

Immunogenic compositions comprising conjugated capsular saccharide antigens and uses thereof

Field of the Invention

5 The present invention relates to the field of immunogenic compositions and vaccines, their manufacture, and the use of such compositions in medicine.

 More particularly, it relates to isolated *Streptococcus pneumoniae* serotype 21 saccharides, glycoconjugates thereof, methods for making *Streptococcus pneumoniae* serotype 21 glycoconjugates and immunogenic composition comprising a *Streptococcus pneumoniae* serotype 21 glycoconjugate.

 The invention also relates to analytical methods to analyze isolated *S. pneumoniae* serotype 21 polysaccharide, reduced serotype 21 polysaccharide or *Streptococcus pneumoniae* serotype 21 glycoconjugates.

 The *Streptococcus pneumoniae* serotype 21 saccharide and glycoconjugates of the invention can be used as a vaccine.

Background of the Invention

 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 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. (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 create amine, amide, urethane, isothiourea, 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.

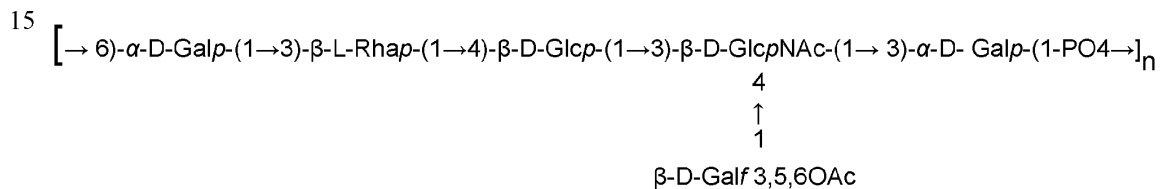
Pneumococcal polysaccharides, in particular capsular polysaccharides, are important immunogens found on the surface of the bacteria. This has led to them being an important component in the design of pneumococcal vaccines. They have proved useful in eliciting immune responses especially when linked to carrier proteins.

Thus, there is a need for antigens which are able to generate a robust immune response to *Streptococcus pneumoniae* serotype 21.

10 The present invention provides in particular *Streptococcus pneumoniae* serotype 21 glycoconjugates.

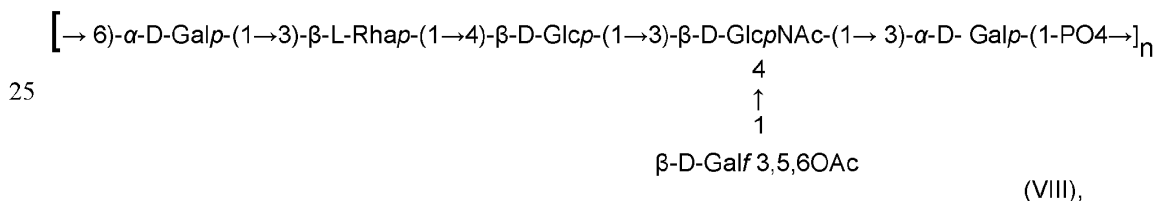
Summary of the Invention

To meet these and other needs, the present invention relates an isolated *S. pneumoniae* serotype 21 saccharide with the following repeating unit:



20 where n represents the number of repeating units.

In an aspect the present invention pertains to an isolated *S. pneumoniae* serotype 21 saccharide with the following repeating unit:



where n represents the number of repeating units, wherein the O-acetyl group at position 3 of β -D-Galf is present in about 50% to about 80% of the repeating units, the O-acetyl group at position 6 of β -D-Galf is present in about 20% to about 40% of the repeating units, wherein the O-acetyl group at position 5 of β -D-Galf is present in about 1% to about 10% of the repeating units.

In certain embodiments, the isolated *S. pneumoniae* serotype 21 saccharide of the invention has between 10 and 5,000 repeating units.

In an aspect, the invention provides a glycoconjugate consisting of a saccharide having the
35 above disclosed repeating unit conjugated to a carrier protein.

In an aspect the invention relates to mode of preparation of such conjugates.

In an embodiment the invention relates to an immunogenic composition comprising a *S. pneumoniae* serotype 21 saccharide of the invention and/or a *Streptococcus pneumoniae* serotype 21 glycoconjugate of the invention.

The invention further pertains to analytical methods of *S. pneumoniae* serotype 21
5 saccharides and conjugates.

Detailed description of the drawings

Figure 1. Schematic of pneumococcal polysaccharide serotype 21 repeat unit organization.

Figure 2. 1D ^1H spectrum of serotype 21 polysaccharide (insert spectrum shows the expanded anomeric region). Inset table shows the normalized and expected peak area for the anomeric and
10 methyl protons. The anomeric and the methyl signals are annotated.

Figure 3. The expanded anomeric region of proton decoupled ^1H - ^{13}C HSQC spectrum of serotype 21 polysaccharide. The coupling constant $^1J_{\text{CH}}$ for each of the sugars is annotated.

Figure 4. Quantitation of O-acetylation migration. Bottom, middle and top panel shows the expanded region of 1D ^1H spectrum of serotype 21 at 0 hr, 9 hr and 15 hr, respectively. All peaks
15 are annotated. The scheme above shows the migration of the O-acetyl from the 3 to 6 position of β -D-Galf sugar.

Figure 5. Quantitation of O-acetylation migration. Bottom, middle and top panel shows the expanded region of 1D ^1H spectrum of serotype 21 at 0, 9 and 14 day(s), respectively. All peaks
20 are annotated. The scheme above shows the migration of the O-acetyl from the 3 to 6 position of β -D-Galf sugar

Figure 6. Expanded anomeric and methyl region 1D ^1H spectra of O-acetylated (bottom panel) and de-O-acetylated (top panel) serotype 21 polysaccharides. The anomeric and the methyl
25 signals are annotated for both spectra. Shaded box denotes the resonances observed due to the O-acetylation of β -D-Galf residue and are absent in the de-O-acetylated serotype 21 polysaccharide.

Figure 7. Chemical shift difference plots of O-acetylated and de-O-acetylated serotype 21 polysaccharide (left panels). The impact of the deacetylation on the structure of serotype 21 polysaccharide is shown by dark balls on the structure of serotype 21 polysaccharide. Arrows
points to bars representing the deacetylation resonances.

Detailed description of the Invention

The present invention is based, in part, on the identification of novel pneumococcal polysaccharide structure(s) by using NMR spectroscopy. It is believed that the structure provided herein is the first identification or the first correct identification of *S. pneumoniae* serotype 21.

The produced (and purified) polysaccharide was used to generate polysaccharide-protein conjugate (glycoconjugates). *S. pneumoniae* serotype 21 has a unique polysaccharide structure,
35 which results in unique consideration when designing conjugate production process. The *S. pneumoniae* serotype 21 glycoconjugates of the invention also have a unique structure and design.

1. Isolated *Streptococcus pneumoniae* serotype 21 saccharide of the invention

As used herein, the term "isolated" in connection with a saccharide refers to isolation of *S. pneumoniae* serotype specific capsular polysaccharide from purified polysaccharide using purification techniques known in the art, including the use of centrifugation, depth filtration, precipitation, ultrafiltration, treatment with activate carbon, diafiltration and/or column chromatography. Generally, an isolated polysaccharide refers to partial removal of proteins, nucleic acids and non-specific endogenous polysaccharide (C-polysaccharide). The isolated polysaccharide contains less than 10%, 8%, 6%, 4%, or 2% protein impurities and/or nucleic acids. The isolated polysaccharide contains less than 20% of C-polysaccharide with respect to type specific polysaccharides.

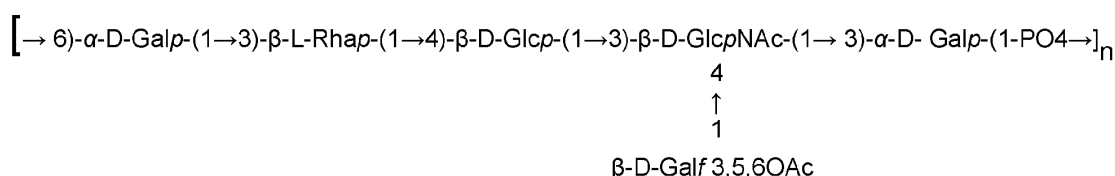
The term "saccharide" throughout this specification may indicate polysaccharide or oligosaccharide and includes both. In preferred embodiments, the saccharide is a polysaccharide, in particular a *S. pneumoniae* serotype 21 capsular polysaccharide.

The term "serotype 21 saccharide", "serotype 21 capsular saccharide", "*S. pneumoniae* serotype 21 saccharide" throughout this specification refers to *S. pneumoniae* serotype 21 capsular saccharide and may be used interchangeably herein.

The structure of *S. pneumoniae* serotype 21 capsular polysaccharide is disclosed for the first time and is shown in Figure 1. The inventors found that the serotype 21 polysaccharide is a hexasaccharide: α -D-galactopyranose (A), β -L-rhamnose (B), β -D-Glucose (C), β -D-glucosamine (D), α -D-galactopyranose (E) and O-acetylated β -D-galactofuranose (F).

The inventors have also found that the O-acetylation of β -D-galactofuranose (residue F) occurs at three different positions: at position 3, 5 and 6. Therefore, three types of repeat units constitute the native serotype 21 polysaccharide, a repeat unit O-acetylated at the C3 position of the β -D-galactofuranose residue (which is the most abundant form of repeat unit in the polysaccharide), a repeat unit O-acetylated at the C6 position of the β -D-galactofuranose residue (the intermediate abundant form of repeat unit in the polysaccharide), a repeat unit O-acetylated at the C5 position of the β -D-galactofuranose residue (the less abundant form of repeat unit in the polysaccharide).

Accordingly, in one embodiment, the present invention provides an isolated *S. pneumoniae* serotype 21 saccharide with the following repeating unit:

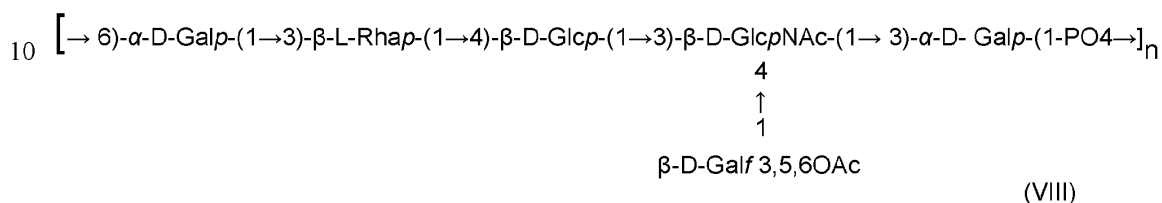


where n represents the number of repeating units.

As shown at figure 1, β -D-galactofuranose (residue F) of serotype 21 polysaccharide is O-acetylated at carbon 3, 5 and 6 position.

The serotype 21 polysaccharide is therefore composed of three types of repeat units: a repeat unit O-acetylated at the C3 position of the β -D-Galf residue, a repeat unit O-acetylated at the C5 position of the β -D-Galf residue and a repeat unit O-acetylated at the C6 position of the β -D-Galf residue.

Accordingly, in one embodiment, the present invention provides an isolated *S. pneumoniae* serotype 21 saccharide with the following repeating unit:



where n represents the number of repeating units, wherein the O-acetyl group at position 3 of β -D-Galp is present in about 50% to about 80% of the repeating units, the O-acetyl group at position 6 of β -D-Galp is present in about 20% to about 40% of the repeating units, wherein the O-acetyl group at position 5 of β -D-Galp is present in about 1% to about 10% of the repeating units. Preferably, all repeating unit bears one O-acetyl group on the β -D-Galp residue.

In one embodiment, the present invention provides an isolated *S. pneumoniae* serotype 20 21 saccharide with the repeating unit of formula (VIII), where n represents the number of repeating units, wherein the O-acetyl group at position 3 of β -D-Galf is present in about 60% of the repeating units, the O-acetyl group at position 6 of β -D-Galf is present in about 30% of the repeating units, wherein the O-acetyl group at position 5 of β -D-Galf is present in about 10% of the repeating units. Preferably, all repeating unit bears one O-acetyl group on the β -D-Galf residue.

25 In one embodiment, the present invention provides an isolated *S. pneumoniae* serotype
21 saccharide with the repeating unit of formula (VIII), where n represents the number of repeating
units, wherein the O-acetyl group at position 3 of β -D-Galf is present in about 64% of the repeating
units, the O-acetyl group at position 6 of β -D-Galf is present in about 30% of the repeating units,
wherein the O-acetyl group at position 5 of β -D-Galf is present in about 6% of the repeating units.
30 Preferably, all repeating unit bears one O-acetyl group on the β -D-Galf residue.

