌(*Clostridium tetani*)引起破伤风。可对破伤风毒素进行处理以得到保护性类毒素。类毒素用于破伤风疫苗中。优选破伤风类毒素为通过甲醛处理制备的那些。破伤风类毒素可通过使破伤风梭菌在生长培养基中生长,随后甲醛处理、超滤及沉淀来获得。接着可通过包含无菌过滤及/或透析之方法处理物质。

[0792] 甲型肝炎病毒抗原:甲型肝炎病毒(HAV)为引起病毒性肝炎之已知因子中之一者。优选HAV组分系基于失活病毒,且失活可通过福尔马林处理来实现。

[0793] 乙型肝炎病毒(HBV)为引起病毒性肝炎之已知因子中之一者。衣壳之主要组分为被称为HBV表面抗原或更常被称为HBsAg之蛋白质,其通常为具有约24kDa之分子量的226-氨基酸多肽。所有现有乙型肝炎疫苗均含有HBsAg,且当此抗原向正常疫苗接种者施用时,其刺激避免HBV感染之抗HBsAg抗体之产生。

[0794] 对于疫苗制造,HBsAg以两种方式制得:纯化来自慢性乙型肝炎携带者之血浆的呈微粒形式之抗原,或通过重组DNA方法表达蛋白质(例如,在酵母细胞中重组表达)。不同于天然HBsAg(即,如在血浆纯化产物中),酵母表达之HBsAg通常未经糖基化,且此为用于本发明之最优选HBsAg形式。

[0795] 缀合之流感嗜血杆菌b型抗原:流感嗜血杆菌b型(Hib)引起细菌性脑膜炎。Hib疫苗通常系基于荚膜糖抗原,其制备已有完备记载。Hib糖可与载体蛋白缀合以增强其免疫原性,尤其在儿童中之免疫原性。典型载体蛋白为破伤风类毒素、白喉类毒素、CRM₁₉₇、流感嗜血杆菌蛋白D及来自血清群B脑膜炎双球菌之外膜蛋白复合物。缀合物之糖部分可包含由Hib细菌制备之全长聚核糖基核糖醇磷酸酯(PRP)及/或全长PRP之片段。Hib缀合物可吸附或不吸附至铝盐佐剂。

[0796] 2.5佐剂

[0797] 在一些实施方案中,本文所公开之免疫原性组合物可进一步包含至少一种、两种或三种佐剂。在一些实施方案中,本文所公开之免疫原性组合物可进一步包含至少一种佐剂。在一些实施方案中,本文所公开之免疫原性组合物可进一步包含一种佐剂。在一些实施方案中,本文所公开之免疫原性组合物可进一步包含两种佐剂。术语“佐剂”系指增强针对

抗原之免疫反应的化合物或混合物。抗原可主要用作递送系统,主要用作免疫调节剂,或具有两者之强力特征。

[0798] 适合的佐剂包括适用于哺乳动物(包括人类)的那些佐剂。已知可在人类中使用之适合的递送系统类型佐剂之实例包括(但不限于)明矾(例如磷酸铝、硫酸铝或氢氧化铝)、磷酸钙、脂质体、水包油乳液(诸如MF59)(4.3%w/v角鲨烯、0.5%w/v聚山梨醇酯80(Tween 80)、0.5%w/v脱水山梨糖醇三油酸酯(Span 85))、油包水乳液(诸如Montanide)及聚(D,L-丙交酯-共聚-乙交酯)(PLG)微米粒子或纳米粒子。

[0799] 在一个实施方案中,本文所公开之免疫原性组合物包含作为佐剂之铝盐(明矾)(例如磷酸铝、硫酸铝或氢氧化铝)。在一个优选实施方案中,本文所公开之免疫原性组合物包含作为佐剂之磷酸铝或氢氧化铝。在一个优选实施方案中,本文所公开之免疫原性组合物包含作为佐剂之磷酸铝。

[0800] 增强如本文所公开之免疫原性组合物之有效性的其他例示性佐剂包括(但不限于):(1)水包油乳液制剂(具有或不具有其他特定免疫刺激剂,诸如胞壁肽(参见下文)或细菌细胞壁组分),诸如(a)SAF,其含有微流化成次微米级乳液或涡旋以产生较大粒径乳液之10%角鲨烯、0.4% Tween 80、5%普流尼克嵌段聚合物L121及thr-MDP,及(b)RIBI™佐剂系统(RAS)(Ribi Immunochem,Hamilton,MT),其含有2%角鲨烯、0.2% Tween 80及一或多种细菌细胞壁组分(诸如单磷酸基脂质A(MPL)、海藻糖二霉菌酸酯(TDM)及细胞壁骨架(CWS),优选地MPL+CWS(DETOX™);(2)可使用皂素佐剂,诸如QS21、STIMULON™(Cambridge Bioscience,Worcester,MA)、ABISCO®(Isconova,Sweden)或ISCOMATRIX®(Commonwealth Serum Laboratories,Australia),或自其产生之颗粒,诸如ISCOMs(免疫刺激复合物),该ISCOMs可不含额外去污剂(例如WO 00/07621);(3)弗氏完全佐剂(CFA)及弗氏不完全佐剂(IFA);(4)细胞因子,诸如白介素(例如IL-1、IL-2、IL-4、IL-5、IL-6、IL-7、IL-12(例如WO 99/44636))、干扰素(例如γ干扰素)、巨噬细胞集落刺激因子(M-CSF)、肿瘤坏死因子(TNF)等;(5)单磷酸基脂质A(MPL)或3-O-去酰化MPL(3dMPL)(参见例如GB-2220221、EP0689454),任选地在与肺炎链球菌糖一起使用时实质上不存在明矾(参见例如WO 00/56358);(6)3dMPL与例如QS21及/或水包油乳液之组合(参见例如EP0835318、EP0735898、EP0761231);(7)聚氧乙烯醚或聚氧乙烯酯(参见例如WO 99/52549);(8)与辛苯昔醇组合之聚氧乙烯脱水山梨糖醇酯表面活性剂(例如WO 01/21207)或与至少一种额外非离子表面活性剂(诸如辛苯昔醇)组合之聚氧乙烯烷基醚或酯表面活性剂(例如WO 01/21152);(9)皂苷及免疫刺激性寡核苷酸(例如CpG寡核苷酸)(例如WO 00/62800);(10)免疫刺激剂及金属盐粒子(参见例如WO 00/23105);(11)皂苷及水包油乳液(例如WO 99/11241);(12)皂苷(例如QS21)+3dMPL+IM2(视情况+固醇)(例如WO 98/57659);(13)充当增强组合物之功效之免疫刺激剂的其他物质。胞壁肽包括N-乙酰基-胞壁酰基-L-苏氨酸基-D-异谷氨酰胺(thr-MDP)、N-25乙酰基-去甲胞壁酰基-L-丙氨酸基-D-异谷氨酰胺(nor-MDP)、N-乙酰基胞壁酰基-L-丙氨酸基-D-异谷氨酰胺基-L-丙氨酸-2-(1'-2'-二棕榈酰基-sn-甘油-3-羟基磷酸基氧基)-乙胺MTP-PE)等。

[0801] 在本发明之一实施方案中,如本文所公开之免疫原性组合物包含作为佐剂之CpG寡核苷酸。如本文中所使用之CpG寡核苷酸系指免疫刺激性CpG寡脱氧核苷酸(CpG ODN),且因此除非另外指示,否则此等术语可互换使用。免疫刺激性CpG寡脱氧核苷酸含有

一或多个免疫刺激性CpG基序,其为未甲基化胞嘧啶-鸟嘌呤二核苷酸,视情况在某些优选的碱基背景内。CpG免疫刺激性基序之甲基化状态通常系指二核苷酸中之胞嘧啶残基。含有至少一个未甲基化CpG二核苷酸之免疫刺激性寡核苷酸为含有通过磷酸酯键与3'鸟嘌呤连接之5'未甲基化胞嘧啶且经由与Toll样受体9(TLR-9)结合而活化免疫系统的寡核苷酸。在另一个实施方案中,免疫刺激性寡核苷酸可含有一或多个甲基化CpG二核苷酸,其将经由TLR9活化免疫系统但不如CpG基序之未甲基化一样强烈。CpG免疫刺激性寡核苷酸可包含一或多个回文序列,其还可包含CpG二核苷酸。CpG寡核苷酸已在多个发布专利、公开专利申请案及其他公开案中描述,包括美国专利第6,194,388号、第6,207,646号、第6,214,806号、第6,218,371号、第6,239,116号;及第6,339,068号。在本发明之一实施方案中,如本文所公开之免疫原性组合物包含W0 2010/125480之第3页第22行至第12页第36行所描述之CpG寡核苷酸中之任一者。

[0802] 已鉴别出不同类别之CpG免疫刺激性寡核苷酸。此等被称为A、B、C及P类,且更详细地描述于W0 2010/125480之第3页第22行至第12页第36行。本发明之方法涵盖此等不同类别之CpG免疫刺激性寡核苷酸之用途。

[0803] 在本发明之一实施方案中,如本文所公开之免疫原性组合物包含A类CpG寡核苷酸。优选地,本发明之“A类”CpG寡核苷酸具有以下核酸序列:5'GGGGACGACGTCGTGGGGGGG 3' (SEQ ID NO:1)。A类寡核苷酸之一些非限制性实例包括:5'G*G*G_G_A_C_G_A_C_G_T_C_G_T_G_G*G*G*G*G*G 3' (SEQ ID NO:2);其中“*”系指硫代磷酸酯键,且“-”系指磷酸二酯键。

[0804] 在本发明之一实施方案中,如本文所公开之免疫原性组合物包含B类CpG寡核苷酸。在一个实施方案中,用于本发明中之CpG寡核苷酸为至少由下式表示之B类CpG寡核苷酸:5'X₁X₂CGX₃X₄3',其中X₁、X₂、X₃及X₄为核苷酸。在一个实施方案中,X₂为腺嘌呤、鸟嘌呤或胸腺嘧啶。在另一个实施方案中,X₃为胞嘧啶、腺嘌呤或胸腺嘧啶。

[0805] 本发明之B类CpG寡核苷酸序列为上文大致描述以及以下文献中所公开的那些:W0 96/02555、W0 98/18810及美国专利第6,194,388号、第6,207,646号、第6,214,806号、第6,218,371号、第6,239,116号及第6,339,068号。例示性序列包括(但不限于)此等后面申请案及专利中所公开的那些。

[0806] 在一个实施方案中,本发明之“B类”CpG寡核苷酸具有以下核酸序列:

[0807] 5'TCGTCGTTTTTCGGTGTCTTTT 3' (SEQ ID NO:3),或

[0808] 5'TCGTCGTTTTTCGGTCGTTTTT 3' (SEQ ID NO:4),或

[0809] 5'TCGTCGTTTTGTCGTTTTGTCGTT 3' (SEQ ID NO:5),或

[0810] 5'TCGTCGTTTCGTCGTTTTGTCGTT 3' (SEQ ID NO:6),或

[0811] 5'TCGTCGTTTTGTCGTTTTTTTCGA 3' (SEQ ID NO:7)。

[0812] 在此等序列中之任一者中,所有连接可皆为硫代磷酸酯键。在另一个实施方案中,在此等序列中之任一者中,连接中之一或多者可为磷酸二酯,优选在CpG基序之“C”与“G”之间,产生半软的CpG寡核苷酸。在此等序列中之任一者中,乙基-尿苷或卤素可取代5'T;卤素取代之实例包括(但不限于)溴-尿苷或碘-尿苷取代。

[0813] B类寡核苷酸之一些非限制性实例包括:

[0814] 5'T*C*G*T*C*G*T*T*T*T*T*C*G*G*T*G*C*T*T*T*T 3' (SEQ ID NO:8),或

[0815] 5'T*C*G*T*C*G*T*T*T*T*T*T*C*G*G*T*C*G*T*T*T*T 3' (SEQ ID NO:9),或

[0816] 5'T*C*G*T*C*G*T*T*T*T*G*T*C*G*T*T*T*T*G*T*C*G*T*T 3'(SEQ ID NO:10),或
[0817] 5'T*C*G*T*C*G*T*T*T*T*C*G*T*C*G*T*T*T*T*G*T*C*G*T*T 3'(SEQ ID NO:11),或
[0818] 5'T*C*G*T*C*G*T*T*T*T*G*T*C*G*T*T*T*T*T*T*T*T*T*C*G*A 3'(SEQ ID NO:12)。

[0819] 其中“*”系指硫代磷酸酯键。

[0820] 在本发明之一实施方案中,如本文所公开之免疫原性组合物包含C类CpG寡核苷酸。在一个实施方案中,本发明之“C类”CpG寡核苷酸具有以下核酸序列:

[0821] 5'TCGCGTCGTTTCGGCGCGCGCCG 3'(SEQ ID NO:13),或
[0822] 5'TCGTCGACGTTTCGGCGCGCGCCG 3'(SEQ ID NO:14),或
[0823] 5'TCGGACGTTTCGGCGCGCGCCG 3'(SEQ ID NO:15),或
[0824] 5'TCGGACGTTTCGGCGCGCGCCG 3'(SEQ ID NO:16),或
[0825] 5'TCGCGTCGTTTCGGCGCGCGCCG 3'(SEQ ID NO:17),或
[0826] 5'TCGACGTTTCGGCGCGCGCCG 3'(SEQ ID NO:18),或
[0827] 5'TCGACGTTTCGGCGCGCGCCG 3'(SEQ ID NO:19),或
[0828] 5'TCGCGTCGTTTCGGCGCGCCG 3'(SEQ ID NO:20),或
[0829] 5'TCGCGACGTTTCGGCGCGCGCCG 3'(SEQ ID NO:21),或
[0830] 5'TCGTCGTTTTTCGGCGCGCGCCG 3'(SEQ ID NO:22),或
[0831] 5'TCGTCGTTTTTCGGCGCGCGCCG 3'(SEQ ID NO:23),或
[0832] 5'TCGTCGTTTTACGGCGCCGTGCCG 3'(SEQ ID NO:24),或
[0833] 5'TCGTCGTTTTTCGGCGCGCGCCGT 3'(SEQ ID NO:25)。

[0834] 在此等序列中之任一者中,所有连接可皆为硫代磷酸酯键。在另一个实施方案中,在此等序列中之任一者中,连接中之一或多者可为磷酸二酯,优选在CpG基序之“C”与“G”之间,产生半软的CpG寡核苷酸。

[0835] C类寡核苷酸之一些非限制性实例包括:

[0836] 5'T*C_G*C_G*T*C_G*T*T*C_G*G*C*G*C_G*C*G*C*C*G3'(SEQ ID NO:26),或
[0837] 5'T*C_G*T*C_G*A*C_G*T*T*C_G*G*C*G*C_G*C*G*C*C*G 3'(SEQ ID NO:27),或
[0838] 5'T*C_G*G*A*C_G*T*T*C_G*G*C*G*C_G*C*G*C*C*G 3'(SEQ ID NO:28),或
[0839] 5'T*C_G*G*A*C_G*T*T*C_G*G*C*G*C*G*C*C*G 3'(SEQ ID NO:29),或
[0840] 5'T*C_G*C_G*T*C_G*T*T*C_G*G*C*G*C*G*C*C*G 3'(SEQ ID NO:30),或
[0841] 5'T*C_G*A*C_G*T*T*C_G*G*C*G*C_G*C*G*C*C*G 3'(SEQ ID NO:31),或
[0842] 5'T*C_G*A*C_G*T*T*C_G*G*C*G*C*G*C*C*G 3'(SEQ ID NO:32),或
[0843] 5'T*C_G*C_G*T*C_G*T*T*C_G*G*C*G*C*C*G 3'(SEQ ID NO:33),或
[0844] 5'T*C_G*C_G*A*C_G*T*T*C_G*G*C*G*C_G*C*G*C*C*G3'(SEQ ID NO:34),或
[0845] 5'T*C*G*T*C*G*T*T*T*T*C*G*G*C*G*C*G*C*G*C*C*G3'(SEQ ID NO:35),或
[0846] 5'T*C*G*T*C*G*T*T*T*T*C*G*G*C*G*G*C*C*G*C*C*G3'(SEQ ID NO:36),或
[0847] 5'T*C*G*T*C_G*T*T*T*T*A*C_G*G*C*G*C*C_G*T*G*C*C*G 3'(SEQ ID NO:37),或
[0848] 5'T*C_G*T*C*G*T*T*T*T*C*G*G*C*G*C*G*C*G*C*C*G*T 3'(SEQ ID NO:38)

[0849] 其中“*”系指硫代磷酸酯键,且“_”系指磷酸二酯键。

[0850] 在此等序列中之任一者中,乙基-尿苷或卤素可取代5'T;卤素取代之实例包括(但不限于)溴-尿苷或碘-尿苷取代。

[0851] 在本发明之一实施方案中,如本文所公开之免疫原性组合物包含P类CpG寡核苷酸。在一个实施方案中,用于本发明中之CpG寡核苷酸为P类CpG寡核苷酸,其含有5' TLR活化域及至少两个回文区域,一个回文区域为至少6个核苷酸长之5'回文区域且其直接或经由间隔子连接至至少8个核苷酸长之3'回文区域,其中寡核苷酸包括至少一个YpR二核苷酸。在一个实施方案中,该寡核苷酸不为T*C_G*T*C_G*A*C_G*T*T*C_G*G*C*G*C_G*C*G*C*C*G (SEQ ID NO:27)。在一个实施方案中,P类CpG寡核苷酸包括至少一个未甲基化CpG二核苷酸。在另一个实施方案中,TLR活化域为TCG、TTCG、TTTCG、TYpR、TTYpR、TTTYpR、UCG、UUCG、UUUCG、TTT或TTTT。在又一个实施方案中,TLR活化域在5'回文区域内。在另一个实施方案中,TLR活化域紧邻5'回文区域之5'。在一个实施方案中,本发明之“P类”CpG寡核苷酸具有以下核酸序列:5'TCGTCGACGATCGGCGCGCGCCG 3' (SEQ ID NO:39)。

[0852] 在所述序列中,所有连接可皆为硫代磷酸酯键。在另一个实施方案中,连接(linkage)中之一或多者可为磷酸二酯,优选在CpG基序之“C”与“G”之间,产生半软的CpG寡核苷酸。在此等序列中之任一者中,乙基-尿苷或卤素可取代5'T;卤素取代之实例包括(但不限于)溴-尿苷或碘-尿苷取代。

[0853] P类寡核苷酸之非限制性实例包括:

[0854] 5'T*C_G*T*C_G*A*C_G*A*T*C_G*G*C*G*C_G*C*G*C*C*G 3' (SEQ ID NO:40)

[0855] 其中“*”系指硫代磷酸酯键,且“-”系指磷酸二酯键。

[0856] 在一个实施方案中,寡核苷酸包括至少一个硫代磷酸酯连接。在另一个实施方案中,寡核苷酸之所有核苷酸间连接皆为硫代磷酸酯连接。在另一个实施方案中,寡核苷酸包括至少一个磷酸二酯样连接。在另一个实施方案中,磷酸二酯样连接为磷酸二酯连接。在另一个实施方案中,亲脂性基团与寡核苷酸缀合。在一个实施方案中,亲脂性基团为胆固醇。

[0857] 在一个实施方案中,本文所公开之CpG寡核苷酸之所有核苷酸间连接为磷酸二酯键(“软”寡核苷酸,如WO 2007/026190中所描述)。在另一个实施方案中,本发明之CpG寡核苷酸抗降解(例如为稳定的)。“稳定寡核苷酸”系指相对抗体内降解(例如经由核酸外切酶或核酸内切酶之体内降解)的寡核苷酸。核酸稳定性可经由主链修饰实现。具有硫代磷酸酯连接之寡核苷酸提供最大活性且防止寡核苷酸由细胞内核酸外切酶及核酸内切酶降解。

[0858] 免疫刺激性寡核苷酸可具有嵌合主链,其具有磷酸二酯连接与硫代磷酸酯连接之组合。出于本发明之目的,嵌合主链系指部分稳定主链,其中至少一个核苷酸间连接为磷酸二酯连接或磷酸二酯样连接,且其中至少一个其他核苷酸间连接为稳定的核苷酸间连接,其中至少一个磷酸二酯连接或磷酸二酯样连接与至少一个稳定连接不同。当磷酸二酯连接优先位于CpG基序内时,此类分子被称为“半软的”,如WO 2007/026190中所描述。

[0859] 其他经修饰寡核苷酸包括磷酸二酯、硫代磷酸酯、甲基磷酸酯、甲基硫代磷酸酯、二硫代磷酸酯及/或对乙氧基连接之组合。

[0860] 混合主链修饰之ODN可如WO 2007/026190中所描述合成。

[0861] CpG寡核苷酸之大小(还即,沿寡核苷酸之长度的核苷酸残基数目)还可促成寡核苷酸之刺激性活性。为了促进至细胞中之吸收,本发明之CpG寡核苷酸优选具有6个核苷酸残基之最小长度。若存在足够的免疫刺激性基序,则大于6个核苷酸之任何大小(甚至许多kb长)之寡核苷酸均能够诱导免疫反应,因为较大寡核苷酸会在细胞内部降解。在某些实施方案中,CpG寡核苷酸为6至100个核苷酸长,优选8至30个核苷酸长。在重要实施方案中,本

发明之核酸及寡核苷酸不为质粒或表达载体。

[0862] 在一个实施方案中,本文所公开之CpG寡核苷酸包含取代或修饰,诸如在如W0 2007/026190之第134至147段所描述的碱基及/或糖之取代或修饰。

[0863] 在一个实施方案中,本发明之CpG寡核苷酸经化学修饰。化学修饰之实例为技术人员已知的,且描述于例如Uhlmann等人(1990) Chem. Rev. 90:543; S. Agrawal编, Humana Press, Totowa, USA 1993; Crooke等人(1996) Annu. Rev. Pharmacol. Toxicol. 36:107-129; 及Hunziker等人(1995) Mod. Synth. Methods 7:331-417中。根据本发明之寡核苷酸可具有一或多个修饰,其中与由天然DNA或RNA构成之具有相同序列之寡核苷酸相比,各修饰位于特定磷酸二酯核苷间桥处及/或特定 β -D-核糖单元处及/或特定天然核苷碱基位置处。

[0864] 在本发明之一些实施方案中,含CpG之核酸可根据本领域技术人员已知之方法简单地与免疫原性载体混合(参见例如W0 03/024480)。

[0865] 在本发明之一个特定实施方案中,本文所公开之免疫原性组合物中之任一者包含2 μ g至100mg之CpG寡核苷酸。在本发明之一个特定实施方案中,本发明之免疫原性组合物包含0.1mg至50mg之CpG寡核苷酸,优选0.2mg至10mg之CpG寡核苷酸,更优选0.3mg至5mg之CpG寡核苷酸。在本发明之一个特定实施方案中,本发明之免疫原性组合物包含0.3mg至5mg之CpG寡核苷酸。甚至优选地,本发明之免疫原性组合物可包含0.5至2mg之CpG寡核苷酸。最优选地,本发明之免疫原性组合物可包含0.75至1.5mg之CpG寡核苷酸。在一个优选实施方案中,本文所公开之免疫原性组合物中之任一者可包含约1mg之CpG寡核苷酸。

[0866] 3制剂

[0867] 本发明之免疫原性组合物可配制成液体形式(还即溶液或悬浮液)或冻干形式。在一个实施方案中,本发明之免疫原性组合物配制成液体形式。在一个实施方案中,本发明之免疫原性组合物配制成冻干形式。液体制剂可有利地自其包装形式直接施用,且因此对于注射而言为理想的,其无需如本发明之冻干组合物那般在水性介质中复原。

[0868] 本发明之免疫原性组合物之配制可使用本领域中公认的方法来实现。举例而言,可将个别多糖及/或缀合物与生理学上可接受之溶媒一起配制以制备组合物。此类溶媒之实例包括(但不限于)水、缓冲盐水、多元醇(例如,甘油、丙二醇、液体聚乙二醇)及右旋糖溶液。

[0869] 本发明提供一种免疫原性组合物,其包含本文所公开之糖缀合物之任何组合及医学上可接受之赋形剂、载体或稀释剂。

[0870] 在一个实施方案中,本发明之免疫原性组合物呈液体形式,优选地呈水性液体形式。

[0871] 本发明之免疫原性组合物可包含以下中之一或多者:缓冲液、盐、二价阳离子、非离子去污剂、低温保护剂(诸如糖)及抗氧化剂(诸如游离基清除剂或螯合剂)或其任何多个组合。

[0872] 在一个实施方案中,本发明之免疫原性组合物包含缓冲剂。在一个实施方案中,该缓冲剂具有约3.5至约7.5之pKa。在一些实施方案中,缓冲剂为磷酸盐、琥珀酸盐、组氨酸或柠檬酸盐。在一些实施方案中,缓冲剂为琥珀酸盐。在一些实施方案中,缓冲剂为组氨酸。在某些实施方案中,缓冲剂为1mM至10mM之最终浓度之琥珀酸盐。在一个特定实施方案中,琥珀酸盐缓冲剂之最终浓度为约5mM。

[0873] 在一个实施方案中,本发明之免疫原性组合物包含盐。在一些实施方案中,盐系选自由以下组成的组:氯化镁、氯化钾、氯化钠及其组合。在一个特定实施方案中,盐为氯化钠。在一个特定实施方案中,本发明之免疫原性组合物包含150mM之氯化钠。

[0874] 在一个实施方案中,本发明之免疫原性组合物包含表面活性剂。在一个实施方案中,表面活性剂系选自由以下组成的组:聚山梨醇酯20 (TWEEN™20)、聚山梨醇酯40 (TWEEN™40)、聚山梨醇酯60 (TWEEN™60)、聚山梨醇酯65 (TWEEN™65)、聚山梨醇酯80 (TWEEN™80)、聚山梨醇酯85 (TWEEN™85)、TRITON™N-101、TRITON™X-100、辛苯昔醇40、壬苯醇醚-9、三乙醇胺、三乙醇胺多肽油酸酯、聚氧乙烯-660羟基硬脂酸酯 (PEG-15、Solutol H 15)、聚氧乙烯-35-蓖麻油酸酯 (CREMOPHOR®EL)、大豆卵磷脂及泊洛沙姆 (poloxamer)。