Furthermore, migration of the O-Acetyl group from 3 position to 6 position of the β -D-Galf sugar has been observed (see Example 3).

Therefore, in one embodiment, the present invention provides an isolated *S. pneumoniae* serotype 21 saccharide with the repeating unit of formula (VIII), where n represents the number of repeating units, wherein the O-acetyl group at position 3 of β -D-Galf is present in about 25% to about 60% of the repeating units, the O-acetyl group at position 6 of β -D-Galf is present in about 30% to about 65% of the repeating units, wherein the O-acetyl group at position 5 of β -D-

Gal β is present in about 5% to about 15% of the repeating units. Preferably, all repeating unit bears one O-acetyl group on the β -D-Gal β residue.

In one embodiment, the present invention provides an isolated *S. pneumoniae* serotype 21 saccharide with the repeating unit of formula (VIII), where n represents the number of repeating units, wherein the O-acetyl group at position 3 of β -D-Gal β is present in about 50% of the repeating units, the O-acetyl group at position 6 of β -D-Gal β is present in about 40% of the repeating units, wherein the O-acetyl group at position 5 of β -D-Gal β is present in about 10% of the repeating units. Preferably, all repeating unit bears one O-acetyl group on the β -D-Gal β residue.

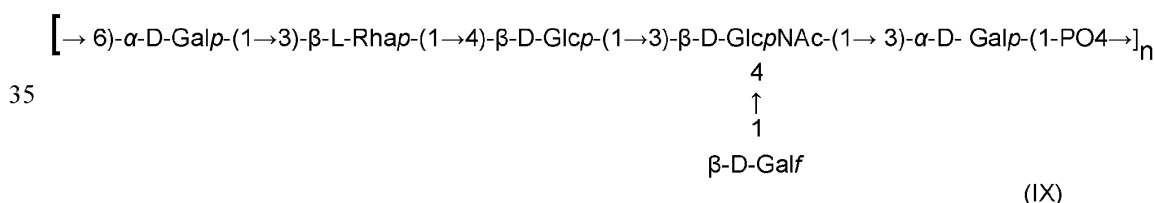
In one embodiment, the present invention provides an isolated *S. pneumoniae* serotype 21 saccharide with the repeating unit of formula (VIII), where n represents the number of repeating units, wherein the O-acetyl group at position 3 of β -D-Gal β is present in about 47% of the repeating units, the O-acetyl group at position 6 of β -D-Gal β is present in about 42% of the repeating units, wherein the O-acetyl group at position 5 of β -D-Gal β is present in about 11% of the repeating units. Preferably, all repeating unit bears one O-acetyl group on the β -D-Gal β residue.

In one embodiment, the present invention provides an isolated *S. pneumoniae* serotype 21 saccharide with the repeating unit of formula (VIII), where n represents the number of repeating units, wherein the O-acetyl group at position 3 of β -D-Gal β is present in about 30% of the repeating units, the O-acetyl group at position 6 of β -D-Gal β is present in about 60% of the repeating units, wherein the O-acetyl group at position 5 of β -D-Gal β is present in about 10% of the repeating units. Preferably, all repeating unit bears one O-acetyl group on the β -D-Gal β residue.

In one embodiment, the present invention provides an isolated *S. pneumoniae* serotype 21 saccharide with the repeating unit of formula (VIII), where n represents the number of repeating units, wherein the O-acetyl group at position 3 of β -D-Gal β is present in about 31% of the repeating units, the O-acetyl group at position 6 of β -D-Gal β is present in about 61% of the repeating units, wherein the O-acetyl group at position 5 of β -D-Gal β is present in about 8% of the repeating units. Preferably, all repeating unit bears one O-acetyl group on the β -D-Gal β residue.

Furthermore, native *S. pneumoniae* serotype 21 saccharide can be deacetylated, for example by treatment with a base (alkaline pH). As shown in the example section, native serotype 21 polysaccharide can be completely deacetylated after incubating with 0.25N NH₄OH at room temperature for 24 hours.

Accordingly, in one embodiment, the present invention provides an isolated *S. pneumoniae* serotype 21 saccharide with the following repeating unit:



where n represents the number of repeating units.

The native *S. pneumoniae* serotype 21 saccharide may be partially deacetylated, for example by gentle treatment with a base (alkaline pH).

Accordingly, in one embodiment, the present invention provides an isolated *S. pneumoniae* serotype 21 saccharide with the repeating unit of formula (VIII), where n represents
5 the number of repeating units, and wherein the O-acetyl group at position 3, 5 or 6 of β -D-Galf is present in about 0% to about 95% of the repeating units.

In one embodiment, the present invention provides an isolated *S. pneumoniae* serotype 21 saccharide with the repeating unit of formula (VIII), where n represents the number of repeating
10 units, and wherein the O-acetyl group at position 3, 5 or 6 of β -D-Galf is present in about 10% to about 90% of the repeating units.

In one embodiment, the present invention provides an isolated *S. pneumoniae* serotype 21 saccharide with the repeating unit of formula (VIII), where n represents the number of repeating
units, and wherein the O-acetyl group at position 3, 5 or 6 of β -D-Galf is present in about 30% to about 60% of the repeating units.

15 In certain embodiments, the isolated *S. pneumoniae* serotype 21 saccharide of the invention has between 10 and 5,000 repeating units. In certain aspects, the isolated saccharide has between 50 and 4,500 repeating units. In certain aspects, the isolated saccharide has between 100 and 4,500 repeating units. In certain aspects, the isolated saccharide has between
150 and 2,000 repeating units.

20 Isolated capsular saccharides from *S. pneumoniae* serotype 21 can be prepared by standard techniques known to those of ordinary skill in the art. Typically capsular polysaccharides are produced by growing a *S. pneumoniae* serotype 21 strain in a medium (e.g., in a soy-based medium), the polysaccharides are then prepared from the bacteria culture. Serotype 21
Streptococcus pneumoniae strains may be obtained from established culture collections (such as
25 for example the Streptococcal Reference Laboratory (Centers for Disease Control and Prevention, Atlanta, GA)) or clinical specimens.

The population of the organism (*S. pneumoniae* serotype 21) is often scaled up from a seed vial to seed bottles and passaged through one or more seed fermentors of increasing
volume until production scale fermentation volumes are reached. At the end of the growth cycle
30 the cells are lysed and the lysate broth is then harvested for downstream (purification) processing (see for example WO 2006/110381 and WO 2008/118752, U.S. Patent App. Pub. Nos. 2006/0228380, 2006/0228381, 2008/0102498 and US2008/0286838). The polysaccharides are typically purified through centrifugation, precipitation, ultra-filtration, and/or column chromatography (see for example WO 2006/110352, WO 2008/118752 and WO2020/170190).

35 The isolated polysaccharide 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 isolated *S. pneumoniae* serotype 21 saccharide of the invention has a weight average molecular weight between 5 kDa and 5000 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 21 saccharide has a weight average molecular weight between 5 kDa and 2000 kDa.

5 In an embodiment, the isolated *S. pneumoniae* serotype 21 polysaccharide has a weight average molecular weight between 50 kDa and 5000 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 21 polysaccharide has a weight average molecular weight between 50 kDa and 2000 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 21 polysaccharide has a weight average molecular weight between 50 kDa and 1000 kDa.

10 In an embodiment, the isolated *S. pneumoniae* serotype 21 polysaccharide has a weight average molecular weight between 100 kDa and 5000 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 21 polysaccharide has a weight average molecular weight between 100 kDa and 2000 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 21 polysaccharide has a weight average molecular weight between 100 kDa and 1000 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 21 polysaccharide has a weight average molecular weight between 100 kDa and 500 kDa.

In an embodiment, the isolated *S. pneumoniae* serotype 21 polysaccharide has a weight average molecular weight between 300 kDa and 5000 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 21 polysaccharide has a weight average molecular weight between 300 kDa and 2000 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 21 polysaccharide has a weight average molecular weight between 300 kDa and 1000 kDa.

In an embodiment, the isolated *S. pneumoniae* serotype 21 polysaccharide has a weight average molecular weight between 500 kDa and 3000 kDa. In an embodiment, the isolated polysaccharide has a weight average molecular weight between 500 kDa and 2000 kDa. In an embodiment, the isolated polysaccharide has a weight average molecular weight between 500 kDa and 1000 kDa.

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.

30 Advantageously, the size of the purified capsular *S. pneumoniae* serotype 21 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 *S. pneumoniae* serotype 21 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 *S. pneumoniae* serotype 21 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 *S. pneumoniae* serotype 21 capsular saccharide is sized to a weight average molecular weight between 10 kDa and 1000 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 21 capsular saccharide is sized to a weight average molecular weight between 50 kDa and 500 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 21 capsular saccharide is sized to a weight average molecular weight between 50 kDa and 400 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 21 capsular saccharide is sized to a weight average molecular weight between 50 kDa and 250 kDa.

In an embodiment, the isolated *S. pneumoniae* serotype 21 capsular saccharide is sized to a weight average molecular weight between 100 kDa and 1000 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 21 capsular saccharide is sized to a weight average molecular weight between 100 kDa and 500 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 21 capsular saccharide is sized to a weight average molecular weight between 100 kDa and 400 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 21 capsular saccharide is sized to a weight average molecular weight between 100 kDa and 250 kDa.

In a preferred embodiment, the isolated *S. pneumoniae* serotype 21 capsular saccharide is sized to a weight average molecular weight between 150 kDa and 400 kDa.

In an embodiment, the isolated *S. pneumoniae* serotype 21 capsular saccharide is sized to a weight average molecular weight between 250 kDa and 1000 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 21 capsular saccharide is sized to a weight average molecular weight between 250 kDa and 500 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 21 capsular saccharide is sized to a weight average molecular weight between 250 kDa and 400 kDa. In a preferred embodiment, the isolated *S. pneumoniae* serotype 21 capsular saccharide is sized to a weight average molecular weight between 200 kDa and 800 kDa.

In an embodiment, the isolated *S. pneumoniae* serotype 21 capsular saccharide capsular saccharide is sized to a weight average molecular weight of about 250 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 21 capsular saccharide is sized to a weight average molecular weight of about 300 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 21 capsular saccharide is sized to a weight average molecular weight of about 350 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 21 capsular saccharide is sized to a weight average molecular weight of about 400 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 21 capsular saccharide is sized to a weight average molecular weight of about 450 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 21 capsular saccharide is sized to a weight average molecular weight of about 500 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 21 capsular saccharide is sized to a weight average molecular weight of about 550 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 21 capsular saccharide is sized to a weight average molecular weight of about 600 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 21 capsular saccharide is sized to a weight average molecular weight of about 700 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 21 capsular saccharide is sized to a weight average molecular weight of about 800 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 21 capsular saccharide is sized to a weight average molecular weight of about 900 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 21 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 and then incorporated into glycoconjugates, as further described herein.

2. *Streptococcus pneumoniae* serotype 21 glycoconjugates of the invention

For the purpose of the invention the term 'glycoconjugate' indicates a capsular saccharide conjugated to a carrier protein via covalent or non-covalent bonds. In an embodiment, the capsular saccharide is conjugated to a carrier protein via non-covalent bonds (such as the rhizavidin/biotin system, see e.g. WO2012155007, WO2020056202). Preferably, the capsular saccharide is conjugated via covalent bonds. In one embodiment the capsular saccharide is conjugated directly to a carrier protein. In a second embodiment the capsular saccharide is conjugated to a carrier protein through a spacer/linker.

The present invention provides glycoconjugates in which saccharides as provided for above are conjugated to a carrier protein. Therefore, in an embodiment, the invention provides a glycoconjugate comprising a saccharide having the above disclosed repeating unit conjugated to a carrier protein.

In an embodiment, the invention provides a glycoconjugate consisting of a saccharide having the above disclosed repeating unit conjugated to a carrier protein.

2.1 Attributes of the *Streptococcus pneumoniae* serotype 21 glycoconjugates of the invention

The isolated polysaccharide described above may be activated (e.g., chemically activated) to make them capable of reacting (e.g. with a linker or directly with the carrier protein) and then incorporated into glycoconjugates, as further described herein.

5 Before activation, the size of the isolated polysaccharide can be reduced while preserving critical features of the structure of the polysaccharide. Mechanical or chemical sizing maybe employed. In an embodiment, the size of the isolated polysaccharide is reduced by chemical hydrolysis. The size of the isolated polysaccharide can also be reduced by mechanical homogenization. In an embodiment, the size of the isolated polysaccharide is reduced by high
10 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 weight average molecular weight (Mw) of the saccharide before conjugation refers to the
15 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 serotype 21 glycoconjugate of the present invention comprises a
20 serotype 21 polysaccharide wherein the weight average molecular weight (Mw) of said polysaccharide before conjugation is between 50 kDa and 1,000 kDa.

In an embodiment, the serotype 21 glycoconjugate of the present invention comprises a serotype 21 polysaccharide wherein the weight average molecular weight (Mw) of said polysaccharide before conjugation is between 100 kDa and 600 kDa.

25 In an embodiment, the serotype 21 glycoconjugate of the present invention comprises a serotype 21 polysaccharide wherein the weight average molecular weight (Mw) of said polysaccharide before conjugation is between 100 kDa and 400 kDa.

In an embodiment, the serotype 21 glycoconjugate of the present invention comprises a serotype 21 polysaccharide wherein the weight average molecular weight (Mw) of said
30 polysaccharide before conjugation is between 150 kDa and 300 kDa.

In some embodiments, the serotype 21 glycoconjugate of the invention has a weight average molecular weight (Mw) of between 250 kDa and 20,000 kDa.

In other embodiments, the serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 500 kDa and 15,000 kDa.

35 In other embodiments, the serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 500 kDa and 10,000 kDa.

In still other embodiments, the serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 750 kDa and 7,500 kDa.

In preferred embodiments, the serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 1,000 kDa and 5,000 kDa.

In a preferred embodiment, the serotype 21 glycoconjugate of the invention prepared by click chemistry has a weight average molecular weight (Mw) of between 750 kDa and 7,500 kDa and even preferably of between 800 kDa and 7,000 kDa. In an embodiment, the carrier protein of said serotype 21 saccharide glycoconjugate is SCP.

In a preferred embodiment, the serotype 21 glycoconjugate of the invention prepared by direct reductive amination has a weight average molecular weight (Mw) of between 2,000 kDa and 10,500 kDa. In an embodiment, the carrier protein of said serotype 21 saccharide glycoconjugate is CRM₁₉₇.

In a preferred embodiment, the serotype 21 glycoconjugate of the invention prepared by click chemistry, comprises a serotype 21 polysaccharide wherein the weight average molecular weight (Mw) of said polysaccharide before conjugation is between 150 kDa and 400 kDa and has a weight average molecular weight (Mw) of between 750 kDa and 7,500 kDa and even preferably of between 800 kDa and 7,000 kDa. In an embodiment, the carrier protein of said serotype 21 saccharide glycoconjugate is SCP.

In a preferred embodiment, the serotype 21 glycoconjugate of the invention prepared by direct reductive amination comprises a serotype 21 polysaccharide wherein the weight average molecular weight (Mw) of said polysaccharide before conjugation is between 100 kDa and 400 kDa and has a weight average molecular weight (Mw) of between 500 kDa and 15,000 kDa and even preferably of between 2,000 kDa and 10,500 kDa. In an embodiment, the carrier protein of said serotype 21 saccharide glycoconjugate is CRM₁₉₇.

Serotype 21 polysaccharide is O-acetylated and the total amount of O-acetylation is approximately one O-acetyl group per polysaccharide repeating unit. The degree of O-acetylation of the polysaccharide can be determined by any method known in the art, for example, by proton NMR or by ion-HPLC analysis (see for example Lemerminier *et al.* (1996) Carbohydrate Research 296:83-96; Jones *et al.* (2002) J. Pharmaceutical and Biomedical Analysis 30:1233-1247; Hestrin, S. (1949) J. Biol. Chem. 180:249-261).

In an embodiment, the serotype 21 glycoconjugate of the present invention comprises a serotype 21 saccharide comprising on average at least 0.5 O-acetyl group per saccharide repeating unit. In a preferred embodiment, the serotype 21 glycoconjugate of the present invention comprises a serotype 21 saccharide comprising on average at least 0.8 O-acetyl group per saccharide repeating unit.

In a preferred embodiment, the serotype 21 glycoconjugate of the present invention comprises a serotype 21 saccharide comprising on average between about 0.8 and about 1 O-acetyl group per saccharide repeating unit.

In an embodiment, the ratio of O-acetyl group per saccharide repeating unit in the glycoconjugate to O-acetyl group per saccharide repeating unit in the isolated polysaccharide is

at least 0.6. In a preferred embodiment, the ratio of O-acetyl group per saccharide repeating unit in the glycoconjugate to O-acetyl group per saccharide repeating unit in the isolated polysaccharide is at least 0.9.

Another way to characterize the serotype 21 glycoconjugates of the invention is by the
5 number of lysine residues in the carrier protein (e.g., CRM₁₉₇, SCP, DT or TT) that become conjugated to 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 polysaccharides, 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
10 recovered compared to the carrier protein starting material used to generate the conjugate materials. In a preferred embodiment, the degree of conjugation of the serotype 21 glycoconjugate of the invention is between 2 and 15. In an embodiment, the degree of conjugation of the serotype 21 glycoconjugate of the invention is between 2 and 10. In an embodiment, the degree of conjugation of the serotype 21 glycoconjugate of the invention is between 3 and 5. In
15 an embodiment, the degree of conjugation of the serotype 21 glycoconjugate of the invention is between 2 and 6. In an embodiment, the degree of conjugation of the serotype 21 glycoconjugate of the invention is between 4 and 10.

The serotype 21 glycoconjugates of the invention may also be characterized by the ratio (weight/weight) of saccharide to carrier protein. In some embodiments, the ratio of serotype 21
20 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
25 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 serotype 21 saccharide to carrier protein in the conjugate is between 0.7 and 1.1. In some such embodiments, the carrier protein is CRM₁₉₇.

30 In a preferred embodiment, for the serotype 21 glycoconjugate of the invention prepared by click chemistry, the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.1. In an embodiment, the carrier protein of said serotype 21 saccharide glycoconjugate is SCP.

In a preferred embodiment, for the serotype 21 glycoconjugate of the invention prepared by
35 direct reductive amination, the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.3. In an embodiment, the carrier protein of said serotype 21 saccharide glycoconjugate is CRM₁₉₇.

In a preferred embodiment, the serotype 21 glycoconjugate of the invention prepared by click chemistry, comprises a serotype 21 polysaccharide wherein the weight average molecular weight (Mw) of said polysaccharide before conjugation is between 150 kDa and 400 kDa and has a weight average molecular weight (Mw) of between 750 kDa and 7,500 kDa and even preferably of between 800 kDa and 7,000 kDa and the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.1. In an embodiment, the carrier protein of said serotype 21 saccharide glycoconjugate is SCP.

In a preferred embodiment, the serotype 21 glycoconjugate of the invention prepared by direct reductive amination comprises a serotype 21 polysaccharide wherein the weight average molecular weight (Mw) of said polysaccharide before conjugation is between 100 kDa and 400 kDa and has a weight average molecular weight (Mw) of between 500 kDa and 15,000 kDa and even preferably of between 2,000 kDa and 10,500 kDa and the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.3. In an embodiment, the carrier protein of said serotype 21 saccharide glycoconjugate is CRM₁₉₇.

The serotype 21 glycoconjugates and immunogenic compositions of the invention may contain free saccharide that is not conjugated to the carrier protein but is nevertheless 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 serotype 21 glycoconjugate comprises less than about 50% of free serotype 21 saccharide compared to the total amount of serotype 21 saccharide. In a preferred embodiment, the serotype 21 glycoconjugate comprises less than about 25% of free serotype 21 saccharide compared to the total amount of serotype 21 saccharide. In an even preferred embodiment, the serotype 21 glycoconjugate comprises less than about 20% of free serotype 21 saccharide compared to the total amount of serotype 21 saccharide. In a yet preferred embodiment, the serotype 21 glycoconjugate comprises less than about 15% of free serotype 21 saccharide compared to the total amount of serotype 21 saccharide.

In a preferred embodiment, the serotype 21 glycoconjugate of the invention prepared by click chemistry, comprises a serotype 21 polysaccharide wherein the weight average molecular weight (Mw) of said polysaccharide before conjugation is between 150 kDa and 400 kDa and has a weight average molecular weight (Mw) of between 750 kDa and 7,500 kDa and even preferably of between 800 kDa and 7,000 kDa, wherein the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.1 and wherein the serotype 21 glycoconjugate comprises less than about 25% of free serotype 21 saccharide compared to the total amount of serotype 21 saccharide. In an embodiment, the carrier protein of said serotype 21 saccharide glycoconjugate is SCP.

In a preferred embodiment, the serotype 21 glycoconjugate of the invention prepared by direct reductive amination comprises a serotype 21 polysaccharide wherein the weight average molecular weight (Mw) of said polysaccharide before conjugation is between 100 kDa and 400

kDa and has a weight average molecular weight (Mw) of between 500 kDa and 15,000 kDa and even preferably of between 2,000 kDa and 10,500 kDa, wherein the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.3 and wherein the serotype 21 glycoconjugate comprises less than about 25% of free serotype 21 saccharide compared to the total amount of serotype 21 saccharide. In an embodiment, the carrier protein of said serotype 21 saccharide glycoconjugate is CRM₁₉₇.

In a preferred embodiment, the serotype 21 glycoconjugate of the invention prepared by click chemistry, comprises a serotype 21 polysaccharide wherein the weight average molecular weight (Mw) of said polysaccharide before conjugation is between 150 kDa and 400 kDa and has a weight average molecular weight (Mw) of between 750 kDa and 7,500 kDa and even preferably of between 800 kDa and 7,000 kDa, wherein the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.1, wherein the degree of conjugation of said serotype 21 glycoconjugate is between 2 and 10 and wherein the serotype 21 glycoconjugate comprises less than about 25% of free serotype 21 saccharide compared to the total amount of serotype 21 saccharide. In an embodiment, the carrier protein of said serotype 21 saccharide glycoconjugate is SCP.

In a preferred embodiment, the serotype 21 glycoconjugate of the invention prepared by direct reductive amination comprises a serotype 21 polysaccharide wherein the weight average molecular weight (Mw) of said polysaccharide before conjugation is between 100 kDa and 400 kDa and has a weight average molecular weight (Mw) of between 500 kDa and 15,000 kDa and even preferably of between 2,000 kDa and 10,500 kDa, wherein the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.3, wherein the degree of conjugation of said serotype 21 glycoconjugate is between 2 and 10 and wherein the serotype 21 glycoconjugate comprises less than about 25% of free serotype 21 saccharide compared to the total amount of serotype 21 saccharide. In an embodiment, the carrier protein of said serotype 21 saccharide glycoconjugate is CRM₁₉₇.

The serotype 21 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 serotype 21 glycoconjugate 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

has a K_d below or equal to 0.3 in a CL-4B column. In a preferred embodiment, at least 60% of the serotype 21 glycoconjugate 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 serotype 21 glycoconjugate 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 serotype 21 glycoconjugate has a K_d below or equal to 0.3 in a CL-4B column.

In a preferred embodiment, the serotype 21 glycoconjugate of the invention prepared by click chemistry, comprises a serotype 21 polysaccharide wherein the weight average molecular weight (Mw) of said polysaccharide before conjugation is between 150 kDa and 400 kDa and has a weight average molecular weight (Mw) of between 750 kDa and 7,500 kDa and even preferably of between 800 kDa and 7,000 kDa, wherein the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.1, wherein the serotype 21 glycoconjugate comprises less than about 25% of free serotype 21 saccharide compared to the total amount of serotype 21 saccharide and wherein between 65% and 80% of the serotype 21 glycoconjugate has a K_d below or equal to 0.3 in a CL-4B column. In an embodiment, the carrier protein of said serotype 21 saccharide glycoconjugate is SCP.

In a preferred embodiment, the serotype 21 glycoconjugate of the invention prepared by direct reductive amination comprises a serotype 21 polysaccharide wherein the weight average molecular weight (Mw) of said polysaccharide before conjugation is between 100 kDa and 400 kDa and has a weight average molecular weight (Mw) of between 500 kDa and 15,000 kDa and even preferably of between 2,000 kDa and 10,500 kDa, wherein the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.3, wherein the serotype 21 glycoconjugate comprises less than about 25% of free serotype 21 saccharide compared to the total amount of serotype 21 saccharide and wherein between 65% and 80% of the serotype 21 glycoconjugate has a K_d below or equal to 0.3 in a CL-4B column. In an embodiment, the carrier protein of said serotype 21 saccharide glycoconjugate is CRM₁₉₇.