[0875] 在一个特定实施方案中,表面活性剂为聚山梨醇酯80。在一些该实施方案中,制剂中之聚山梨醇酯80之最终浓度为至少0.0001%至10%重量/重量 (w/w) 聚山梨醇酯80。在一些所述实施方案中,制剂中之聚山梨醇酯80之最终浓度为至少0.001%至1%重量/重量 (w/w) 聚山梨醇酯80。在一些所述实施方案中,制剂中之聚山梨醇酯80之最终浓度为至少0.01%至1%重量/重量 (w/w) 聚山梨醇酯80。在其他实施方案中,制剂中之聚山梨醇酯80之最终浓度为0.01%、0.02%、0.03%、0.04%、0.05%、0.06%、0.07%、0.08%、0.09%或0.1% (w/w) 聚山梨醇酯80。在另一个实施方案中,制剂中之聚山梨醇酯80之最终浓度为0.02% (w/w) 聚山梨醇酯80。在另一个实施方案中,制剂中之聚山梨醇酯80之最终浓度为0.01% (w/w) 聚山梨醇酯80。在另一个实施方案中,制剂中之聚山梨醇酯80之最终浓度为0.03% (w/w) 聚山梨醇酯80。在另一个实施方案中,制剂中之聚山梨醇酯80之最终浓度为0.04% (w/w) 聚山梨醇酯80。在另一个实施方案中,制剂中之聚山梨醇酯80之最终浓度为0.05% (w/w) 聚山梨醇酯80。在另一个实施方案中,制剂中之聚山梨醇酯80之最终浓度为1% (w/w) 聚山梨醇酯80。

[0876] 在一个特定实施方案中,表面活性剂为聚山梨醇酯20。在一些该实施方案中,制剂中之聚山梨醇酯20之最终浓度为至少0.0001%至10%重量/重量 (w/w) 聚山梨醇酯20。在一些所述实施方案中,制剂中之聚山梨醇酯20之最终浓度为至少0.001%至1%重量/重量 (w/w) 聚山梨醇酯20。在一些所述实施方案中,制剂中之聚山梨醇酯20之最终浓度为至少0.01%至1%重量/重量 (w/w) 聚山梨醇酯20。在其他实施方案中,制剂中之聚山梨醇酯20之最终浓度为0.01%、0.02%、0.03%、0.04%、0.05%、0.06%、0.07%、0.08%、0.09%或0.1% (w/w) 聚山梨醇酯20。在另一个实施方案中,制剂中之聚山梨醇酯20之最终浓度为0.02% (w/w) 聚山梨醇酯20。在另一个实施方案中,制剂中之聚山梨醇酯20之最终浓度为0.01% (w/w) 聚山梨醇酯20。在另一个实施方案中,制剂中之聚山梨醇酯20之最终浓度为0.03% (w/w) 聚山梨醇酯20。在另一个实施方案中,制剂中之聚山梨醇酯20之最终浓度为0.04% (w/w) 聚山梨醇酯80。在另一个实施方案中,制剂中之聚山梨醇酯20之最终浓度为0.05% (w/w) 聚山梨醇酯20。在另一个实施方案中,制剂中之聚山梨醇酯20之最终浓度为1% (w/w) 聚山梨醇酯20。

[0877] 在一个特定实施方案中,表面活性剂为聚山梨醇酯40。在一些该实施方案中,制剂中之聚山梨醇酯40之最终浓度为至少0.0001%至10%重量/重量 (w/w) 聚山梨醇酯40。在一些所述实施方案中,制剂中之聚山梨醇酯40之最终浓度为至少0.001%至1%重量/重量 (w/

w) 聚山梨醇酯40。在一些所述实施方案中,制剂中之聚山梨醇酯40之最终浓度为至少0.01%至1%重量/重量(w/w)聚山梨醇酯40。在其他实施方案中,制剂中之聚山梨醇酯40之最终浓度为0.01%、0.02%、0.03%、0.04%、0.05%、0.06%、0.07%、0.08%、0.09%或0.1% (w/w) 聚山梨醇酯40。在另一个实施方案中,制剂中之聚山梨醇酯40之最终浓度为1% (w/w) 聚山梨醇酯40。

[0878] 在一个特定实施方案中,表面活性剂为聚山梨醇酯60。在一些该实施方案中,制剂中之聚山梨醇酯60之最终浓度为至少0.0001%至10%重量/重量(w/w)聚山梨醇酯60。在一些所述实施方案中,制剂中之聚山梨醇酯60之最终浓度为至少0.001%至1%重量/重量(w/w)聚山梨醇酯60。在一些所述实施方案中,制剂中之聚山梨醇酯60之最终浓度为至少0.01%至1%重量/重量(w/w)聚山梨醇酯60。在其他实施方案中,制剂中之聚山梨醇酯60之最终浓度为0.01%、0.02%、0.03%、0.04%、0.05%、0.06%、0.07%、0.08%、0.09%或0.1% (w/w) 聚山梨醇酯60。在另一个实施方案中,制剂中之聚山梨醇酯60之最终浓度为1% (w/w) 聚山梨醇酯60。

[0879] 在一个特定实施方案中,表面活性剂为聚山梨醇酯65。在一些该实施方案中,制剂中之聚山梨醇酯65之最终浓度为至少0.0001%至10%重量/重量(w/w)聚山梨醇酯65。在一些所述实施方案中,制剂中之聚山梨醇酯65之最终浓度为至少0.001%至1%重量/重量(w/w)聚山梨醇酯65。在一些所述实施方案中,制剂中之聚山梨醇酯65之最终浓度为至少0.01%至1%重量/重量(w/w)聚山梨醇酯65。在其他实施方案中,制剂中之聚山梨醇酯65之最终浓度为0.01%、0.02%、0.03%、0.04%、0.05%、0.06%、0.07%、0.08%、0.09%或0.1% (w/w) 聚山梨醇酯65。在另一个实施方案中,制剂中之聚山梨醇酯65之最终浓度为1% (w/w) 聚山梨醇酯65。

[0880] 在一个特定实施方案中,表面活性剂为聚山梨醇酯85。在一些该实施方案中,制剂中之聚山梨醇酯85之最终浓度为至少0.0001%至10%重量/重量(w/w)聚山梨醇酯85。在一些所述实施方案中,制剂中之聚山梨醇酯85之最终浓度为至少0.001%至1%重量/重量(w/w)聚山梨醇酯85。在一些所述实施方案中,制剂中之聚山梨醇酯85之最终浓度为至少0.01%至1%重量/重量(w/w)聚山梨醇酯85。在其他实施方案中,制剂中之聚山梨醇酯85之最终浓度为0.01%、0.02%、0.03%、0.04%、0.05%、0.06%、0.07%、0.08%、0.09%或0.1% (w/w) 聚山梨醇酯85。在另一个实施方案中,制剂中之聚山梨醇酯85之最终浓度为1% (w/w) 聚山梨醇酯85。

[0881] 在某些实施方案中,本发明之免疫原性组合物具有5.5至7.5之pH,更优选5.6至7.0之pH,甚至更优选5.8至6.0之pH。

[0882] 在一个实施方案中,本发明提供一种填充有本文所公开之免疫原性组合物中之任一者的容器。在一个实施方案中,容器系选自由以下组成的组:小瓶、注射器、烧瓶、发酵罐、生物反应器、袋子、广口瓶、安瓿、药筒及一次性笔。在某些实施方案中,容器为硅化的。

[0883] 在一个实施方案中,本发明之容器系由玻璃、金属(例如钢、不锈钢、铝等)及/或聚合物(例如热塑性塑料、弹性体、热塑性弹性体)制成。在一个实施方案中,本发明之容器系由玻璃制成。

[0884] 在一个实施方案中,本发明提供一种填充有本文所公开之免疫原性组合物中之任一者的注射器。在某些实施方案中,注射器为硅化的及/或系由玻璃制成。

[0885] 用于注射之本发明之免疫原性组合物的典型剂量具有0.1mL至2mL之体积。在一个实施方案中,用于注射之本发明之免疫原性组合物具有0.2mL至1mL之体积,甚至更优选约0.5mL之体积。

[0886] 4本发明之糖缀合物及免疫原性组合物之用途

[0887] 本文所公开之糖缀合物可用作抗原。举例而言,其可为疫苗之一部分。

[0888] 因此,在一个实施方案中,本发明之免疫原性组合物适用作药物。

[0889] 在一个实施方案中,本发明之免疫原性组合物适用作疫苗。

[0890] 因此,在一个实施方案中,本文所描述之免疫原性组合物适用于在个体中产生免疫反应。在一个方面中,个体为哺乳动物,诸如人类、非人类灵长类动物、猫、绵羊、猪、马、牛科动物或狗。在一个方面中,个体为人类。

[0891] 本文所描述之免疫原性组合物可用于预防、治疗或改善个体之细菌感染、疾病或病状之治疗性或预防性方法中。因此,在一个方面中,本发明提供一种预防、治疗或改善个体之与细菌感染相关之感染、疾病或病状的方法,其包含向该个体施用免疫有效量的本发明之免疫原性组合物。

[0892] 本发明之免疫原性组合物可用于保护或治疗易受细菌感染影响之人类,其系借助于经由全身或黏膜途径来施用该免疫原性组合物。在一个实施方案中,本发明之免疫原性组合物通过肌肉内、腹膜内、皮内或皮下途径施用。在一个实施方案中,本发明之免疫原性组合物通过肌肉内、腹膜内、皮内或皮下注射施用。在一个实施方案中,本发明之免疫原性组合物通过肌肉内或皮下注射施用。在一个实施方案中,本发明之免疫原性组合物通过肌肉内注射施用。在一个实施方案中,本发明之免疫原性组合物通过皮下注射施用。

[0893] 5待用本发明之免疫原性组合物治疗的个体

[0894] 如本文所公开,本文所描述之免疫原性组合物可用于预防、治疗或改善个体之细菌感染、疾病或病状之各种治疗性或预防性方法中。

[0895] 在一个优选实施方案中,该个体为人类。在一个最优选实施方案中,该个体为新生儿(还即低于三月龄)、婴儿(还即年龄在3个月至一岁)或幼儿(还即一岁至四岁)。

[0896] 在一个实施方案中,本文所公开之免疫原性组合物适用作疫苗。

[0897] 在此类实施方案中,待进行疫苗接种之个体可小于1岁。举例而言,待进行疫苗接种之个体可为约1、约2、约3、约4、约5、约6、约7、约8、约9、约10、约11或约12个月龄。在一个实施方案中,待进行疫苗接种之个体为约2、约4或约6个月龄。在另一个实施方案中,待进行疫苗接种之个体小于2岁。举例而言,待进行疫苗接种之个体可为约12个月龄至约15个月龄。在一些情况下,需要少至一次剂量之根据本发明之免疫原性组合物,但在一些情况下,可给予第二次、第三次或第四次剂量(参见下方部分8)。

[0898] 在本发明之一实施方案中,待进行疫苗接种之个体为50岁或更大之人类成人,更优选55岁或更大之人类成人。在一个实施方案中,待进行疫苗接种之个体为65岁或更大、70岁或更大、75岁或更大、或80岁或更大之人类成人。

[0899] 在一个实施方案中,待进行疫苗接种之个体为免疫功能不全个体,尤其人类。免疫功能不全个体一般定义为呈现建立对于感染剂刺激之正常体液或细胞防御之能力减弱或降低的人。

[0900] 在本发明之一实施方案中,待进行疫苗接种之免疫功能不全个体罹患削弱免疫

系统且引起不足以防止肺炎链球菌疾病或治疗肺炎链球菌疾病之抗体反应的疾病或病状。

[0901] 在一个实施方案中,该疾病为原发性免疫缺乏病症。优选地,该原发性免疫缺乏病症系选自由以下组成的组:组合性T细胞及B细胞免疫缺乏症、抗体缺乏症、界定明确的综合征、免疫调节异常疾病、吞噬细胞病症、先天性免疫缺乏症、自体发炎性病症及补体缺乏症。在一个实施方案中,该原发性免疫缺乏病症系选自WO 2010/125480之第24页第11行至第25页第19行所公开之病症。

[0902] 在本发明之一个特定实施方案中,待进行疫苗接种之免疫功能不全个体罹患选自由以下组成的组之疾病:HIV感染、获得性免疫缺乏综合征(AIDS)、癌症、慢性心脏病或肺病、充血性心脏衰竭、糖尿病、慢性肝病、酒精中毒、肝硬化、脊髓液渗漏、心肌病、慢性支气管炎、肺气肿、慢性阻塞性肺病(COPD)、脾功能障碍(诸如镰状细胞疾病)、脾功能缺乏(无脾症)、血液恶性疾病、白血病、多发性骨髓瘤、霍奇金病(Hodgkin's disease)、淋巴瘤、肾衰竭、肾病综合征及哮喘。

[0903] 在本发明之一个实施方案中,待进行疫苗接种之免疫功能不全个体罹患营养不良。

[0904] 在本发明之一个特定实施方案中,待进行疫苗接种之免疫功能不全个体服用减少身体对于感染之抵抗的药物或进行减少身体对于感染之抵抗的治疗。在一个实施方案中,该药物系选自WO 2010/125480之第26页第33行至第26页第4行所公开之药物。