In a preferred embodiment, the serotype 21 glycoconjugate of the invention prepared by click chemistry, comprises a serotype 21 polysaccharide wherein the weight average molecular weight (Mw) of said polysaccharide before conjugation is between 150 kDa and 400 kDa and has a weight average molecular weight (Mw) of between 750 kDa and 7,500 kDa and even preferably of between 800 kDa and 7,000 kDa, wherein the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.1, wherein the degree of conjugation of said serotype 21 glycoconjugate is between 2 and 10, wherein the serotype 21 glycoconjugate comprises less than about 25% of free serotype 21 saccharide compared to the total amount of serotype 21 saccharide and wherein between 65% and 80% of the serotype 21 glycoconjugate has a K_d below or equal to 0.3 in a CL-4B column. In an embodiment, the carrier protein of said serotype 21 saccharide glycoconjugate is SCP.

In a preferred embodiment, the serotype 21 glycoconjugate of the invention prepared by direct reductive amination comprises a serotype 21 polysaccharide wherein the weight average

molecular weight (Mw) of said polysaccharide before conjugation is between 100 kDa and 400 kDa and has a weight average molecular weight (Mw) of between 500 kDa and 15,000 kDa and even preferably of between 2,000 kDa and 10,500 kDa, wherein the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.3, wherein the degree of conjugation of said serotype 21 glycoconjugate is between 2 and 10, wherein the serotype 21 glycoconjugate comprises less than about 25% of free serotype 21 saccharide compared to the total amount of serotype 21 saccharide and wherein between 65% and 80% of the serotype 21 glycoconjugate has a K_d below or equal to 0.3 in a CL-4B column. In an embodiment, the carrier protein of said serotype 21 saccharide glycoconjugate is CRM₁₉₇.

10 2.2 Mode of preparation of the *Streptococcus pneumoniae* serotype 21 glycoconjugates of the invention

The serotype 21 glycoconjugate of the present invention can be prepared by any coupling technique known to those of ordinary skill in the art.

15 In an embodiment, the serotype 21 saccharide is coupled to the carrier protein via non-covalent bonds (see e.g. WO2012155007, WO2020056202).

In an embodiment, the serotype 21 saccharide is conjugated via covalent bonds. In one embodiment the capsular saccharide is conjugated directly to a carrier protein. In a second embodiment the capsular saccharide is conjugated to a carrier protein through a spacer/linker.

20 In an embodiment, the serotype 21 glycoconjugate of the present invention is conjugated to the carrier protein via a linker, for instance a bifunctional linker. The linker is optionally heterobifunctional or homobifunctional, having for example a reactive amino group and a reactive carboxylic acid group, two reactive amino groups or two reactive carboxylic acid groups. The linker has for example between 4 and 20, 4 and 12, 5 and 10 carbon atoms.

25 A possible linker is adipic acid dihydrazide (ADH). Other linkers include B-propionamide (WO 00/10599), nitrophenyl-ethylamine (Gever et al (1979) Med. Microbiol. Immunol. 165; 171-288), haloalkyl halides (US4057685), glycosidic linkages (US4673574, US4808700), hexane diamine and 6-aminocaproic acid (US4459286).

In an embodiment, the serotype 21 glycoconjugate of the present invention is conjugated directly to the carrier protein (without a linker).

30 In general the following types of chemical groups on a protein carrier can be used for coupling / conjugation:

1) Amino group (for instance via lysine). In one embodiment this group is linked to carboxyl groups on saccharides directly or to a carboxyl group on a linker with carbodiimide chemistry e.g. with EDAC (1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide). In another embodiment this group is linked to hydroxyl groups activated with CDAP or CNBr on saccharides directly or to such groups on a linker; to saccharides or linkers having an aldehyde group; to saccharides or linkers having a succinimide ester group.

2) Carboxyl (for instance via aspartic acid or glutamic acid). In one embodiment this group is linked to amino groups on saccharides directly or to an amino group on a linker with carbodiimide chemistry e.g. with EDAC.

3) Sulphydryl (for instance via cysteine). In one embodiment this group is linked to a bromo or chloro acetylated saccharide or linker with maleimide chemistry. In one embodiment this group is activated/modified with bis diazobenzidine.

4) Hydroxyl group (for instance via tyrosine). In one embodiment this group is activated/modified with bis diazobenzidine.

5) Imidazolyl group (for instance via histidine). In one embodiment this group is activated/modified with bis diazobenzidine.

6) Guanidyl group (for instance via arginine).

7) Indolyl group (for instance via tryptophan).

On the serotype 21 saccharide, in general the following groups can be used for a coupling: OH, COOH or NH₂. Aldehyde groups can be generated after different treatments known in the art such as: periodate, acid hydrolysis, hydrogen peroxide, etc.

In an embodiment, the serotype 21 glycoconjugate of the present invention is prepared using CDAP chemistry. In said embodiment, the serotype 21 saccharide is activated with 1-cyano-4-dimethylamino pyridinium tetrafluoroborate (CDAP) to form a cyanate ester. The activated saccharide can then be coupled directly or via a spacer (linker) group to an amino group on the carrier protein. For example, the spacer could be cystamine or cysteamine to give a thiolated polysaccharide which can be coupled to the carrier via a thioether linkage obtained after reaction with a maleimide-activated carrier protein (for example using N-[γ-maleimidobutyroxy]succinimide ester (GMBS)) or a haloacetylated carrier protein (for example using iodoacetamide, N-succinimidyl bromoacetate (SBA; SIB), N-succinimidyl(4-iodoacetyl)aminobenzoate (SIAB), sulfosuccinimidyl(4-iodoacetyl)aminobenzoate (sulfo-SIAB), N-succinimidyl iodoacetate (SIA), or succinimidyl 3-[bromoacetamido]propionate (SBAP)).

In a preferred embodiment, the cyanate ester of the activated saccharide is coupled with hexane diamine or adipic acid dihydrazide (ADH) and the amino-derivatised saccharide is conjugated to the carrier protein using carbodiimide (e.g., EDAC or EDC) chemistry via a carboxyl group on the protein carrier. Such conjugates are described for example in WO 93/15760, WO 95/08348 and WO 96/129094.

In an embodiment, the serotype 21 glycoconjugate of the present invention is prepared using carbodiimides, hydrazides, active esters, norborane, p-nitrobenzoic acid, N-hydroxysuccinimide, S--NHS, EDC, TSTU. Many are described in International Patent Application Publication No. WO 98/42721. Conjugation may involve a carbonyl linker which may be formed by reaction of a free hydroxyl group of the saccharide with CDI (see Bethell et al. (1979) 1. Biol. Chern. 254:2572-2574; Hearn et al. (1981) J. Chromatogr. 218:509-518) followed by reaction with a protein to form a carbamate linkage. This may involve reduction of the anomeric terminus to a

primary hydroxyl group, optional protection/deprotection of the primary hydroxyl group, reaction of the primary hydroxyl group with CDI to form a CDI carbamate intermediate and coupling the CDI carbamate intermediate with an amino group on a protein.

Direct reductive amination

5 In an embodiment, the serotype 21 glycoconjugate of the present invention is prepared by direct reductive amination (see e.g. US 4365170, US 4673574, WO2006/110381, WO2008/079653, WO2008/143709, WO2008/079732, WO2011/110531, WO2012/119972, WO2015110941, WO2015110940, WO2018/144439, WO2018/156491).

According to the present invention, reductive amination involves two steps, (1) oxidation
10 (activation) of the serotype 21 purified saccharide, (2) reduction of the activated saccharide and the carrier protein (e.g., CRM₁₉₇, TT or SCP) to form a glycoconjugate.

As mentioned above, before oxidation, sizing of the serotype 21 saccharide to a target molecular weight (MW) range can be performed. Therefore, in an embodiment, the isolated polysaccharide is sized before oxidation.

15 In an embodiment, the serotype 21 saccharide of the invention is conjugated to a carrier protein by a process comprising the step of:

- (a) reacting said serotype 21 saccharide with an oxidizing agent;
- (b) compounding the activated saccharide of step (a) with a carrier protein; and
- (c) reacting the compounded activated saccharide and carrier protein with a reducing agent to
20 form a glycoconjugate.

In an embodiment, the serotype 21 saccharide of the invention is conjugated to a carrier protein by a process comprising the step of:

- (a) reacting said serotype 21 saccharide with an oxidizing agent;
- (a') quenching the oxidation reaction by addition of a quenching agent;
- 25 (b) compounding the activated saccharide of step (a') with a carrier protein; and
- (c) reacting the compounded activated saccharide and carrier protein with a reducing agent to form a glycoconjugate.

Following the oxidation step (a) the saccharide is said to be activated and is referred to as "activated saccharide".

30 In an embodiment, the oxidizing agent is any oxidizing agent which oxidizes a terminal hydroxyl group to an aldehyde. In an embodiment, the oxidizing agent is periodate. For the purpose of the present invention, the term "periodate" includes both periodate and periodic acid; the term also includes both metaperiodate (IO₄⁻) and orthoperiodate (IO₆⁵⁻) and the various salts of periodate (e.g., sodium periodate and potassium periodate).

35 In an embodiment, the oxidizing agent is periodate in the presence of bivalent cations (see WO2008/143709).

In an embodiment, the oxidizing agent is periodic acid. In an embodiment, the oxidizing agent is periodic acid in the presence of bivalent cations. In an embodiment, the oxidizing agent

is periodic acid in the presence of Mg^{2+} . In an embodiment, the oxidizing agent is periodic acid in the presence of Ca^{2+} . In an embodiment, the oxidizing agent is orthoperiodate.

In a preferred embodiment, the oxidizing agent is sodium periodate. In an embodiment, the periodate used for the oxidation is metaperiodate. In an embodiment the periodate used for the oxidation is sodium metaperiodate.

When a polysaccharide reacts with periodate, periodate oxidises vicinal hydroxyl groups to form carbonyl or aldehyde groups and causes cleavage of a C-C bond. For this reason, the term "reacting a polysaccharide with periodate" includes oxidation of vicinal hydroxyl groups by periodate.

In one embodiment step a) comprises reacting the polysaccharide with 0.01-2 molar equivalents of periodate. In one embodiment step a) comprises reacting the polysaccharide with 0.1-1.0 molar equivalents of periodate. In one embodiment step a) comprises reacting the polysaccharide with 0.1-0.5 molar equivalents of periodate.

In an embodiment, the oxidizing agent is a mixture of a stable nitroxyl radical compound with an oxidant (see WO2014097099).

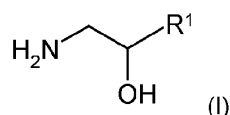
In an aspect, said stable nitroxyl radical compound is a molecule bearing a TEMPO or a PROXYL (2,2,5,5-tetramethyl-1-pyrrolidinyloxy) moiety. Preferably said molecule has the ability to selectively oxidize primary alcohol in the presence of an oxidant, to generate aldehyde groups, without affecting secondary hydroxyl groups. More preferably said molecule has the ability to selectively oxidize primary alcohol in the presence of an oxidant, to generate aldehyde groups, without over oxidation to carboxyl groups. In an aspect, said stable nitroxyl radical compound is TEMPO, 2,2,6,6-Tetramethyl-4-(methylsulfonyloxy)-1-piperidinoxy, 4-Phosphonooxy-TEMPO, 4-Oxo-TEMPO, 4-Methoxy-TEMPO, 4-Isothiocyanato-TEMPO, 4-(2-Iodoacetamido)-TEMPO free radical, 4-Hydroxy-TEMPO, 4-Cyano-TEMPO, 4-Carboxy-TEMPO, 4-(2-Bromoacetamido)-TEMPO or 4-Amino-TEMPO, 4-Acetamido-2,2,6,6-tetramethylpiperidine 1-oxyl. Preferably said stable nitroxyl radical compound is TEMPO. In an aspect, said stable nitroxyl radical compound is selected from the groups consisting of TEMPO, 2,2,6,6-Tetramethyl-4-(methylsulfonyloxy)-1-piperidinoxy, 4-Phosphonooxy-TEMPO, 4-Oxo-TEMPO, 4-Methoxy-TEMPO, 4-Isothiocyanato-TEMPO, 4-(2-Iodoacetamido)-TEMPO free radical, 4-Hydroxy-TEMPO, 4-Cyano-TEMPO, 4-Carboxy-TEMPO, 4-(2-Bromoacetamido)-TEMPO, 4-Amino-TEMPO, 4-Acetamido-2,2,6,6-tetramethylpiperidine 1-oxyl. Preferably said stable nitroxyl radical compound is TEMPO. In a further aspect, said stable nitroxyl radical compound is 3β -DOXYL-5 α -cholestane, 5-DOXYL-stearic acid, 16-DOXYL-stearic acid, Methyl 5-DOXYL-stearate, 3-(Aminomethyl)-PROXYL, 3-Carbamoyl-PROXYL, 3-Carbamoyl-2,2,5,5-tetramethyl-3-pyrrolin-1-oxyl, 3-Carboxy-PROXYL or 3-Cyano-PROXYL. In a further aspect, said stable nitroxyl radical compound is selected from the groups consisting of 3β -DOXYL-5 α -cholestane, 5-DOXYL-stearic acid, 16-DOXYL-stearic acid, Methyl 5-DOXYL-stearate, 3-(Aminomethyl)-PROXYL, 3-Carbamoyl-PROXYL, 3-Carbamoyl-2,2,5,5-tetramethyl-3-pyrrolin-1-oxyl, 3-Carboxy-PROXYL, 3-Cyano-PROXYL.

In an aspect, the oxidant is a molecule bearing a N-halo moiety. Preferably said molecule has the ability to selectively oxidize primary alcohol in the presence of a nitroxyl radical compound. In an aspect, said oxidant is N-Chlorosuccinimide, N-Bromosuccinimide, N-Iodosuccinimide, Dichloroisocyanuric acid, 1,3,5-trichloro-1,3,5-triazinane-2,4,6-trione, Dibromoisocyanuric acid, 1,3,5-tribromo-1,3,5-triazinane-2,4,6-trione, Diiodoisocyanuric acid or 1,3,5-triiodo-1,3,5-triazinane-2,4,6-trione. In an aspect, said oxidant is selected from the group consisting of N-Chlorosuccinimide, N-Bromosuccinimide, N-Iodosuccinimide, Dichloroisocyanuric acid, 1,3,5-trichloro-1,3,5-triazinane-2,4,6-trione, Dibromoisocyanuric acid, 1,3,5-tribromo-1,3,5-triazinane-2,4,6-trione, Diiodoisocyanuric acid and 1,3,5-triiodo-1,3,5-triazinane-2,4,6-trione. Preferably said oxidant is N-Chlorosuccinimide.

In an aspect, said stable nitroxyl radical compound is 2,2,6,6-Tetramethyl-1-piperidinyloxy free radical (TEMPO) and said oxidant is N-Chlorosuccinimide (NCS).

In one embodiment, the quenching agent of step a') is selected from vicinal diols, 1,2-aminoalcohols, amino acids, glutathione, sulfite, bisulfate, dithionite, metabisulfite, thiosulfate, phosphites, hypophosphites or phosphorous acid.

In one embodiment, the quenching agent is a 1,2-aminoalcohols of formula (I):



wherein R¹ is selected from H, methyl, ethyl, propyl or isopropyl.

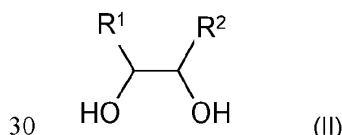
In one embodiment, the quenching agent is selected from sodium and potassium salts of sulfite, bisulfate, dithionite, metabisulfite, thiosulfate, phosphites, hypophosphites or phosphorous acid.

In one embodiment, the quenching agent is an amino acid. In such embodiments, said amino acid may be selected from serine, threonine, cysteine, cystine, methionine, proline, hydroxyproline, tryptophan, tyrosine, and histidine.

In one embodiment, the quenching agent is a sulfite such as bisulfate, dithionite, metabisulfite, thiosulfate.

In one embodiment, the quenching agent is a compound comprising two vicinal hydroxyl groups (vicinal diols), i.e., two hydroxyl groups covalently linked to two adjacent carbon atoms.

Preferably, the quenching agent is a compound of formula (II):



wherein R¹ and R² are each independently selected from H, methyl, ethyl, propyl or isopropyl.

In a preferred embodiment, the quenching agent is glycerol, ethylene glycol, propan-1,2-diol, butan-1,2-diol or butan-2,3-diol, or ascorbic acid. In an even preferred embodiment, the quenching agent is butan-2,3-diol.

In a preferred embodiment the degree of oxidation (also named "degree of activation" in the present document) of the activated serotype 21 saccharide is between 2 and 30. In an embodiment the degree of oxidation (DO) of the activated serotype 21 polysaccharide is between 10 and 25.

In a very preferred embodiment the degree of oxidation of the activated serotype 21 saccharide is between 10 and 21.

In one embodiment the activated saccharide and the carrier protein are lyophilised before step b).

In an embodiment the initial input ratio (weight by weight) of activated serotype 21 saccharide to carrier protein at step b) is between 4:1 and 0.1:1. In an embodiment the initial input ratio (weight by weight) of activated serotype 21 saccharide to carrier protein at step b) is between 1.5:1 and 0.5:1.

In an embodiment, the reduction reaction (c) is carried out in aqueous solvent. In another embodiment, the reduction reaction (c) is carried out in aprotic solvent.

In an embodiment, the reduction reaction (c) is carried out in the presence of dimethylsulphoxide (DMSO) or dimethylformamide (DMF). In an embodiment, the reduction reaction (c) is carried out in the presence of dimethylformamide (DMF). In an embodiment, the reduction reaction (c) is carried out in the presence of dimethylsulphoxide (DMSO).

In one embodiment the reduction reaction (c) is carried out in a solution consisting essentially of dimethylsulphoxide (DMSO) or dimethylformamide (DMF). In one embodiment the reduction reaction (c) is carried out in a solution consisting essentially of dimethylformamide (DMF). In one embodiment the reduction reaction (c) is carried out in a solution consisting essentially of dimethylsulphoxide (DMSO).

In an embodiment, the reduction reaction (c) is carried out in DMSO (dimethylsulfoxide) or in DMF (dimethylformamide) solvent. In an embodiment, the reduction reaction (c) is carried out in DMSO (dimethylsulfoxide) solvent.

In an embodiment, the reducing agent is sodium cyanoborohydride, sodium triacetoxyborohydride, sodium or zinc borohydride in the presence of Bronsted or Lewis acids, amine boranes such as pyridine borane, 2-Picoline Borane, 2,6-diborane-methanol, dimethylamine-borane, t-BuMePrN-BH₃, benzylamine-BH₃ or 5-ethyl-2-methylpyridine borane (PEMB). In an embodiment, the reducing agent is sodium triacetoxyborohydride. In a preferred embodiment, the reducing agent is sodium cyanoborohydride. In an embodiment, the reducing agent is sodium cyanoborohydride in the present of nickel (see WO2018144439).

In one embodiment between 0.2 and 20 molar equivalents of reducing agent is used at step c). In one embodiment between 0.5 and 10 molar equivalents of reducing agent is used at

step c). In one embodiment between 1.0 and 5 molar equivalents of reducing agent is used at step c).

At the end of the reduction reaction, there may be unreacted aldehyde groups remaining in the conjugates, these may be capped using a suitable capping agent. In one embodiment this capping agent is sodium borohydride (NaBH₄). In an embodiment capping is achieved by mixing the product of step c) with 1 to 20 molar equivalents of sodium borohydride. In an embodiment capping is achieved by mixing the product of step c) with 1 to 10 molar equivalents of sodium borohydride. In an embodiment capping is achieved by mixing the product of step c) with 1 to 5 molar equivalents of sodium borohydride.

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.

CDI and/or CDT chemistry

In an embodiment, the serotype 21 glycoconjugate of the present invention is prepared by CDI and/or CDT chemistry as disclosed in WO2022249107.

CDI and/or CDT chemistry involves two steps, (1) reacting the serotype 21 saccharide with CDI and/or CDT in an aprotic solvent to produce an activated saccharide (activation), (2) reacting the activated saccharide with a carrier protein (e.g. CRM₁₉₇, TT or SCP) to form a glycoconjugate.

In an embodiment, the activating agent of step (1) is 1,1'-carbonyldiimidazole (CDI). In an embodiment, the activating agent of step (1) is 1,1'-Carbonyl-di-(1,2,4-triazole) (CDT).

As mentioned above, before activation with CDI and/or CDT, sizing of the serotype 21 saccharide to a target molecular weight (MW) range can be performed.

Therefore, in an embodiment, the serotype 21 saccharide is sized before activation with CDI. In an embodiment, the isolated polysaccharide is sized before activation with CDT. In an embodiment, the serotype 21 saccharide is sized to any of the target molecular weight (MW) range defined above.

Therefore, in an embodiment, the serotype 21 saccharide is conjugated to a carrier protein by a process comprising the step of:

(a) reacting said isolated polysaccharide with CDI and/or CDT in an aprotic solvent;

(b) reacting the activated polysaccharide of step (a) with a carrier protein in an aprotic solvent to form a glycoconjugate.

Following step (a) the polysaccharide is said to be activated and is referred to as "activated polysaccharide".

In one embodiment step a) comprises reacting the serotype 21 saccharide with CDI.

In one embodiment step a) comprises reacting the serotype 21 saccharide with an amount of CDI that is between 0.5-10 molar equivalent to the amount of serotype 21 saccharide present in the reaction mixture.

5 In one embodiment step a) comprises reacting the serotype 21 saccharide with CDT.

In one embodiment step a) comprises reacting the serotype 21 saccharide with an amount of CDT that is between 0.5-10 molar equivalent to the amount of serotype 21 saccharide present in the reaction mixture.

10 In an embodiment, the activating reaction a) is carried out in the presence of dimethylsulphoxide (DMSO), dimethylformamide (DMF), dimethylacetamide, N-methyl-2-pyrrolidone or hexamethylphosphoramide (HMPA). In an embodiment, the activating reaction a) is carried out in the presence of dimethylsulphoxide (DMSO).

In one embodiment the activating reaction a) is carried out in a solution consisting essentially of dimethylsulphoxide (DMSO) or dimethylformamide (DMF). In one embodiment the activating reaction a) is carried out in a solution consisting essentially of dimethylsulphoxide (DMSO).

15 In an embodiment, the conjugation reaction b) is carried out in the presence of dimethylsulphoxide (DMSO), dimethylformamide (DMF), dimethylacetamide, N-methyl-2-pyrrolidone or hexamethylphosphoramide (HMPA).

20 In an embodiment, the conjugation reaction b) is carried out in the presence of dimethylsulphoxide (DMSO).

In one embodiment the conjugation reaction b) is carried out in a solution consisting essentially of dimethylsulphoxide (DMSO) or dimethylformamide (DMF). In one embodiment the conjugation reaction b) is carried out in a solution consisting essentially of dimethylsulphoxide (DMSO).

25 In one embodiment, weak organic base can be added to the reaction mixture after the activating reaction a) but before the conjugation reaction b). The weak organic base can be added before or after the carrier protein is introduced to the reaction mixture. Therefore, in one embodiment, the weak organic base is added to the reaction mixture before the carrier protein is introduced. In another embodiment, the weak organic base is added to the reaction mixture after the carrier protein is introduced. Weak organic base can be selected from alkanamines, imidazole, triazole, pyridine, histidine and guanidine. Alkanamines include alkyl primary amines such as methyl amine, ethylamine, propylamine, isopropylamine; alkyl secondary amines such as dimethyl amine, diethylamine, dipropylamine, diisopropylamine; alkyl tertiary amines such as trimethyl amine, triethylamine, tri-isopropylamine, di-N,N'-isopropylethylamine, et al. In an embodiment, the weak organic base is an alkanamine. In an embodiment, the weak organic base is an imidazole. In an embodiment, the weak organic base is a triazole. In an embodiment, the

weak organic base is pyridine. In an embodiment, the weak organic base is histidine. In an embodiment, the weak organic base is guanidine.