[0905] 在本发明之一个特定实施方案中,待进行疫苗接种之免疫功能不全个体为吸烟者。

[0906] 在本发明之一个特定实施方案中,待进行疫苗接种之免疫功能不全个体具有以下之白血球计数(白细胞计数):低于 5×10^9 个细胞/升、或低于 4×10^9 个细胞/升、或低于 3×10^9 个细胞/升、或低于 2×10^9 个细胞/升、或低于 1×10^9 个细胞/升、或低于 0.5×10^9 个细胞/升、或低于 0.3×10^9 个细胞/升、或低于 0.1×10^9 个细胞/升。

[0907] 白血球计数(白细胞计数):血液中之白血球(WBC)之数目。WBC通常作为CBC(全血球计数)之一部分测量。白血球为血液中之感染对抗细胞,且不同于被称为红血球之红色(含氧)血细胞。存在不同类型的白血球,包括嗜中性粒细胞(多形核白细胞;PMN)、带状细胞(略微不成熟的嗜中性粒细胞)、T型淋巴细胞(T细胞)、B型淋巴细胞(B细胞)、单核细胞、嗜酸性粒细胞及嗜碱性粒细胞。所有类型之白血球均在白血球计数中反映。白血球计数之正常范围通常在4,300与10,800个细胞/立方毫米血液之间。此还可被称作白细胞计数,且可以国际单位形式表述为 $4.3-10.8 \times 10^9$ 个细胞/升。

[0908] 在本发明之一个特定实施方案中,待进行疫苗接种之免疫功能不全个体罹患嗜中性粒细胞减少症。在本发明之一个特定实施方案中,待进行疫苗接种之免疫功能不全个体具有以下之嗜中性粒细胞计数:低于 2×10^9 个细胞/升、或低于 1×10^9 个细胞/升、或低于 0.5×10^9 个细胞/升、或低于 0.1×10^9 个细胞/升、或低于 0.05×10^9 个细胞/升。

[0909] 低白血球计数或“嗜中性粒细胞减少症”为特征在于在循环血液中异常低含量之嗜中性粒细胞的病状。嗜中性粒细胞为帮助预防及对抗感染之一种特定种类之白血球。癌症患者经历嗜中性粒细胞减少症之最常见原因为作为化学疗法之副作用。化学疗法诱发的嗜中性粒细胞减少症增加患者之感染风险且干扰癌症治疗。

[0910] 在本发明之一个特定实施方案中,待进行疫苗接种之免疫功能不全个体具有低于

500/mm³之CD4+细胞计数、或低于300/mm³之CD4+细胞计数、或低于200/mm³之CD4+细胞计数、低于100/mm³之CD4+细胞计数、低于75/mm³之CD4+细胞计数、或低于50/mm³之CD4+细胞计数。

[0911] CD4细胞测试通常报导为mm³中之细胞数目。正常CD4计数在500与1,600之间,且CD8计数在375与1,100之间。CD4计数在患有HIV之人中大大降低。

[0912] 在本发明之一个实施方案中,本文所公开之免疫功能不全个体中之任一者为男性人类。在本发明之一个实施方案中,本文所公开之免疫功能不全个体中之任一者为女性人类。

[0913] 6方案

[0914] 在一些情况下,需要少至一次剂量之根据本发明之免疫原性组合物,但在一些情形下,诸如较高免疫缺乏之条件下,可给予第二次、第三次或第四次剂量。在初次疫苗接种之后,个体可接受经恰当间隔之一次或若干次加强免疫接种。

[0915] 在一个实施方案中,根据本发明之免疫原性组合物之疫苗接种之时程为单次剂量。在一个特定实施方案中,该单次剂量时程系针对至少2岁之健康人群。

[0916] 在一个实施方案中,根据本发明之免疫原性组合物之疫苗接种之时程为多剂量时程。在一个特定实施方案中,该多剂量时程由相隔约1个月至约2个月之时间间隔的一系列2次剂量组成。在一个特定实施方案中,该多剂量时程由相隔约1个月之时间间隔的一系列2次剂量组成,或由相隔约2个月之时间间隔的一系列2次剂量组成。

[0917] 在另一个实施方案中,该多剂量时程由相隔约1个月至约2个月之时间间隔的一系列3次剂量组成。在另一个实施方案中,该多剂量时程由相隔约1个月之时间间隔的一系列3次剂量组成,或由相隔约2个月之时间间隔的一系列3次剂量组成。

[0918] 在另一个实施方案中,该多剂量时程由以下组成:相隔约1个月至约2个月之时间间隔的一系列3次剂量,及随后在第一次剂量之后约10个月至约13个月之第四次剂量。在另一个实施方案中,该多剂量时程由以下组成:相隔约1个月之时间间隔的一系列3次剂量及随后在第一次剂量之后约10个月至约13个月之第四次剂量组成,或相隔约2个月之时间间隔的一系列3次剂量及随后在第一次剂量之后约10个月至约13个月之第四次剂量。

[0919] 在一个实施方案中,多剂量时程由以下组成:在一岁中之至少一次剂量(例如1、2或3次剂量)及随后至少一次幼儿剂量。

[0920] 在一个实施方案中,多剂量时程由以下组成:自2个月龄开始相隔约1个月至约2个月之时间间隔的一系列2或3次剂量(例如剂量之间相隔28至56天),及随后在12至18个月龄时之幼儿剂量。在一个实施方案中,该多剂量时程由以下组成:自2个月龄开始相隔约1个月至约2个月之时间间隔的一系列3次剂量(例如剂量之间相隔28至56天),及随后在12至15个月龄时之幼儿剂量。在另一个实施方案中,该多剂量时程由以下组成:自2个月龄开始相隔约2个月之时间间隔的一系列2次剂量,及随后在12至18个月龄时之幼儿剂量。

[0921] 在一个实施方案中,该多剂量时程由在2、4、6及12至15个月龄时之4次剂量系列疫苗组成。

[0922] 在一个实施方案中,在第0天给予初次剂量,且以在约2至约24周范围内之间隔,优选地以4至8周之给药间隔,给予一或多次加强剂量。

[0923] 在一个实施方案中,在第0天给予初次剂量,且在约3个月后给予加强剂量。

[0924] 7. 本发明还提供如以下编号段落1至96中所定义之以下实施方案

[0925] 1.一种制备荚膜糖糖缀合物之方法,其包含以下步骤:

[0926] (a)使经分离荚膜糖与碳酸衍生物及叠氮基接头在非质子性溶剂中反应以产生经活化之叠氮基糖,

[0927] (b)使载体蛋白与带有N-羟基琥珀酰亚胺(NHS)部分及炔烃基之试剂反应,其中NHS部分与氨基反应以形成酰胺键,从而获得炔烃官能化之载体蛋白,

[0928] (c)通过 Cu^{+1} 介导之叠氮化物-炔烃环加成反应使步骤(a)之经活化之叠氮基糖与步骤(b)之经活化之炔烃-载体蛋白反应以形成糖缀合物。

[0929] 2.如段落1之方法,其中经分离糖在活化步骤(a)之前经尺寸设定。

[0930] 3.如段落2之方法,其中经分离荚膜糖经尺寸设定为200kDa与800kDa之间的重量平均分子量。

[0931] 4.如段落1至3中任一者之方法,该碳酸衍生物系选自以下组成的组:1,1'-羰基二咪唑(CDI)、1,1'-羰基-二-(1,2,4-三唑)(CDT)、二琥珀酰亚胺碳酸酯(DSC)及N-羟基琥珀酰亚胺氯甲酸酯。

[0932] 5.如段落1至3中任一者之方法,其中该碳酸衍生物为1,1'-羰基二咪唑(CDI)。

[0933] 6.如段落1至3中任一者之方法,其中该碳酸衍生物为1,1'-羰基-二-(1,2,4-三唑)(CDT)。

[0934] 7.如段落1至6中任一者之方法,其中该叠氮基接头为式(I)化合物,

[0935] $\text{H}_2\text{N}-\text{X}-\text{N}_3$ (I)

[0936] 其中X系选自以下组成的组: $\text{CH}_2(\text{CH}_2)_n$ 、 $(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$ 、 $\text{NHCO}(\text{CH}_2)_n$ 、 $\text{NHCO}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$ 、 $0\text{CH}_2(\text{CH}_2)_n$ 及 $0(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$;其中n系选自1至10且m系选自1至4。

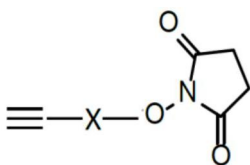
[0937] 8.如段落1至6中任一者之方法,其中该叠氮基接头为式(II)化合物,

[0938] $\text{H}_2\text{N}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{N}_3$ (II)

[0939] 9.如段落1至8中任一者之方法,其中该带有N-羟基琥珀酰亚胺(NHS)部分及炔烃基之试剂为带有N-羟基琥珀酰亚胺(NHS)部分及末端炔烃之试剂。

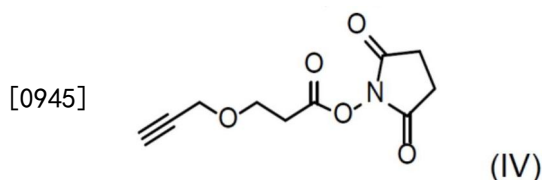
[0940] 10.如段落1至8中任一者之方法,其中该带有N-羟基琥珀酰亚胺(NHS)部分及炔烃基之试剂为带有N-羟基琥珀酰亚胺(NHS)部分及环炔烃之试剂。

[0941] 11.如段落1至8中任一者之方法,其中该带有N-羟基琥珀酰亚胺(NHS)部分及炔烃基之试剂为式(III)化合物,

[0942]  (III)

[0943] 其中X系选自以下组成的组: $\text{CH}_2\text{O}(\text{CH}_2)_n\text{CH}_2\text{C}=\text{O}$ 及 $\text{CH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_m(\text{CH}_2)_n\text{CH}_2\text{C}=\text{O}$,其中n系选自0至10且m系选自0至4。

[0944] 12.如段落1至8中任一者之方法,其中该带有N-羟基琥珀酰亚胺(NHS)部分及炔烃基之试剂为式(IV)化合物:



[0946] 13. 如段落1至12中任一者之方法, 其中步骤a) 包含在非质子性溶剂中, 使荚膜糖与碳酸衍生物反应, 随后使经碳酸衍生物活化之荚膜糖与叠氮基接头反应, 以产生经活化之叠氮基荚膜糖。

[0947] 14. 如段落1至13中任一者之方法, 其中在步骤a) 处, 经分离荚膜糖与该碳酸衍生物在非质子性溶剂中反应。

[0948] 15. 如段落1至13中任一者之方法, 其中在步骤a) 处, 经分离荚膜糖与碳酸衍生物在基本上由二甲亚砜(DMSO)组成之溶液中反应。

[0949] 16. 如段落1至14中任一者之方法, 其中在步骤a) 处, 经分离荚膜糖与CDI在包含0.1%至1% (v/v) 水之非质子性溶剂中反应。

[0950] 17. 如段落1至14中任一者之方法, 其中在步骤a) 处, 经分离荚膜糖与CDI在包含0.1%至1% (v/v) 水之DMSO中反应。

[0951] 18. 如段落1至17中任一者之方法, 其中在步骤a) 处, 在碳酸衍生物活化之后添加水。

[0952] 19. 如段落18之方法, 其中添加水以使混合物中之总水含量达到约1%至约10% (v/v) 之间。

[0953] 20. 如段落1至19中任一者之方法, 其中步骤a) 进一步包含使经碳酸衍生物活化之糖与相对于经活化糖之糖重复单元的量在0.01至10摩尔当量之间的量的叠氮基接头反应。

[0954] 21. 如段落1至20中任一者之方法, 其中步骤a) 之后经活化糖之活化程度在0.5%至50%之间。

[0955] 22. 如段落1至21中任一者之方法, 其中步骤b) 包含使载体蛋白与相对于载体上之赖氨酸0.1至10摩尔当量之量的带有N-羟基琥珀酰亚胺(NHS) 部分及炔烃基之试剂反应。

[0956] 23. 如段落1至22中任一者之方法, 其中步骤b) 之后经活化载体之活化程度在1与50之间。

[0957] 24. 如段落1至23中任一者之方法, 其中缀合反应c) 系在水性缓冲液中以作为催化剂之铜(I) 存在下进行。

[0958] 25. 如段落1至23中任一者之方法, 其中缀合反应c) 系在水性缓冲液中以氧化剂及作为催化剂之铜(I) 存在下进行。

[0959] 26. 如段落1至23中任一者之方法, 其中缀合反应c) 系在水性缓冲液中以作为催化剂之铜(I) 及作为氧化剂之抗坏血酸盐存在下进行, 其中反应混合物进一步包含THPTA(三(3-羟丙基三唑基甲基) 胺) 及氨基胍。

[0960] 27. 如段落1至26中任一者之方法, 其中步骤c) 处之经活化叠氮基糖与经活化炔烃-载体之初始输入比率(重量/重量) 在0.1与3之间。

[0961] 28. 如段落1至27中任一者之方法, 其中在步骤c) 之后, 该方法进一步包含用叠氮基封端剂对缀合物中保留之未反应叠氮基进行封端的步骤。

[0962] 29. 如段落28之方法, 其中该叠氮基封端剂为式(V) 化合物,

[0963] $\equiv -X-OH$ (V)