In one embodiment following the conjugation reaction b) unconjugated reactive sites of the activated polysaccharide are hydrolyzed. In one embodiment unconjugated reactive sites are hydrolyzed by addition to the conjugation solution of an aqueous solution. In one embodiment unconjugated reactive sites are hydrolyzed by addition to the conjugation solution of an aqueous buffered solution. In one embodiment unconjugated reactive sites are hydrolyzed by addition to the conjugation solution of an aqueous buffered solution and adjustment of the pH to between about 3.0 to about 10.0. In one embodiment unconjugated reactive sites are hydrolyzed by addition to the conjugation solution of an aqueous buffered solution and adjustment of the pH to between about 7.0 to about 10.0. In one embodiment unconjugated reactive sites are hydrolyzed by addition to the conjugation solution of an aqueous buffered solution and adjustment of the pH to between about 3.0 to about 7.0. In one embodiment unconjugated reactive sites are hydrolyzed by addition to the conjugation solution of an aqueous buffered solution and adjustment of the pH to about 4.0. In one embodiment unconjugated reactive sites are hydrolyzed by addition to the conjugation solution of an aqueous buffered solution and adjustment of the pH to about 9.0.

eTEC chemistry

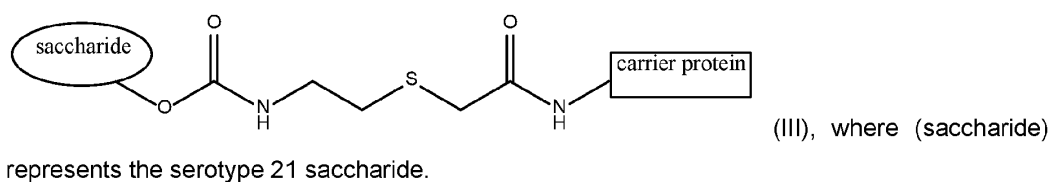
In an embodiment, the serotype 21 glycoconjugate of the present invention is prepared by eTEC chemistry as disclosed WO2014027302.

The eTEC spacer includes seven linear atoms (i.e., $-\text{C}(\text{O})\text{NH}(\text{CH}_2)_2\text{SCH}_2\text{C}(\text{O})-$) and provides stable thioether and amide bonds between the saccharide and carrier protein. Synthesis of the eTEC linked glycoconjugate involves reaction of an activated hydroxyl group of the saccharide with the amino group of a thioalkylamine reagent, e.g., cystamine or cysteamine or a salt thereof, forming a carbamate linkage to the saccharide to provide a thiolated saccharide. Generation of one or more free sulfhydryl groups is accomplished by reaction with a reducing agent to provide an activated thiolated saccharide. Reaction of the free sulfhydryl groups of the activated thiolated saccharide with an activated carrier protein having one or more α -haloacetamide groups on amine containing residues generates a thioether bond to form the conjugate, wherein the carrier protein is attached to the eTEC spacer through an amide bond.

Therefore, in an embodiment, the serotype 21 glycoconjugate of the present invention comprises a serotype 21 saccharide covalently conjugated to a carrier protein through a (2-((2-oxoethyl)thio)ethyl)carbamate (eTEC) spacer.

In an embodiment, the serotype 21 glycoconjugate of the present invention comprises a serotype 21 saccharide conjugated to a carrier protein through a (2-((2-oxoethyl)thio)ethyl)carbamate (eTEC) spacer, wherein the saccharide is covalently linked to the eTEC spacer through a carbamate linkage, and wherein the carrier protein is covalently linked to the eTEC spacer through an amide linkage.

The eTEC linked glycoconjugates of the invention may be represented by the general formula (III):

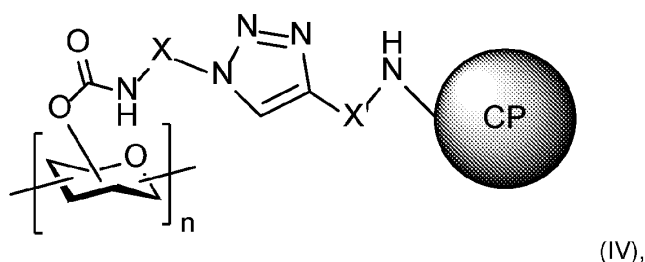


- 5 Formula (III) is a schematic representation of glycoconjugates of the invention. It should not be understood that only one linkage is present between the saccharide and the carrier protein. Rather, an individual carrier protein (CP) molecule may be linked to more than one serotype 21 saccharide molecule and an individual saccharide molecule can be linked to more than one individual carrier protein (CP) molecule. Additionally, a majority of the saccharide repeating unit
10 remains unmodified and covalent linkages between the carrier protein and the saccharide is for a minority of the saccharide repeat units.

Click chemistry

In an embodiment, the serotype 21 glycoconjugate of the present invention is prepared by click chemistry (see e.g. PCT/IB2023/050202).

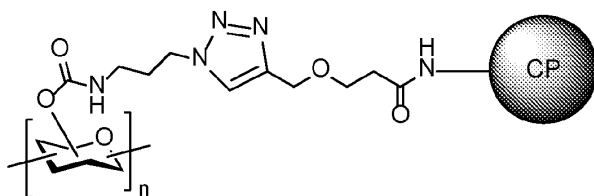
- 15 Therefore, in an embodiment, the serotype 21 glycoconjugate of the present invention comprises a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (IV):



- wherein X is selected from the group consisting of $\text{CH}_2(\text{CH}_2)_{n'}$, $(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$,
20 $\text{NHCO}(\text{CH}_2)_{n'}$, $\text{NHCO}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$, $\text{OCH}_2(\text{CH}_2)_{n'}$ 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,
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,
25 wherein the structure in square bracket represents a repeat unit of the serotype 21 saccharide and wherein n represents the number of repeating units.

- In a particular aspect, the invention is directed to a serotype 21 glycoconjugate comprising a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (IV), wherein X is $\text{CH}_2(\text{CH}_2)_{n'}$, where n' is 2 and wherein X' is
30 $\text{CH}_2\text{O}(\text{CH}_2)_{n''}\text{CH}_2\text{C}=\text{O}$ where n'' is 1.

In a particular aspect, the invention pertains to a serotype 21 glycoconjugate comprising a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (V),



(V)

- 5 wherein the structure in square bracket represents a repeat unit of the serotype 21 saccharide and wherein n represents the number of repeating units.

Formulas (IV), (V) are schematic representations 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
 10 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 serotype 21 saccharide.

- 15 In an embodiment, the invention provides a serotype 21 glycoconjugate comprising a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (IV), wherein X is $\text{CH}_2(\text{CH}_2)_{n'}$, 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
 20 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
 25 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
 30 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.
- 10 In an embodiment, the invention provides a serotype 21 glycoconjugate comprising a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (IV), wherein X is $\text{CH}_2(\text{CH}_2)_{n'}$, where n' is selected from 1 to 10 and wherein $\text{CH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_{m'}(\text{CH}_2)_{n''}\text{CH}_2\text{C}=\text{O}$, where n'' is selected from 0 to 10 and m' is selected from 0 to 4.
- 15 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.
- 20 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.
- 25 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.
- 30 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.
- 35 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.

10 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.

20 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.

25 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.

35 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.

5 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.

10 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.

15 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.

20 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.

25 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.

30 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.

35 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.

In an embodiment, the invention provides a serotype 21 glycoconjugate comprising a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and

- having the general formula (IV), 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.
- 20 In an embodiment, the invention provides a serotype 21 glycoconjugate comprising a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (IV), 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.
- 25 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.
- 30 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.
- 35 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.

10 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.

15 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.

25 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.

30 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.

10 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.

25 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.

In an embodiment, the invention provides a serotype 21 glycoconjugate comprising a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (IV), 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.

In an embodiment, the invention provides a serotype 21 glycoconjugate comprising a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (IV), 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.

10 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.

20 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.

25 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.

35 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 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.

10 In an embodiment, the invention provides a serotype 21 glycoconjugate comprising a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (IV), 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.

30 In an embodiment, the invention provides a serotype 21 glycoconjugate comprising a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (IV), 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.

35 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.

10 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.

15 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.

25 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.

30 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.

In an embodiment, the invention provides a serotype 21 glycoconjugate comprising a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and

having the general formula (IV), 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.

In an embodiment, the invention provides a serotype 21 glycoconjugate comprising a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (IV), 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.

10 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.

20 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.

25 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.

35 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.

10 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.

20 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.

25 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.

35 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.

10 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.

15 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.

20 In an embodiment, the invention provides a serotype 21 glycoconjugate comprising a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (IV), 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.

In an embodiment, the invention provides a serotype 21 glycoconjugate comprising a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and
5 having the general formula (IV), 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
10 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
15 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
20 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
25 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
30 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,
35 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.

10 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.

20 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.

25 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.

10 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.

In a very preferred embodiment of the present invention, the serotype 21 glycoconjugate of the present invention are prepared using click chemistry. The invention also relates to a method
15 of making serotype 21 glycoconjugate, as disclosed herein above.

In an embodiment, click chemistry may comprise three steps, (a) reacting an isolated serotype 21 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
20 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⁺ 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
25 "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 may be performed. Therefore, in an embodiment, the isolated serotype 21 saccharide is sized before activation with a carbonic acid derivative and an azido
30 linker. In an embodiment, the isolated serotype 21 saccharide is sized to any of the target molecular weight (MW) range defined above. In an embodiment, the isolated serotype 21 saccharide is not sized before activation with a carbonic acid derivative and an azido linker.

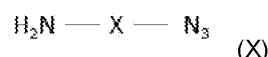
In an embodiment, said carbonic acid derivative is selected from the group consisting of 1,1'-carbonyldiimidazole (CDI), 1,1'-carbonyl-di-(1,2,4-triazole) (CDT), N,N'-Disuccinimidyl carbonate (DSC) and N-hydroxysuccinimidyl chloroformate.
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In an embodiment,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

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



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 (X), 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 (X), 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 (X), 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 (X), 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 (X), 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 (X), 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 (XI),

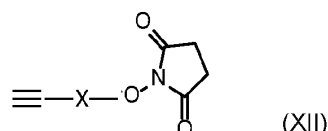


In a preferred embodiment, said azido linker is 3-azido-propylamine.

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 (XII),



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 (XII), 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.

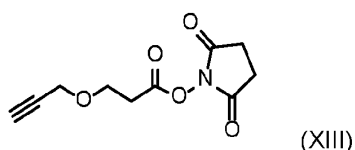
In an embodiment, said agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group is a compound of formula (XII), wherein X is $CH_2O(CH_2CH_2O)_m(CH_2)_nCH_2C=O$,

- where n is selected from 0 to 10 and m is selected from 0 to 4. In an embodiment, 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, 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 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.
- 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 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 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 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 (XIII):



10

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 0.1-10 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 0.5-5 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 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.

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 0.1-5 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 0.5-2 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 0.01 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 0.1 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 of about 0.2 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 of about 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 of about 1 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 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 of about 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 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.

30 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).

35 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).

5 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).

10 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 0.5% (v/v) water. 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 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 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 isolated saccharide is reacted with CDI in an aprotic solvent comprising about 0.9% (v/v) water.

35 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 0.5% (v/v) water. In one embodiment the isolated saccharide is reacted with CDI in DMSO comprising 0.1% to 0.2% (v/v) water.

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.

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. 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% to about 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 2% (v/v). In an embodiment, water is added to bring the total water content in the mixture to about 5% (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 0.1-5 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 0.5-2 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 1-5 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

In the above embodiments, said carbonic acid derivative may be CDI. In another embodiment, said carbonic acid derivative is CDT. In a preferred embodiment, said carbonic acid derivative is DSC (N,N'-Disuccinimidyl carbonate).

In one embodiment the degree of activation of the activated saccharide following step a) is between 1.0 to 100%. The degree of activation of the azido saccharide being defined as the percentage of Repeating Unit linked to an azido linker.

5 In one embodiment the degree of activation of the activated saccharide following step a) is between 5 to 70%. In another embodiment the degree of activation of the activated saccharide following step a) is between 5 to 50%.

In a preferred embodiment the degree of activation of the activated saccharide following step a) is between 15 to 50%.

10 In another embodiment the degree of activation of the activated saccharide following step a) is between 10 to 40%.

In another embodiment the degree of activation of the activated saccharide following step a) is between 5 to 15%.

In another embodiment the degree of activation of the activated saccharide following step a) is between 15 to 35%.

15 In a preferred embodiment the degree of activation of the activated saccharide following step a) is between 15 to 50%.

In a very preferred embodiment the degree of activation of the activated saccharide following step a) is between 10 to 40%.

20 In an embodiment the degree of activation of the activated saccharide following step a) is about 25%.

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

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 0.1-10 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.

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 1-5 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.

35 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.

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.

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 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 about 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 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 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.

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 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 .

25 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 25. In another embodiment the degree of activation of the activated carrier (SCP) following step b) is between 10 to 25. In another embodiment the degree of activation of the activated carrier (SCP) following step b) is between 17 to 22. 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.

5 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.

20 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.