[0964] 其中X为 $(CH_2)_n$,其中n系选自1至15。

[0965] 30.如段落28之方法,其中该叠氮基封端剂为炔丙醇。

[0966] 31.如段落28至30中任一者之方法,其中未反应叠氮基之封端系用相对于经活化糖之糖重复单元的量在0.05至20摩尔当量之间的量的封端剂进行。

[0967] 32.如段落1至31中任一者之方法,其中在步骤c)之后,该方法进一步包含用炔烃基封端剂对缀合物中保留之未反应炔烃基进行封端的步骤。

[0968] 33.如段落32之方法,其中该炔烃基封端剂为带有叠氮基之试剂。

[0969] 34.如段落33之方法,其中该炔烃基封端剂为式(VI)化合物,

[0970] N_3-X-OH (VI)

[0971] 其中X为 $(CH_2)_n$,其中n系选自1至15。

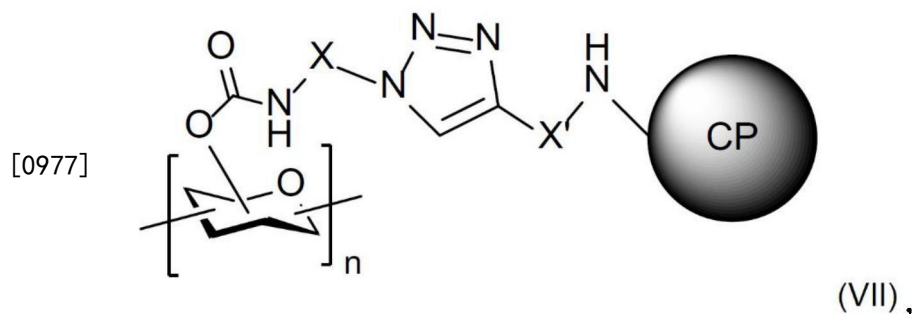
[0972] 35.如段落32之方法,其中该炔烃基封端剂为3-叠氮基-1-丙醇。

[0973] 36.如段落32至35中任一者之方法,其中未反应炔烃基之封端系用相对于经活化糖之糖重复单元的量在0.05至20摩尔当量之间的量的封端剂进行。

[0974] 37.如段落1至36中任一者之方法,其中该方法进一步包含在产生糖缀合物之后对其进行纯化的步骤。

[0975] 38.一种荚膜糖糖缀合物,其系根据如段落1至37之方法中之任一者产生。

[0976] 39.一种荚膜糖糖缀合物,其包含经由间隔子与载体蛋白(CP)共价缀合之荚膜糖且具有通式(VII):

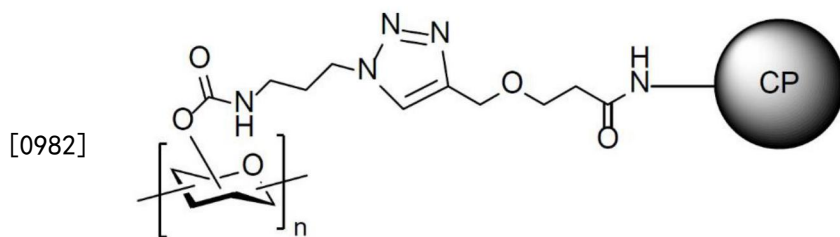


[0978] 其中X系选自由以下组成的组: $CH_2(CH_2)_{n'}$ 、 $(CH_2CH_2O)_mCH_2CH_2$ 、 $NHCO(CH_2)_{n'}$ 、 $NHCO(CH_2CH_2O)_mCH_2CH_2$ 、 $OCH_2(CH_2)_{n'}$ 及 $O(CH_2CH_2O)_mCH_2CH_2$;其中 n' 系选自1至10且 m 系选自1至4,

[0979] 且其中 X' 系选自由以下组成的组: $CH_2O(CH_2)_{n''}CH_2C=O$ 、 $CH_2O(CH_2CH_2O)_m(CH_2)_{n''}CH_2C=O$,其中 n'' 系选自0至10且 m' 系选自0至4。

[0980] 40.一种荚膜糖糖缀合物,其包含经由间隔子与载体蛋白(CP)共价缀合之荚膜糖且具有通式(VII),其中X为 $CH_2(CH_2)_{n'}$,其中 n' 为2,且其中 X' 为 $CH_2O(CH_2)_{n''}CH_2C=O$,其中 n'' 为1。

[0981] 41.一种荚膜糖糖缀合物,其包含经由间隔子与载体蛋白(CP)共价缀合之荚膜糖且具有通式(VIII),



(VIII)。

[0983] 42. 如段落38至41中任一者之荚膜糖糖缀合物, 其包含荚膜糖, 其中该荚膜糖在缀合之前的重量平均分子量 (M_w) 在10kDa与2,000kDa之间。

[0984] 43. 如段落38至41中任一者之荚膜糖糖缀合物, 其包含荚膜糖, 其中该荚膜糖在缀合之前的重量平均分子量 (M_w) 在50kDa与1,000kDa之间。

[0985] 44. 如段落38至41中任一者之荚膜糖糖缀合物, 其包含荚膜糖, 其中该荚膜糖在缀合之前的重量平均分子量 (M_w) 在200kDa与750kDa之间。

[0986] 45. 如段落38至44中任一者之荚膜糖糖缀合物, 其具有250kDa与20,000kDa之间的重量平均分子量 (M_w)。

[0987] 46. 如段落38至44中任一者之荚膜糖糖缀合物, 其具有500kDa与5,000kDa之间的重量平均分子量 (M_w)。

[0988] 47. 如段落38至44中任一者之荚膜糖糖缀合物, 其具有750kDa与2,500kDa之间的重量平均分子量 (M_w)。

[0989] 48. 如段落38至47中任一者之荚膜糖糖缀合物, 其中荚膜糖糖缀合物之缀合程度在2与15之间。

[0990] 49. 如段落38至48中任一者之荚膜糖糖缀合物, 其中糖缀合物中荚膜糖与载体蛋白之比率 (w/w) 在0.5与3.0之间。

[0991] 50. 如段落38至49中任一者之荚膜糖糖缀合物, 其中该荚膜糖糖缀合物针对糖之每4个糖重复单元在载体蛋白与糖之间包含至少一个共价连接。

[0992] 51. 如段落38至49中任一者之荚膜糖糖缀合物, 其中该荚膜糖糖缀合物针对糖之每25个糖重复单元在载体蛋白与糖之间包含至少一个共价连接。

[0993] 52. 如段落38至49中任一者之荚膜糖糖缀合物, 其中该荚膜糖糖缀合物针对糖之每5至10个糖重复单元在载体蛋白与糖之间包含至少一个共价连接。

[0994] 53. 如段落38至56中任一者之荚膜糖糖缀合物, 其中该载体蛋白为CRM₁₉₇。

[0995] 54. 如段落38至53中任一者之荚膜糖糖缀合物, 其中该载体蛋白为SCP。

[0996] 55. 如段落38至53中任一者之荚膜糖糖缀合物, 其中该载体蛋白为无酶促活性的SCP。

[0997] 56. 如段落38至53中任一者之荚膜糖糖缀合物, 其中该载体蛋白为无酶促活性的来自GBS之SCP (SCP_B)。

[0998] 57. 如段落38至53中任一者之荚膜糖糖缀合物, 其中该载体蛋白为SCP_B之片段。

[0999] 58. 如段落38至53中任一者之荚膜糖糖缀合物, 其中该载体蛋白为SCP之片段, 其包含蛋白酶结构域、蛋白酶相关结构域 (PA域) 及三个纤连蛋白III型 (Fn) 结构域但不包含输出信号前序列、原序列及细胞壁锚定域。

[1000] 59. 如段落38至53中任一者之荚膜糖糖缀合物, 其中该载体蛋白为无酶促活性的

SCP之片段,其中该无酶促活性的SCP之片段包含蛋白酶结构域、蛋白酶相关结构域(PA域)及三个纤连蛋白III型(Fn)结构域但不包含输出信号前序列、原序列及细胞壁锚定域。

[1001] 60.如段落38至53中任一者之荚膜糖糖缀合物,其中该载体蛋白为无酶促活性的SCP之片段,其包含蛋白酶结构域、蛋白酶相关结构域(PA域)及三个纤连蛋白III型(Fn)结构域但不包含输出信号前序列、原序列及细胞壁锚定域,其中该失活系通过置换野生型序列之至少一个氨基酸来实现,且其中该置换系选自D130A、H193A、N295A及S512A组成的组,其中数目指示根据W000/34487之SEQ ID NO:1之编号的肽酶中之氨基酸残基位置。

[1002] 61.如段落38至53中任一者之荚膜糖糖缀合物,其中该载体蛋白为无酶促活性的SCP之片段,其包含蛋白酶结构域、蛋白酶相关结构域(PA域)及三个纤连蛋白III型(Fn)结构域但不包含输出信号前序列、原序列及细胞壁锚定域,其中该失活系通过置换野生型序列之至少两个氨基酸来实现,其中该至少两个氨基酸置换为D130A及S512A,其中数目指示根据W000/34487之SEQ ID NO:1之编号的肽酶中之氨基酸残基位置。

[1003] 62.如段落38至53中任一者之荚膜糖糖缀合物,其中该载体蛋白为由与SEQ ID NO:41具有至少95%同一性之多肽组成的无酶促活性的SCP之片段。

[1004] 63.如段落38至53中任一者之荚膜糖糖缀合物,其中该载体蛋白为由与SEQ ID NO:42具有至少95%同一性之多肽组成的无酶促活性的SCP之片段。

[1005] 64.如段落38至53中任一者之荚膜糖糖缀合物,其中该载体蛋白为由SEQ ID NO:41组成的无酶促活性的SCP之片段。

[1006] 65.如段落38至53中任一者之荚膜糖糖缀合物,其中该载体蛋白为由SEQ ID NO:42组成的无酶促活性的SCP之片段。

[1007] 66.如段落38至53中任一者之荚膜糖糖缀合物,其中该载体蛋白为DT(白喉类毒素)。

[1008] 67.如段落38至53中任一者之荚膜糖糖缀合物,其中该载体蛋白为TT(破伤风类毒素)。

[1009] 68.如段落38至53中任一者之荚膜糖糖缀合物,其中该载体蛋白为PD(流感嗜血杆菌蛋白D)。

[1010] 69.如段落1至37中任一者之制备荚膜糖糖缀合物之方法或如段落38至68中任一者之荚膜糖糖缀合物,其中该荚膜糖为来自病原性细菌之荚膜糖。

[1011] 70.如段落1至37中任一者之制备荚膜糖糖缀合物之方法或如段落38至68中任一者之荚膜糖糖缀合物,其中该荚膜糖为来自病原性链球菌属、病原性葡萄球菌属、病原性肠球菌属、病原性芽孢杆菌属、病原性棒状杆菌属、病原性利斯特菌属、病原性丹毒丝菌属、病原性梭菌属、病原性嗜血杆菌属、病原性奈瑟氏菌属或病原性埃希氏菌属之荚膜糖。

[1012] 71.如段落1至37中任一者之制备荚膜糖糖缀合物之方法或如段落38至68中任一者之荚膜糖糖缀合物,其中该荚膜糖为来自粪肠球菌、大肠杆菌、金黄色葡萄球菌或链球菌之荚膜糖。

[1013] 72.如段落1至37中任一者之制备荚膜糖糖缀合物之方法或如段落38至68中任一者之荚膜糖糖缀合物,其中该荚膜糖为来自流感嗜血杆菌、脑膜炎奈瑟氏菌、肺炎链球菌、酿脓链球菌、无乳链球菌、C组及G组链球菌或大肠杆菌之荚膜糖。

[1014] 73.如段落1至37中任一者之制备荚膜糖糖缀合物之方法或如段落38至68中任一

者之荚膜糖糖缀合物,其中该荚膜糖为来自脑膜炎奈瑟氏菌、肺炎链球菌、无乳链球菌或大肠杆菌之荚膜糖。

[1015] 74.如段落1至37中任一者之制备荚膜糖糖缀合物之方法或如段落38至68中任一者之荚膜糖糖缀合物,其中该荚膜糖为来自肺炎链球菌或无乳链球菌之荚膜糖。

[1016] 75.如段落1至37中任一者之制备荚膜糖糖缀合物之方法或如段落38至68中任一者之荚膜糖糖缀合物,其中该荚膜糖为来自肺炎链球菌之荚膜糖。

[1017] 76.如段落1至37中任一者之制备荚膜糖糖缀合物之方法或如段落38至68中任一者之荚膜糖糖缀合物,其中该荚膜糖为来自金黄色葡萄球菌之荚膜糖。

[1018] 77.如段落1至37中任一者之制备荚膜糖糖缀合物之方法或如段落38至68中任一者之荚膜糖糖缀合物,其中该荚膜糖为来自粪肠球菌之荚膜糖。

[1019] 78.如段落1至37中任一者之制备荚膜糖糖缀合物之方法或如段落38至68中任一者之荚膜糖糖缀合物,其中该荚膜糖为来自流感嗜血杆菌b型之荚膜糖。

[1020] 79.如段落1至37中任一者之制备荚膜糖糖缀合物之方法或如段落38至68中任一者之荚膜糖糖缀合物,其中该荚膜糖为来自脑膜炎奈瑟氏菌之荚膜糖。

[1021] 80.如段落1至37中任一者之制备荚膜糖糖缀合物之方法或如段落38至68中任一者之荚膜糖糖缀合物,其中该荚膜糖为来自大肠杆菌之荚膜糖。

[1022] 81.如段落1至37中任一者之制备荚膜糖糖缀合物之方法或如段落38至68中任一者之荚膜糖糖缀合物,其中该荚膜糖为来自粪肠球菌之荚膜糖。

[1023] 82.如段落1至37中任一者之制备荚膜糖糖缀合物之方法或如段落38至68中任一者之荚膜糖糖缀合物,其中该荚膜糖为来自无乳链球菌(B组链球菌(GBS))之荚膜糖。