30 In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at step 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 step 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 step 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 step 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 step c) is about 0.5. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at step c) is about

0.6. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at step c) is about 0.7. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at step c) is about 0.8. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at step c) is about 0.9. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at step c) is about 1. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at step c) is about 1.1. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at step c) is about 1.2. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at step c) is about 1.3. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at step c) is about 1.4. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at step c) is about 1.5. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at step c) is about 1.6. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at step c) is about 1.7. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at step c) is about 1.8. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at step c) is about 1.9. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at step c) is about 2.

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 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 (XIV),



wherein X is $(\text{CH}_2)_n$ wherein n is selected from 1 to 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 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 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.

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.

10 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 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.

15 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.

20 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.

25 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.

30 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.

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.

35 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.

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



wherein X is $(\text{CH}_2)_n$ wherein n is selected from 1 to 15.

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

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.

15 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.

20 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.

25 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.

30 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.

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.

35 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.

5 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.

10 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.

15 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.

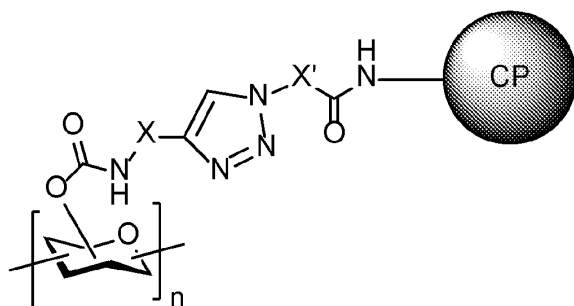
20 Following conjugation to the carrier protein, the glycoconjugate can be purified (enriched with respect to the amount of saccharide-protein conjugate) by a variety of techniques known to the skilled person. These techniques include dialysis, concentration/diafiltration operations, tangential flow 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.

25 In an aspect, the invention provides a serotype 21 glycoconjugate produced according to any of the methods disclosed herein.

Alternative click chemistry

30 In an embodiment of the present invention, the serotype 21 glycoconjugate of the present invention is prepared by alternative click chemistry as disclosed e.g. in application No. PCT/IB2024/051122 (filed on February 07, 2024).

Therefore, in an embodiment, the serotype 21 glycoconjugate of the present invention comprises a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (XVI):



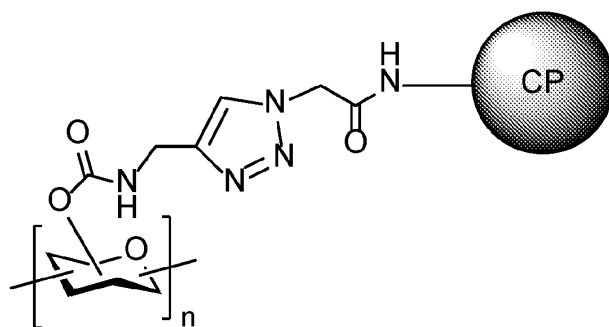
(XVI),

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 0 to 10 and m is selected from 1 to 4,

- 5 wherein X' is selected from the group consisting of $\text{CH}_2(\text{CH}_2)_{n''}$, $\text{CH}_2\text{O}(\text{CH}_2)_{n''}\text{CH}_2$, $\text{CH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_{m'}(\text{CH}_2)_{n''}\text{CH}_2$, where n'' is selected from 0 to 10 and m' is selected from 0 to 4 and wherein the structure in square bracket represents a repeat unit of the serotype 21 saccharide and wherein n represents the number of repeating units.

- 10 In a particular aspect, the invention is directed to a serotype 21 glycoconjugate comprising a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (XVI), wherein X is $\text{CH}_2(\text{CH}_2)_{n'}$, where n' is 0 and wherein X' is $\text{CH}_2(\text{CH}_2)_{n''}$ where n'' is 0.

- 15 In a particular aspect, the invention pertains to a serotype 21 glycoconjugate comprising a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (XVII),



(XVII),

wherein the structure in square bracket represents a repeat unit of the serotype 21 saccharide and wherein n represents the number of repeating units.

- 20 Formulas (XVI) and (XVII) are schematic representations 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 serotype 21 saccharide.

In an embodiment, the invention provides a serotype 21 glycoconjugate comprising a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (XVI), wherein X is $\text{CH}_2(\text{CH}_2)_{n'}$, where n' is selected from 0 to 10 and wherein X' is $\text{CH}_2(\text{CH}_2)_{n''}$ where n'' is selected from 0 to 10. In an embodiment, n' is selected from 0 to 5 and n'' is selected from 0 to 10. In an embodiment, n' is selected from 0 to 5 and n'' is selected from 0 to 5. In an embodiment, n' is selected from 0 to 3 and n'' is selected from 0 to 3.

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

In an embodiment, the invention provides a serotype 21 glycoconjugate comprising a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (XVI), wherein X is $\text{CH}_2(\text{CH}_2)_{n'}$, where n' is selected from 0 to 10 and

- wherein X' is $\text{CH}_2\text{O}(\text{CH}_2)_{n'}\text{CH}_2$ where n' is selected from 0 to 10. In an embodiment, n' is selected from 0 to 5 and n'' is selected from 0 to 10. In an embodiment, n' is selected from 0 to 5 and n'' is selected from 0 to 5. In an embodiment, n' is selected from 0 to 3 and n'' is selected from 0 to 3. In an embodiment, n' is selected from 0 to 2 and n'' is selected from 0 to 2. In a particular
- 5 embodiment, n' is 0 and n'' is 0. 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 0 and n'' is 1. In a particular
- 10 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 0 and n'' is 2. In a particular embodiment, n' is 1 and n'' is 2. In another
- 15 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 0 and n'' is 3. 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
- 20 embodiment, n' is 0 and n'' is 4. 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 0 and n'' is 5. 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
- 25 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 0 and n'' is 6. 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.
- 30 In an embodiment, the invention provides a serotype 21 glycoconjugate comprising a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (XVI), wherein X is $\text{CH}_2(\text{CH}_2)_{n'}$, where n' is selected from 0 to 10 and wherein X' is $\text{CH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_{m'}(\text{CH}_2)_{n''}\text{CH}_2$, where n'' is selected from 0 to 10 and m' is selected from 0 to 4.
- 35 In an embodiment, n' is selected from 0 to 5, m' is selected from 0 to 4 and n'' is selected from 0 to 10. In an embodiment, n' is selected from 0 to 5, m' is selected from 0 to 4 and n'' is selected from 0 to 5. In an embodiment, n' is selected from 0 to 3, m' is selected from 0 to 2 and n'' is selected from 0 to 3. In an embodiment, n' is selected from 0 to 2, m' is selected from 0 to 2 and

n'' is selected from 0 to 1. In an embodiment, n' is selected from 0 to 1, m' is selected from 0 to 1 and n'' is selected from 0 to 1.

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

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

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

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.

10 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.

15 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.

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

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.

25 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.

30 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.

35 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 0, m' is 0 and n'' is 4. In a particular embodiment, n' is 0, m' is 1 and n'' is 4. In a particular embodiment, n' is 0, m' is 2 and n'' is 4. In a particular embodiment, n' is 0, m' is 3 and n'' is 4.

5 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.

10 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.

15 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.

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

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.

25 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.

30 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.

35 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.

In an embodiment, the invention provides a serotype 21 glycoconjugate comprising a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and

having the general formula (XVI), 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 $\text{CH}_2(\text{CH}_2)_{n''}$, 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.

In an embodiment, the invention provides a serotype 21 glycoconjugate comprising a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (XVI), 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 $\text{CH}_2\text{O}(\text{CH}_2)_{n''}\text{CH}_2$, 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.

In an embodiment, the invention provides a serotype 21 glycoconjugate comprising a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and
5 having the general formula (XVI), wherein X is $(CH_2CH_2O)_mCH_2CH_2$, where m is selected from 1 to 4 and wherein X' is $CH_2O(CH_2CH_2O)_m(CH_2)_{n''}CH_2$, 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
10 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
15 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.
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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.
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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.
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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.
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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.

10 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.

15 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.

20 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.

25 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.

30 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.

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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.

In an embodiment, the invention provides a serotype 21 glycoconjugate comprising a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (XVI), wherein X is $\text{NHCO}(\text{CH}_2)_{n'}$, where n' is selected from 1 to 10 and wherein X' is $\text{CH}_2(\text{CH}_2)_{n''}$, 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.

In an embodiment, the invention provides a serotype 21 glycoconjugate comprising a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and
 5 having the general formula (XVI), wherein X is $\text{NHCO}(\text{CH}_2)_{n'}$, where n' is selected from 1 to 10 and wherein X' is $\text{CH}_2\text{O}(\text{CH}_2)_{n''}\text{CH}_2$, 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
 10 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
 15 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
 20 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
 25 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
 30 further embodiment, n' is 5 and n'' is 6. In yet a further embodiment, n' is 6 and n'' is 6.

In an embodiment, the invention provides a serotype 21 glycoconjugate comprising a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and
 having the general formula (XVI), wherein X is $\text{NHCO}(\text{CH}_2)_{n'}$, where n' is selected from 1 to 10 and wherein X' is $\text{CH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_m(\text{CH}_2)_{n''}\text{CH}_2$, where n'' is selected from 0 to 10 and m' is
 35 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 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.

10 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.

15 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.

In an embodiment, the invention provides a serotype 21 glycoconjugate comprising a
 20 serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (XVI), 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 $\text{CH}_2(\text{CH}_2)_{n'}$, 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
 25 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
 30 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
 35 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.

In an embodiment, the invention provides a serotype 21 glycoconjugate comprising a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (XVI), 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 $\text{CH}_2\text{O}(\text{CH}_2)_{n''}\text{CH}_2$, 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.

In an embodiment, the invention provides a serotype 21 glycoconjugate comprising a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (XVI), 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 $\text{CH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_{m'}(\text{CH}_2)_{n''}\text{CH}_2$, 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.

10 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.

25 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.

In an embodiment, the invention provides a serotype 21 glycoconjugate comprising a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (XVI), wherein X is $\text{OCH}_2(\text{CH}_2)_{n'}$, where n' is selected from 1 to 10 and wherein X' is $\text{CH}_2(\text{CH}_2)_{n''}$, 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.

In an embodiment, the invention provides a serotype 21 glycoconjugate comprising a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (XVI), wherein X is $\text{OCH}_2(\text{CH}_2)_{n'}$, where n' is selected from 1 to 10 and wherein X' is $\text{CH}_2\text{O}(\text{CH}_2)_{n''}\text{CH}_2$, 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.
- 15 In an embodiment, the invention provides a serotype 21 glycoconjugate comprising a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (XVI), wherein X is $\text{OCH}_2(\text{CH}_2)_{n'}$, where n' is selected from 1 to 10 and wherein X' is $\text{CH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_{m'}(\text{CH}_2)_{n''}\text{CH}_2$, where n'' is selected from 0 to 10 and m' is selected from 0 to 4.
- 20 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.
- 25 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.
- 30 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.
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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.

10 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.

15 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.

25 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.

30 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.

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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.

10 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.

20 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.

25 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.

35 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.

In an embodiment, the invention provides a serotype 21 glycoconjugate comprising a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (XVI), wherein X is $O(CH_2CH_2O)_mCH_2CH_2$, where m is selected from 1 to 4 and wherein X' is $CH_2(CH_2)_{n''}$, 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.

In an embodiment, the invention provides a serotype 21 glycoconjugate comprising a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (XVI), wherein X is $O(CH_2CH_2O)_mCH_2CH_2$, where m is selected from 1 to 4 and wherein X' is $CH_2O(CH_2)_{n''}CH_2$, 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.

In an embodiment, the invention provides a serotype 21 glycoconjugate comprising a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (XVI), wherein X is $O(CH_2CH_2O)_mCH_2CH_2$, where m is selected from 1 to 4 and wherein X' is $CH_2O(CH_2CH_2O)_{m'}(CH_2)_{n''}CH_2$, 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.

10 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.

20 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.

25 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.

5 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.

10 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.

15 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.

20 In an embodiment of the present invention, the serotype 21 glycoconjugate of the present invention are prepared using the alternative click chemistry of the present section. The invention also relates to a method of making serotype 21 glycoconjugate, as disclosed herein above.

In an embodiment, click chemistry may comprise three steps, (a) reacting an isolated serotype 21 saccharide with a carbonic acid derivative and an alkyne linker in an aprotic solvent to produce an activated alkynyl saccharide (activation of the saccharide), (b) reacting a carrier
25 protein with an agent bearing an N-Hydroxysuccinimide (NHS) moiety and an azido group where the NHS moiety reacts with the amino groups to form an amide linkage thereby obtaining an azido functionalized carrier protein (activation of the carrier protein), (c) reacting the activated alkynyl saccharide of step (a) with the activated azido-carrier protein of step (b) by Cu⁺¹ mediated azide-alkyne cycloaddition reaction to form a glycoconjugate.