[1024] 83.如段落1至37中任一者之制备荚膜糖糖缀合物之方法或如段落38至68中任一者之荚膜糖糖缀合物,其中该荚膜糖为来自GBS Ia、Ib、II、III、IV、V、VI、VII或VIII型之荚膜糖。

[1025] 84.如段落1至37中任一者之制备荚膜糖糖缀合物之方法或如段落38至68中任一者之荚膜糖糖缀合物,其中该荚膜糖为来自选自以下组成的组之肺炎链球菌血清型的荚膜糖:血清型1、2、4、5、6A、6B、6C、7C、7F、8、9V、9N、10A、10B、11A、12F、14、15A、15B、15C、16F、17F、18C、19A、19F、20、21、22A、22F、23A、23B、23F、24B、24F、27、29、31、33B、33F、34、35B、35F、38、72及73。

[1026] 85.如段落1至37中任一者之制备荚膜糖糖缀合物之方法或如段落38至68中任一者之荚膜糖糖缀合物,其中该荚膜糖为来自肺炎链球菌血清型35B之荚膜糖。

[1027] 86.如段落1至37中任一者之制备荚膜糖糖缀合物之方法或如段落38至68中任一者之荚膜糖糖缀合物,其中该荚膜糖为来自肺炎链球菌血清型29之荚膜糖。

[1028] 87.如段落1至37中任一者之制备荚膜糖糖缀合物之方法或如段落38至68中任一者之荚膜糖糖缀合物,其中该荚膜糖为来自肺炎链球菌血清型15A之荚膜糖。

[1029] 88.如段落1至37中任一者之制备荚膜糖糖缀合物之方法或如段落38至68中任一者之荚膜糖糖缀合物,其中该荚膜糖为来自肺炎链球菌血清型24F之荚膜糖。

[1030] 89.如段落1至37中任一者之制备荚膜糖糖缀合物之方法或如段落38至68中任一者之荚膜糖糖缀合物,其中该荚膜糖为来自肺炎链球菌血清型23A之荚膜糖。

[1031] 90.如段落1至37中任一者之制备荚膜糖糖缀合物之方法或如段落38至68中任一

者之荚膜糖糖缀合物,其中该荚膜糖为来自肺炎链球菌血清型23B之荚膜糖。

[1032] 91.如段落1至37中任一者之制备荚膜糖糖缀合物之方法或如段落38至68中任一者之荚膜糖糖缀合物,其中该荚膜糖为来自肺炎链球菌血清型6B之荚膜糖。

[1033] 92.如段落1至37中任一者之制备荚膜糖糖缀合物之方法或如段落38至68中任一者之荚膜糖糖缀合物,其中该荚膜糖为来自肺炎链球菌血清型19A之荚膜糖。

[1034] 93.如段落1至37中任一者之制备荚膜糖糖缀合物之方法或如段落38至68中任一者之荚膜糖糖缀合物,其中该荚膜糖为来自肺炎链球菌血清型19F之荚膜糖。

[1035] 94.如段落1至37中任一者之制备荚膜糖糖缀合物之方法或如段落38至68中任一者之荚膜糖糖缀合物,其中该荚膜糖为来自肺炎链球菌血清型23F之荚膜糖。

[1036] 95.如段落1至94中任一者之制备荚膜糖糖缀合物之方法或荚膜糖糖缀合物,其中该荚膜糖不为来自肺炎链球菌血清型3之荚膜糖。

[1037] 96.一种免疫原性组合物,其包含如段落38至95中任一者之荚膜糖糖缀合物。

[1038] 如本文所使用,术语“约”意味在值之统计学上有意义的范围内,诸如所陈述之浓度范围、时间范围、分子量、温度或pH。此类范围可在给定值或范围之一数量级内,通常在20%内,更通常在10%内,且甚至更通常在5%内或在1%内。有时,此类范围可能在用于测量及/或测定给定值或范围之标准方法的典型实验误差内。术语“约”所涵盖之容许偏差将取决于所研究之特定系统,且可由本领域技术人员容易地理解。每当在本申请案中叙述一个范围时,还考虑该范围内之每个数目作为本发明之一实施方案。

[1039] 本发明人预期,在各情况下,本文中之术语“包含(comprising)”、“包含(comprise)”及“包含(comprises)”视情况可分别替换为术语“基本上由…组成(consisting essentially of)”、“基本上由…组成(consist essentially of)”、“基本上由…组成(consists essentially of)”、“由…组成(consisting of)”、“由…组成(consist of)”及“由…组成(consists of)”。

[1040] “免疫原性量”、“免疫有效量”、“治疗有效量”、“预防有效量”或“剂量”中之各者在本文中可互换使用,通常系指通过本领域技术人员已知之标准分析所测量,足以诱发免疫反应,还即细胞(T细胞)或体液(B细胞或抗体)反应或两种反应的抗原或免疫原性组合物之量。

[1041] 考虑本文件之范围中之任一者内之任何全数整数作为本发明之一实施方案。

[1042] 在本专利说明书内引用之所有参考文献或专利申请案均以引用的方式并入本文中。

[1043] 在随附实施例中说明本发明。除非另外详细描述,否则使用本领域技术人员所熟知且常用的标准技术进行以下实施例。实施例为说明性的,而非限制本发明。

实施例

[1044] 实施例1:使用点击化学缀合肺炎链球菌血清型35B荚膜多糖(参见图3)

[1045] 1.用叠氮基接头活化血清型35B荚膜多糖

[1046] 将血清型35B荚膜多糖(200mg)与咪唑(600mg)混合,且随后冷冻并冻干。

[1047] 在冻干3天之后,用无水DMSO(100mL)复原冻干之多糖。随后使反应混合物升温至45°C,且添加669μL(2MEq)之CDI(100mg/mL于DMSO中)。将反应混合物在45°C下搅拌2小时。

在将反应混合物冷却至23°C之后,添加2mL (2% v/v) 之WFI以淬灭游离CDI,且在23°C下搅拌30分钟。随后,添加41 μ L (2MEq) 之3-叠氮基-1-丙胺,且随后在23°C下搅拌20小时。在反应20小时之后,用400mL含10mM磷酸钠缓冲液 (SPB) 之盐水 (pH 7) 将反应混合物稀释 (5 $\times$, v/v) 至冷却 (5°C)。随后通过UF/DF使用10K MWC PES膜 (Millipore Pellicon 2Mini) 相对于含10mM SPB之盐水 (pH 7) (30 $\times$, v/v) 纯化经稀释之反应混合物。

[1048] 2. 用炔烃NHS酯将CRM₁₉₇及SCP活化为炔烃-CRM₁₉₇

[1049] CRM₁₉₇之活化:

[1050] 向CRM₁₉₇溶液 (1000mg) 中添加57mL WFI及50mL之0.5M磷酸钠缓冲液 (pH 8.3)。在冷却至8°C之后,向反应混合物中逐滴添加3.8mL (相对于CRM₁₉₇上之赖氨酸0.5MEq) 之3-炔丙氧基-丙酸NHS酯 (POPS) (20mg/mL于DMSO中),将反应温度维持在8 $\pm$ 3°C。将反应混合物在8°C下搅拌2小时之后,通过UF/DF使用10K MWC PES膜 (Millipore Pellicon 2Mini) 相对于含100mM磷酸钠缓冲液之盐水 (pH 7.0) (30 $\times$ 渗滤体积) 进行纯化。UF/DF之后,添加23g (15% v/v) 蔗糖。

[1051] SCP之活化:

[1052] SCP之活化类似于CRM₁₉₇之活化,不同之处在于调节接头之量。接头之最终量为相对于SCP 0.5MEq (每SCP 0.092w/w接头)。

[1053] 点击缀合:

[1054] 经活化之叠氮基聚合物及炔烃CRM₁₉₇或SCP系通过Cu+1介导之叠氮化物-炔烃环加成反应缀合,该环加成反应被称为“点击反应”。

[1055] 在23°C下,向用叠氮基接头活化之血清型35B荚膜多糖 (参见上文步骤1) 及炔烃-CRM₁₉₇或炔烃-SCP (参见上文步骤2) (于含100mM磷酸钠缓冲液 (SPB) 之盐水 (pH 7.0) 中) 之混合物中添加5mM硫酸铜 (CuSO₄) (1mL) 及25mM三 (3-羟丙基三唑基甲基) 胺 (THPTA) (1mL) 之混合物,且接着添加100mM氨基胍 (2mL) 及100mM抗坏血酸钠 (2mL)。将反应混合物在23°C下搅拌2小时之后,在23°C下用炔丙醇 (1MEq) 对未反应的叠氮基进行封端,持续2小时,且在第一次封端之后,在23°C下通过3-叠氮基-1-丙醇 (2MEq) 对未反应炔烃基进行封端,持续2小时。随后,通过UF/DF使用100K MWC PES膜相对于含10mM EDTA+10mM SPB之盐水 (pH 7.0) (30 $\times$ 渗滤体积) 且接着相对于含5mM琥珀酸盐之盐水 (pH 6.0) (30 $\times$ 渗滤体积) 来纯化反应混合物。

[1056] 表1. 使用点击化学获得之Pn35B缀合物之属性

[1057]	缀合物编号	1	2	3
	多糖			
	多糖MW, kDa	816	252	252
	经活化多糖之属性			
	活化程度(DoA)	~18	~20	~26
	经活化多糖MW, kDa	898	326	306
	经活化载体之属性			
	活化程度(DoA)	15	17	~26
	经活化载体MW, kDa	58	58	101
	缀合物属性			
	载体蛋白	CRM ₁₉₇	CRM ₁₉₇	SCP
	产率(%)	56	79	73
	SPR比率	1.2	0.9	0.8
	游离糖, %	6	<5	<5
[1058]	缀合物MW, kDa	1729	1093	939

[1059] MW:分子量;SPR:糖与蛋白质之比率

[1060] 点击化学允许产生游离糖含量极低且产率良好之35B缀合物。此外,其允许获得包含接近天然多糖之多糖的35B缀合物(与用高碘酸盐活化相反,在活化步骤期间未观测到尺寸减小,参见WO 2020/247299)。

[1061] 实施例2:使用点击化学缀合肺炎链球菌血清型29荚膜多糖(参见图3)

[1062] 1. 用叠氮基接头活化血清型29荚膜多糖

[1063] 将血清型29荚膜多糖(200mg)与咪唑(600mg)混合,且随后冷冻并冻干。

[1064] 在冻干3天之后,用无水DMSO(100mL)复原冻干之多糖。随后使反应混合物升温至45°C,且添加1050μL(3MEq)之CDI(100mg/mL于DMSO中)。将反应混合物在45°C下搅拌(300rpm)3小时。在将反应混合物冷却至23°C之后,添加2mL(2%v/v)之WFI以淬灭游离CDI,且在23°C下搅拌30分钟。随后,添加42μL(2MEq)之3-叠氮基-1-丙胺,接着添加60μL三乙胺,且随后在23°C下搅拌20小时。在反应20小时之后,用400mL含10mM磷酸钠缓冲液(SPB)之盐水(pH 7)(5×,v/v)将反应混合物稀释至冷却(5°C)。随后通过UF/DF使用10K MWCO PES膜(Millipore Pellicon 2Mini)相对于含10mM SPB之盐水(pH 7)(30×,v/v)纯化经稀释之反应混合物。

[1065] 2. 用炔烃NHS酯将CRM₁₉₇活化为炔烃-CRM₁₉₇

[1066] 向CRM₁₉₇溶液(1000mg)中添加57mL WFI及50mL之0.5M磷酸钠缓冲液(pH 8.3)。在冷却至8°C之后,向反应混合物中逐滴添加3.8mL(相对于CRM₁₉₇上之赖氨酸0.5MEq)之3-炔丙氧基-丙酸NHS酯(POPS)(20mg/mL于DMSO中),将反应温度维持在8±3°C。将反应混合物在8°C下搅拌2小时之后,通过UF/DF使用10K MWCO PES膜(Millipore Pellicon 2Mini)相对

于含100mM磷酸钠缓冲液之盐水 (pH 7.0) (30×渗滤体积) 进行纯化。UF/DF之后,添加23g (15% v/v) 蔗糖。

[1067] 3. 点击缀合: 经活化之叠氮基聚合物及炔烃CRM₁₉₇系通过Cu+1介导之叠氮化物-炔烃环加成反应缀合, 该环加成反应被称为“点击反应”

[1068] 在23℃下, 向用叠氮基接头活化之血清型29荚膜多糖 (参见上文步骤1) 及炔烃-CRM₁₉₇ (参见上文步骤2) (于含100mM磷酸钠缓冲液 (SPB) 之盐水 (pH 7.0) 中) 之混合物中添加5mM硫酸铜 (CuSO₄) (1mL) 及25mM三 (3-羟丙基三唑基甲基) 胺 (THPTA) (1mL) 之混合物, 且接着添加100mM氨基胍 (2mL) 及100mM抗坏血酸钠 (2mL)。将反应混合物在23℃下搅拌2小时之后, 在23℃下用炔丙醇 (1MEq) 对未反应叠氮基进行封端, 持续2小时, 且在第一次封端之后, 在23℃下通过3-叠氮基-1-丙醇 (2MEq) 对未反应炔烃基进行封端, 持续2小时。随后, 通过UF/DF使用100K MWC PES膜相对于含10mM EDTA+10mM SPB之盐水 (pH 7.0) (30×渗滤体积) 且接着相对于含5mM琥珀酸盐之盐水 (pH 6.0) (30×渗滤体积) 来纯化反应混合物。

[1069] 表2. 使用点击化学获得之Pn29缀合物之属性

[1070]	缀合物编号	1
	起始多糖	
	多糖MW, kDa	674
	经活化多糖之属性	
	活化程度(DoA)	~9
	经活化多糖MW, kDa	755
	缀合物属性	
	载体蛋白	CRM ₁₉₇
	产率(%)	56
	SPR比率	1.6
[1071]	游离糖, %	11
	缀合物MW, kDa	116 5

[1072] MW: 分子量; SPR: 糖与蛋白质之比率

[1073] 点击化学允许产生游离糖含量较低且产率良好之29缀合物。此外, 其允许获得包含接近天然多糖之多糖的血清型29缀合物 (与用高碘酸盐活化相反, 在活化步骤期间未观测到尺寸减小, 参见WO 2020/247299)。

[1074] 实施例3. 使用点击化学缀合之血清型35B缀合物之免疫原性

[1075] 在标准条件下测定与-CRM₁₉₇或SCP之血清型35B缀合物在小鼠中之调理吞噬活性(OPA)效价。使用点击化学(点击)来产生所测试之缀合物(参见表1处之属性)。

[1076] 在第0周,经由皮下途径用0.01μg/动物、0.05μg/动物或0.1μg/动物之测试缀合物对6至8周龄雌性瑞士韦伯斯特(Swiss Webster)小鼠组进行免疫接种(250μL)。在第3周用相同剂量之缀合物对小鼠进行加强,且随后在第5周抽血。对第5周血清样品进行血清型特异性OPA。

[1077] 结果呈现于表3处。

表3 -- 用与-CRM ₁₉₇ 或SCP点击缀合之血清型35B抗原进行疫苗接种后之OPA效价。使用缀合之血清型35B在佐剂存在下对小鼠进行疫苗接种。雌性瑞士韦伯斯特小鼠, 6至8周龄; 剂量: 0.01、0.05及0.1 μg/动物 + AlPO ₄ ; 疫苗接种: 第0周及第3周; 第5周抽血读数: OPA				
	剂量(μg/动物)	<u>0.01</u>	<u>0.05</u>	<u>0.1</u>
[1078]	点击	平均值(OPA效价)	21	341
	-CRM ₁₉₇	小鼠总数	45	25
	(天然)	无反应者数目	31	5
	表1之缀合物编号			
	1	无反应者%	69	20
				0
	点击	平均值(OPA效价)	NA	44
	-CRM ₁₉₇	小鼠总数	NA	25
[1079]	(尺寸设定的)	无反应者数目	NA	14
	表1之缀合物编号2			
		无反应者%	NA	56
	点击-SCP	平均值(OPA效价)	27	182
	表1之缀合物编号3	小鼠总数	25	25
		无反应者数目	13	5
		无反应者%	52	20
				12

[1080] NA: ‘未获得’

[1081] 表3之数据指示所有血清型35B缀合物均在鼠类免疫原性模型中引起剂量依赖性OPA效价。

[1082] 实施例4:使用点击化学缀合肺炎链球菌血清型23A及23B荚膜多糖(参见图3)

[1083] 使用用于血清型35B及29之类似方法(参见实施例1及2),用叠氮基接头(3-叠氮基-1-丙胺)活化血清型23A及23B荚膜多糖。使用实施例1及2之类似方法,用炔烃NHS酯将CRM₁₉₇及SCP活化为炔烃-CRM₁₉₇或炔烃-SCP,且将经活化之叠氮基多糖23A及23B与炔烃

CRM₁₉₇或SCP点击缀合(通过Cu+1介导之叠氮化物-炔烃环加成反应缀合)。

[1084] 已获得之缀合物之属性呈现于表4及5处。

[1085] 表4.使用点击化学获得之Pn23A缀合物之属性

[1086]	缀合物编号	1	2
	多糖		
	多糖MW, kDa	196	196
	经活化多糖之属性		
	活化程度(DoA)	~10-20	~10-20
	经活化多糖MW, kDa	248	248
	经活化载体之属性		
	活化程度(DoA)	15	26
	经活化载体MW, kDa	65	99.3
	缀合物属性		
[1087]	载体蛋白	CRM ₁₉₇	SCP
	产率(%)	57	77
	SPR比率	1.2	1
	游离糖, %	<5	<5
	缀合物MW, kDa	1469	1412

[1088] MW:分子量;SPR:糖与蛋白质之比率

[1089] 点击化学允许产生游离糖含量极低且产率良好之23A缀合物。

[1090] 表5.使用点击化学获得之Pn23B缀合物之属性

[1091]	缀合物编号	1	2
	多糖		
	多糖MW, kDa	157	157
	经活化多糖之属性		
	活化程度(DoA)	18	18
	经活化多糖MW, kDa	165	165
	经活化载体之属性		
	活化程度(DoA)	17	26
	经活化载体MW, kDa	58	101
	缀合物属性		
	载体蛋白	CRM ₁₉₇	SCP
	产率(%)	58	63
	SPR比率	0.91	0.85
	游离糖, %	10.6	11.5
	缀合物MW, kDa	1113	919

[1092] MW:分子量;SPR:糖与蛋白质之比率

[1093] 点击化学允许产生游离糖百分比较低且产率良好之23B缀合物。

[1094] 实施例5:使用点击化学缀合肺炎链球菌血清型15A荚膜多糖(参见图3)

[1095] 使用用于血清型35B及29之类似方法(参见实施例1及2),用叠氮基接头(3-叠氮基-1-丙胺)活化血清型15A荚膜多糖。使用实施例1及2之类似方法,用炔烃NHS酯将CRM₁₉₇及SCP活化为炔烃-CRM₁₉₇或炔烃-SCP,且将经活化之叠氮基多糖15A与炔烃CRM₁₉₇或SCP点击缀合(通过Cu+1介导之叠氮化物-炔烃环加成反应缀合)。

[1096] 已获得之缀合物之属性呈现于表6处。

[1097] 表6.使用点击化学获得之Pn15A缀合物之属性

[1098]	缀合物编号	1	2
	多糖		
	多糖MW, kDa	175	229
	经活化多糖之属性		
	活化程度(DoA)	~15	~17
	经活化多糖MW, kDa	244	320
	经活化载体之属性		
	活化程度(DoA)	17	~26
	经活化载体MW, kDa	58	101
	缀合物属性		
	载体蛋白	CRM ₁₉₇	SCP
	产率(%)	25	64
	SPR比率	1.0	1.1
	游离糖, %	6	9
	缀合物MW, kDa	2249	802

[1099] MW:分子量;SPR:糖与蛋白质之比率

[1100] 点击化学允许产生游离糖含量较低且针对SPC之产率良好之15A缀合物。

[1101] 实施例6:使用点击化学缀合肺炎链球菌血清型24F荚膜多糖(参见图3)

[1102] 使用用于血清型35B及29之类似方法(参见实施例1及2),用叠氮基接头(3-叠氮基-1-丙胺)活化血清型24F荚膜多糖。使用实施例1及2之类似方法,用炔烃NHS酯将CRM₁₉₇及SCP活化为炔烃-CRM₁₉₇或炔烃-SCP,且将经活化之叠氮基多糖24F与炔烃CRM₁₉₇或SCP点击缀合(通过Cu+1介导之叠氮化物-炔烃环加成反应缀合)。

[1103] 已获得之缀合物之属性呈现于表7。

[1104] 表7.使用点击化学获得之Pn24F缀合物之属性

[1105]	缀合物编号	1	2
	多糖		
	多糖MW, kDa	194	194
	经活化多糖之属性		
	活化程度(DoA)	6~10	6~10
	经活化多糖MW, kDa	282	282
	经活化载体之属性		
	活化程度(DoA)	15	20
	经活化载体MW, kDa	65	101
	缀合物属性		
	载体蛋白	CRM ₁₉₇	SCP
	产率(%)	52	82
	SPR比率	1.1	0.9
	游离糖, %	7	<5
	缀合物MW, kDa	1195	2206

[1106] MW:分子量;SPR:糖与蛋白质之比率

[1107] 点击化学允许产生游离糖含量较低且产率良好之24F缀合物。

[1108] 实施例7:使用点击化学缀合肺炎链球菌血清型6B荚膜多糖(参见图3)

[1109] 使用用于血清型35B及29之类似方法(参见实施例1及2),用叠氮基接头(3-叠氮基-1-丙胺)活化血清型6B荚膜多糖。使用实施例1及2之类似方法,用炔烃NHS酯将CRM₁₉₇及SCP活化为炔烃-CRM₁₉₇或炔烃-SCP,且将经活化之叠氮基多糖6B与炔烃CRM₁₉₇或SCP点击缀合(通过Cu+1介导之叠氮化物-炔烃环加成反应缀合)。

[1110] 已获得之缀合物之属性呈现于表8处。

[1111] 表8.使用点击化学获得之6B缀合物之属性

[1112]

缀合物编号	1	2
经活化多糖之属性		
活化程度(DoA)	20	20
经活化载体之属性		
活化程度(DoA)	15	26
缀合物属性		
载体蛋白	CRM ₁₉₇	SCP
SPR比率	1.20	1.10
游离糖, %	<5	<5
游离蛋白, %	<1	<1
缀合物MW, kDa	1919	1927

[1113] MW:分子量;SPR:糖与蛋白质之比率

[1114] 点击化学允许产生游离糖及游离蛋白含量极低之6B缀合物。

[1115] 实施例8:使用点击化学缀合肺炎链球菌血清型19A荚膜多糖(参见图3)

[1116] 使用用于血清型35B及29之类似方法(参见实施例1及2),用叠氮基接头(3-叠氮基-1-丙胺)活化血清型19A荚膜多糖。使用实施例1及2之类似方法,用炔烃NHS酯将CRM₁₉₇及SCP活化为炔烃-CRM₁₉₇或炔烃-SCP,且将经活化之叠氮基多糖19A与炔烃CRM₁₉₇或SCP点击缀合(通过Cu+1介导之叠氮化物-炔烃环加成反应缀合)。

[1117] 已获得之缀合物之属性呈现于表9处。

[1118] 表9.使用点击化学获得之19A缀合物之属性

[1119]

缀合物编号	1	2
经活化多糖之属性		
活化程度(DoA)	17	17
经活化载体之属性		
活化程度(DoA)	15	26

[1120]	缀合物属性		
	载体蛋白	CRM ₁₉₇	SCP
	SPR比率	1.50	1.20
	游离糖, %	14	<5
	游离蛋白, %	<1	<1
	缀合物MW, kDa	1360	994

[1121] MW:分子量;SPR:糖与蛋白质之比率

[1122] 点击化学允许产生游离糖含量较低且游离蛋白含量极低之19A缀合物。

[1123] 实施例9:使用点击化学缀合肺炎链球菌血清型19F荚膜多糖(参见图3)

[1124] 使用用于血清型35B及29之类似方法(参见实施例1及2),用叠氮基接头(3-叠氮基-1-丙胺)活化血清型19F荚膜多糖。使用实施例1及2之类似方法,用炔烃NHS酯将CRM₁₉₇及SCP活化为炔烃-CRM₁₉₇或炔烃-SCP,且将经活化之叠氮基多糖19F与炔烃CRM₁₉₇或SCP点击缀合(通过Cu+1介导之叠氮化物-炔烃环加成反应缀合)。

[1125] 已获得之缀合物之属性呈现于表10处。

[1126] 表10.使用点击化学获得之19F缀合物之属性

[1127]	缀合物编号	1	2
	经活化多糖之属性		
	活化程度(DoA)	24	24
	经活化载体之属性		
	活化程度(DoA)	15	26
	缀合物属性		
	载体蛋白	CRM ₁₉₇	SCP
	SPR比率	0.88	0.92
	游离糖, %	7	1
	游离蛋白, %	<1	<1
	缀合物MW, kDa	1775	2099

[1128] MW:分子量;SPR:糖与蛋白质之比率

[1129] 点击化学允许产生游离糖含量较低且游离蛋白含量极低之19F缀合物。

[1130] 实施例10:使用点击化学缀合肺炎链球菌血清型23F荚膜多糖(参见图3)

[1131] 使用用于血清型35B及29之类似方法(参见实施例1及2),用叠氮基接头(3-叠氮基-1-丙胺)活化血清型23F荚膜多糖。使用实施例1及2之类似方法,用炔烃NHS酯将CRM₁₉₇及SCP活化为炔烃-CRM₁₉₇或炔烃-SCP,且将经活化之叠氮基多糖23F与炔烃CRM₁₉₇或SCP点击缀合

合(通过Cu+1介导之叠氮化物-炔烃环加成反应缀合)。

[1132] 已获得之缀合物之属性呈现于表11处。

[1133] 表11.使用点击化学获得之23F缀合物之属性

[1134]	缀合物编号	1	2
	经活化多糖之属性		
	活化程度(DoA)	26	28
	经活化载体之属性		
	活化程度(DoA)	15	26
	缀合物属性		
	载体蛋白	CRM ₁₉₇	SCP
	SPR比率	1.20	1.10
	游离糖, %	5	8
	游离蛋白, %	<1	<1
	缀合物MW, kDa	2111	1798

[1135] MW:分子量;SPR:糖与蛋白质之比率

[1136] 点击化学允许产生游离糖含量较低且游离蛋白含量极低之23F缀合物。

[1137] 实施例11.使用点击化学缀合之血清型19F缀合物之免疫原性

[1138] 在标准条件下测定与-CRM₁₉₇或SCP之血清型19F缀合物在小鼠中之调理吞噬活性(OPA)效价。使用点击化学(点击)来产生所测试之缀合物(参见实施例9表10处之属性)。

[1139] 在第0周,经由皮下途径用0.05μg/动物或0.1μg/动物之测试缀合物对6至8周龄雌性瑞士韦伯斯特小鼠组进行免疫接种(250μL)。在第3周用相同剂量之缀合物对小鼠进行加强,且随后在第5周抽血。对第5周血清样品进行血清型特异性OPA。

[1140] 结果呈现于表12处。

表12 -- 用与-CRM₁₉₇或SCP点击缀合之血清型19F抗原进行疫苗接种后之OPA效价。使用缀合之血清型19F在佐剂存在下对小鼠进行疫苗接种。雌性瑞士韦伯斯特小鼠，6至8周龄；剂量：0.1及0.05 μg/动物 + AlPO₄；疫苗接种：第0周及第3周；第5周抽血读数：OPA

	剂量(μg/动物)	0.05		0.1
[1141] 点击 -CRM ₁₉₇ 表10之缀合物 编号1	平均值	102		143
	小鼠总数	50		50
	无反应者数目	8		8
	无反应者%	16		16
点击-SCP 表10之缀合物 编号2	平均值	306		328
	小鼠总数	50		50
	无反应者数目	0		1
	无反应者%	0		2

[1142] 实施例12. 使用点击化学缀合之血清型29缀合物之免疫原性

[1143] 在标准条件下测定与-CRM₁₉₇之血清型29缀合物在小鼠中之调理吞噬活性 (OPA) 效价。使用点击化学 (点击) 来产生所测试之缀合物 (参见实施例2表2处之属性)。

[1144] 在第0周, 经由皮下途径用0.01μg/动物、0.05μg/动物或0.1μg/动物之测试缀合物对6至8周龄雌性瑞士韦伯斯特小鼠组进行免疫接种 (250μL)。在第3周用相同剂量之缀合物对小鼠进行加强, 且随后在第5周抽血。对第5周血清样品进行血清型特异性OPA。

[1145] 结果呈现于表13处。

表13 -- 用通过点击化学与-CRM₁₉₇缀合之血清型29抗原进行疫苗接种后之OPA效价。使用缀合之血清型29在佐剂存在下对小鼠进行疫苗接种。雌性

瑞士韦伯斯特小鼠，6至8周龄；剂量：0.01、0.05及0.1 μg/动物 + AlPO4；疫苗接种：第0周及第3周；第5周抽血读数：OPA				
	剂量(μg/动物)	<u>0.01</u>	<u>0.05</u>	<u>0.1</u>
[1147] 点击 -CRM ₁₉₇	平 均 值 (OPA 效价)	28	1332	2271
	小鼠总数	25	25	25
	无反应者数目	12	1	0
	无反应者%	48	4	0

[1148] 表13之数据指示血清型29缀合物在鼠类免疫原性模型中引起剂量依赖性OPA效价。

[1149] 实施例13. 使用点击化学缀合之血清型19A缀合物之免疫原性

[1150] 在标准条件下测定与-CRM₁₉₇或SCP之血清型19A缀合物在小鼠中之调理吞噬活性(OPA)效价。使用点击化学(点击)来产生所测试之缀合物(参见实施例8表9处之属性)。

[1151] 在第0周,经由皮下途径用0.05μg/动物或0.1μg/动物之测试缀合物对6至8周龄雌性瑞士韦伯斯特小鼠组进行免疫接种(250μL)。在第3周用相同剂量之缀合物对小鼠进行加强,且随后在第5周抽血。对第5周血清样品进行血清型特异性OPA。

[1152] 结果呈现于表14处。

表14 -- 用与-CRM ₁₉₇ 或SCP点击缀合之血清型19A抗原进行疫苗接种后之OPA效价。使用缀合之血清型19A在佐剂存在下对小鼠进行疫苗接种。雌性瑞士韦伯斯特小鼠, 6至8周龄; 剂量: 0.05及0.1 μg/动物 + AlPO ₄ ; 疫苗接种: 第0周及第3周; 第5周抽血读数: OPA			
	剂量(μg/动物)	<u>0.05</u>	<u>0.1</u>
[1153]	点击 -CRM ₁₉₇ 表9之缀合 物编号1	平均值	25
		小鼠总数	50
		无反应者数目	24
		无反应者%	48
	点击-SCP 表9之缀合	平均值	126
		小鼠总数	50
[1154]	物编号2	无反应者数目	4
		无反应者%	8

[1155] 实施例14. 使用点击化学缀合之血清型6B缀合物之免疫原性

[1156] 在标准条件下测定与-CRM₁₉₇或SCP之血清型6B缀合物在小鼠中之调理吞噬活性(OPA)效价。使用点击化学(点击)来产生所测试之缀合物(参见实施例7表8处之属性)。

[1157] 在第0周,经由皮下途径用0.05μg/动物、0.1μg/动物或0.2μg/动物之测试缀合物对6至8周龄雌性瑞士韦伯斯特小鼠组进行免疫接种(250μL)。在第3周用相同剂量之缀合物对小鼠进行加强,且随后在第5周抽血。对第5周血清样品进行血清型特异性OPA。

[1158] 结果呈现于表15处。

[1159]

表15 -- 用与-CRM ₁₉₇ 或SCP点击缀合之血清型6B抗原进行疫苗接种后之OPA效价。使用缀合之血清型6B在佐剂存在下对小鼠进行疫苗接种。雌性瑞士韦伯斯特小鼠，6至8周龄；剂量0.2、0.1及0.05 µg/动物 + AlPO ₄ ；疫苗接种：第0周及第3周；第5周抽血读数：OPA				
	剂量(µg/动物)	<u>0.05</u>	<u>0.1</u>	<u>0.2</u>
点击-CRM ₁₉₇ 表8之缀合物编号1	平均值	76	255	217
	小鼠总数	50	50	50
	无反应者数目	15	9	7
	无反应者%	30	18	14
点击-SCP 表8之缀合物编号2	平均值	54	70	202
	小鼠总数	48	50	49
	无反应者数目	18	16	7
	无反应者%	38	32	14

[1160] 实施例15. 使用点击化学缀合之血清型15A缀合物之免疫原性

[1161] 在标准条件下测定与-CRM₁₉₇或SCP之血清型15A缀合物在小鼠中之调理吞噬活性(OPA)效价。使用点击化学(点击)来产生所测试之缀合物(参见实施例5表6处之属性)。

[1162] 在第0周, 经由皮下途径用0.01µg/动物、0.1µg/动物或1.0µg/动物之测试缀合物对6至8周龄雌性瑞士韦伯斯特小鼠组进行免疫接种(250µL)。在第3周用相同剂量之缀合物对小鼠进行加强, 且随后在第5周抽血。对第5周血清样品进行血清型特异性OPA。

[1163] 结果呈现于表16处。

[1164]

表16 -- 用与-CRM ₁₉₇ 或SCP点击缀合之血清型15A抗原进行疫苗接种后之OPA效价。使用缀合之血清型15A在佐剂存在下对小鼠进行疫苗接种。雌性瑞士韦伯斯特小鼠，6至8周龄；剂量：1.0、0.1及0.01 µg/动物 + AlPO ₄ ；疫苗接种：第0周及第3周；第5周抽血读数：OPA				
	剂量(µg/动物)	<u>0.01</u>	<u>0.1</u>	<u>1.0</u>
点击-CRM ₁₉₇ 表6之缀合物编号1	平均值	135	6457	1662 4
	小鼠总数	23	25	25
	无反应者数目	5	0	0
	无反应者%	22	0	0
点击-SCP 表6之缀合物编号2	平均值	463	3294	5578
	小鼠总数	25	25	25
	无反应者数目	2	0	1
	无反应者%	8	0	4

[1165] 本说明书中提及之所有出版物及专利申请指示本发明所属领域之技术人员的水平。所有出版物及专利申请均在此以引用之方式并入,如同各个别出版物或专利申请特定且单独地指示为以引用之方式并入一般。

[1166] 尽管出于清晰理解的目的已经藉助于说明及实施例相当详细地描述了前述发明,但可在所附权利要求的范围内实施某些改变及修改。



图1



图2

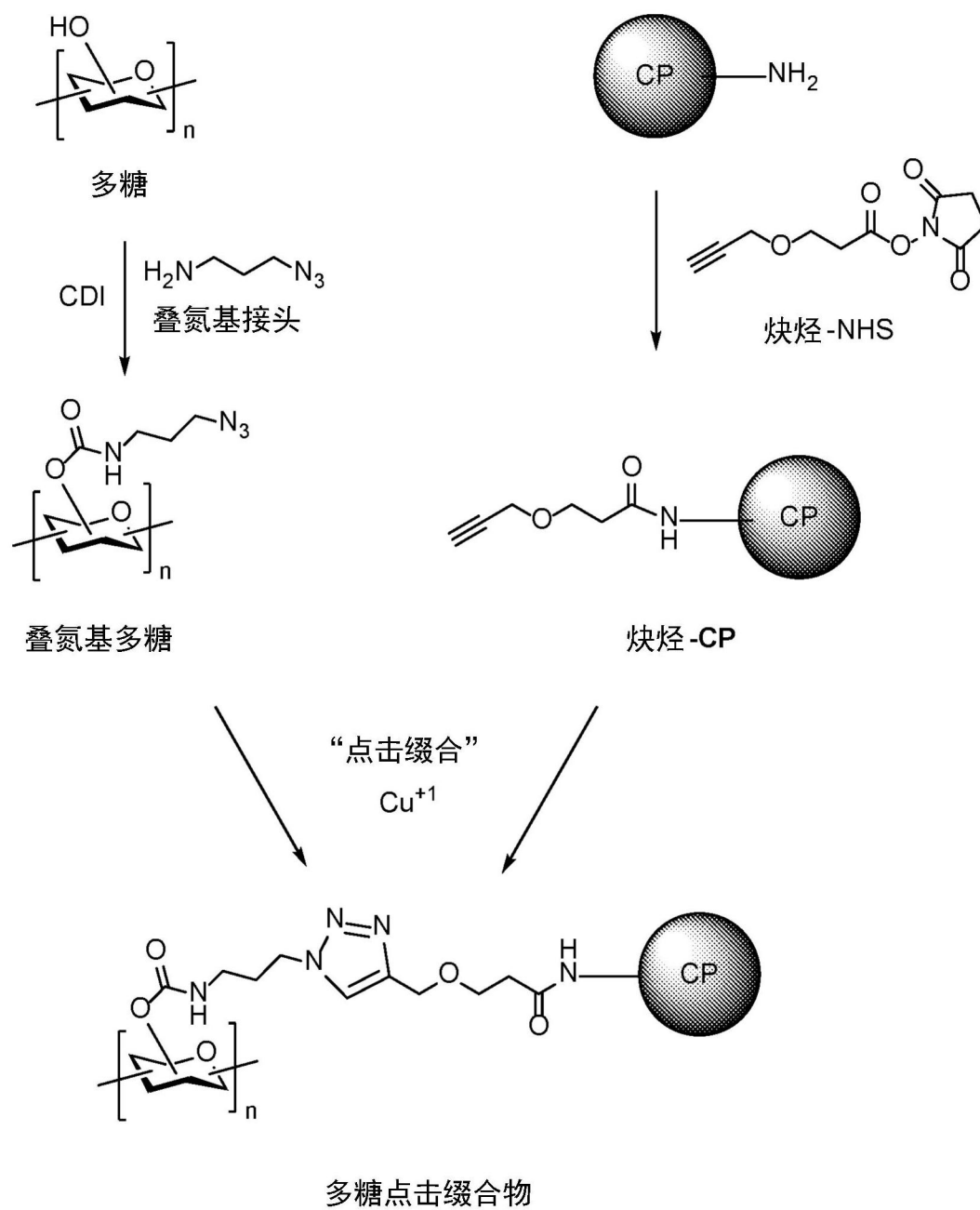


图3