30 Following step (a) the saccharide is said to be activated and is referred to herein as "activated saccharide" or "activated alkynyl saccharide".

Following step (b) the carrier is said to be activated and is referred to as "activated carrier" or "activated azido-carrier".

35 As mentioned above, before the activation (a), sizing of the serotype 21 saccharide to a target molecular weight (MW) range may be performed. Therefore, in an embodiment, the isolated serotype 21 saccharide is sized before activation with a carbonic acid derivative and an alkyne linker. In an embodiment, the isolated serotype 21 saccharide is sized to any of the target

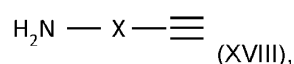
molecular weight (MW) range defined above. In an embodiment, the isolated serotype 21 saccharide is not sized before activation with a carbonic acid derivative and an alkyne linker.

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

In an embodiment, said alkyne linker is a compound of formula (XVIII),



wherein X is selected from the group consisting of CH_2 , $\text{CH}_2(\text{CH}_2)_n$, $(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$, $\text{NHCO}(\text{CH}_2)_n$, $\text{NHCO}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$, $\text{OCH}_2(\text{CH}_2)_n$ 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 alkyne linker is a compound of formula (XVIII), wherein X is CH_2 .

In an embodiment, said alkyne linker is a compound of formula (XVIII), 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 alkyne linker is a compound of formula (XVIII), 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 alkyne linker is a compound of formula (XVIII), 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

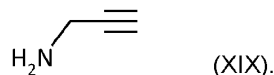
embodiment, n is 5. In yet a further embodiment, n is 6. In yet a further embodiment, n is 7. In yet a further embodiment, n is 8. In yet a further embodiment, n is 9. In yet a further embodiment, n is 10.

In an embodiment, said alkyne linker is a compound of formula (XVIII), wherein X is
 5 $\text{NHCO}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$, 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 alkyne linker is a compound of formula (XVIII), wherein X is
 10 $\text{OCH}_2(\text{CH}_2)_n$, 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
 15 a further embodiment, n is 8. In yet a further embodiment, n is 9. In yet a further embodiment, n is 10.

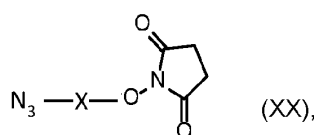
In an embodiment, said alkyne linker is a compound of formula (XVIII), wherein X is
 $\text{O}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$, where m is selected from 1 to 4. In an embodiment, m is selected from 1 to 3. In an embodiment, m is selected from 1 to 2. In a particular embodiment,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

In an embodiment, said alkyne linker is a compound of formula (XIX),



Hence in a preferred embodiment, said alkyne linker is propargylamine.

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



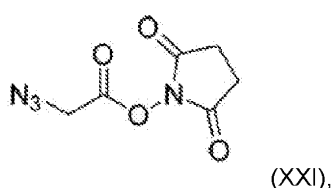
30 where X is selected from the group consisting of $(\text{CH}_2)_n\text{CH}_2\text{C}=\text{O}$ and $(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2=\text{O}$ where n is selected from 0 to 10 and m is selected from 0 to 4.

In an embodiment, said agent bearing an N-Hydroxysuccinimide (NHS) moiety and an azido group is a compound of formula (XX), wherein X is $(\text{CH}_2)_n\text{CH}_2\text{C}=\text{O}$, where n is selected from 0 to 10. In an embodiment, n is selected from 0 to 5. In an embodiment, n is selected from
 35 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 azido group is a compound of formula (XX), wherein X is $(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2=\text{O}$, where m is selected from 0 to 4. In an embodiment, m is selected from 0 to 3. In an embodiment, m is selected from 0 to 2. In an embodiment, m is selected from 0 to 1. In a particular embodiment, m is 0. 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 agent bearing an N-Hydroxysuccinimide (NHS) moiety and an azido group is a compound of formula (XXI):



Hence in an embodiment, said agent bearing an N-Hydroxysuccinimide (NHS) moiety and an azido group is (2,5-Dioxopyrrolidin-1-yl) 2-azidoacetate.

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 alkyne linker in an aprotic solvent to produce an activated alkynyl 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 0.1-10 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 0.5-5 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 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.

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 0.1-5 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 0.5-2 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 of about 0.01 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 0.1 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.

15 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 in the reaction mixture.

In one embodiment step a) comprises reacting the saccharide with an amount of carbonic acid derivative of about 2 molar equivalents 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 equivalents 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 10 molar equivalents to the amount of saccharide present in the reaction mixture.

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

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

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

5 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).

10 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).

15 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

20 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).

25 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.

30 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

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

Preferably, 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
15 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 with CDI in an aprotic
20 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 isolated saccharide is reacted with CDI in an aprotic
25 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 saccharide is reacted with CDI in DMSO comprising 0.1% to 0.5% (v/v) water. In one embodiment the isolated saccharide
30 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.

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
35 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.

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.

5 Preferably, in one embodiment the isolated 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 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
10 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)
15 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.

In one embodiment the free carbonic acid derivative is then quenched by the addition of water before the addition of the alkyne linker. Water can inactivate free CDI.
20 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% to about 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
25 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 5% (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 alkyne linker.

30 In one embodiment step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of alkyne 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 alkyne linker that is between 0.1-5 molar equivalent to the
35 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 alkyne linker that is between 0.1-5 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 alkyne linker that is between 0.5-2 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 alkyne linker that is between 1-5 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

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

10 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 alkynyl saccharide being defined as the percentage of Repeating Unit linked to an alkyne linker.

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%.

20 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%. In a preferred embodiment the degree of activation of the activated saccharide following step a) is between 15 to 50%.

25 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%.

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

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

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

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

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

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

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

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

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

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

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

In one embodiment the degree of activation of the activated carrier following step b) is
5 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 azido group.

In an embodiment, the carrier protein is CRM₁₉₇, which contains 39 lysine residues. In said
10 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
15 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
20 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
25 (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
30 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
35 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 a preferred embodiment the degree of activation of the activated carrier (SCP) following step b) is between 15 to 25. 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 of 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 alkynyl saccharide to activated azido-carrier at step c) is between 0.1 and 3. In an embodiment the initial input ratio (weight by weight) of activated alkynyl saccharide to activated azido-carrier at step c) is between 0.5 and 2. In an embodiment the initial input ratio (weight by weight) of activated alkynyl saccharide to activated azido-carrier at step c) is between 0.6 and 1.5. In an embodiment the initial input ratio (weight by weight) of activated alkynyl saccharide to activated azido-carrier at step c) is between 0.8 and 1. In a preferred embodiment the initial input ratio (weight by weight) of activated alkynyl saccharide to activated azido-carrier at step c) is between 0.8 and 1.2. In an embodiment the initial input ratio (weight by weight) of activated alkynyl saccharide to activated azido-carrier at step c) is about 0.5. In an embodiment the initial input ratio (weight by weight) of activated alkynyl saccharide to activated azido-carrier at step c) is about 0.6. In an embodiment the initial input ratio (weight by weight) of activated alkynyl saccharide to activated azido-carrier at step c) is about 0.7. In an embodiment the initial input ratio (weight by weight) of activated alkynyl saccharide to activated azido-carrier at step c) is about 0.8. In an embodiment the initial input ratio (weight by weight) of activated alkynyl saccharide to activated azido-carrier at step c) is about 0.9. In an embodiment the initial input ratio (weight by weight) of activated alkynyl saccharide to activated azido-carrier at step c) is about 1. In an embodiment the initial input ratio (weight by weight) of activated alkynyl saccharide to activated azido-carrier at step c) is about 1.1. In an embodiment the initial input ratio (weight by weight) of activated alkynyl saccharide to activated azido-carrier at step c) is about 1.2. In an embodiment the initial input ratio (weight by weight) of activated alkynyl saccharide to activated azido-carrier at step c) is about 1.3. In an embodiment the initial input ratio (weight by weight) of activated alkynyl saccharide to activated

azido-carrier at step c) is about 1.4. In an embodiment the initial input ratio (weight by weight) of activated alkynyl saccharide to activated azido-carrier at step c) is about 1.5. In an embodiment the initial input ratio (weight by weight) of activated alkynyl saccharide to activated azido-carrier at step c) is about 1.6. In an embodiment the initial input ratio (weight by weight) of activated alkynyl saccharide to activated azido-carrier at step c) is about 1.7. In an embodiment the initial input ratio (weight by weight) of activated alkynyl saccharide to activated azido-carrier at step c) is about 1.8. In an embodiment the initial input ratio (weight by weight) of activated alkynyl saccharide to activated azido-carrier at step c) is about 1.9. In an embodiment the initial input ratio (weight by weight) of activated alkynyl saccharide to activated azido-carrier at step c) is about 2.

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.

In an embodiment, 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.

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

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.

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.

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.

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 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 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.

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.

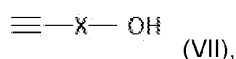
In an embodiment the capping of the unreacted alkyne groups is performed with an amount of capping agent that about 2 molar equivalents to the amount of polysaccharide repeat unit of the activated saccharide.

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 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 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 (VII),



wherein X is $(\text{CH}_2)_n$ wherein n is selected from 1 to 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.

5 In an embodiment 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 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 10 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 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 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.

15 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.

20 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.

25 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.

30 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.

35 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 conjugation to the carrier protein, the serotype 21 glycoconjugate of the invention 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 serotype 21 glycoconjugate produced according to any of the methods disclosed herein.

2.3 Carrier Proteins

A component of the glycoconjugate is a carrier protein to which the serotype 21 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 serotype 21 saccharide glycoconjugate 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, Maecel 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). Another suitable carrier protein is rhizavidin [aa 45-179J-GGGGSSS-SP1500- AAA-SP0785] (CP1) (WO2020056202). Another suitable carrier protein is Rhavi-linker-PdT(G294P)-linker-SP0435 [aa 62-185] fusion protein (SPP2), see WO2023039223. WO2020/056202 and WO2023/039223 are incorporated by reference. SPP2 is described in particular at sections [0245] to [250] of WO2023/039223.

In a preferred embodiment, the carrier protein of the serotype 21 saccharide glycoconjugate of the invention is selected from the group consisting of TT, DT, DT mutants (such as CRM₁₉₇), and a C5a peptidase from *Streptococcus* (SCP).

In an embodiment, the carrier protein of the serotype 21 saccharide glycoconjugate is DT (Diphtheria Toxoid). In another embodiment, the carrier protein of the serotype 21 saccharide glycoconjugate is TT (Tetanus Toxoid).

In another embodiment, the carrier protein of the serotype 21 saccharide glycoconjugate is PD (*H. influenzae* protein D; see, e.g., EP0594610 B).

In a preferred embodiment, the carrier protein of the serotype 21 saccharide glycoconjugate is CRM₁₉₇ or a C5a peptidase from *Streptococcus* (SCP).

In another embodiment, the carrier protein of the serotype 21 saccharide glycoconjugate is rhizavidin [aa 45-179J-GGGGSSS-SP1500- AAA-SP0785] (CP1).

In another embodiment, the carrier protein of the serotype 21 saccharide glycoconjugate is Rhavi-linker-PdT(G294P)-linker-SP0435 [aa 62-185] fusion protein (SPP2). In an embodiment, said SPP2 has the amino acid sequence as set forth at SEQ ID NO: 19 of WO2023/039223.

In a preferred embodiment, the serotype 21 saccharide is conjugated to CRM₁₉₇ protein. 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 serotype 21 saccharide is conjugated to CRM₁₉₇ protein. In an embodiment, the serotype 21 saccharide is conjugated to CRM₁₉₇ protein or the A chain of CRM₁₉₇ (see CN103495161). In an embodiment, the serotype 21 saccharide is conjugated the A chain of CRM₁₉₇ obtained via expression by genetically recombinant *E. coli* (see CN103495161).

- 5 In other preferred embodiments, the carrier protein of the serotype 21 saccharide 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
10 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.*,
15 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
20 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
25 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
30 wall anchor domain (commonly residues 1029/1030/1031 to the C-terminus).

In an embodiment, the carrier protein of the serotype 21 saccharide 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 serotype 21 saccharide glycoconjugate of the invention is an SCP from GAS (SCPA).
35

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 serotype 21 saccharide glycoconjugate of the invention is an enzymatically inactive SCP.

In other preferred embodiments, the carrier protein of the serotype 21 saccharide glycoconjugate of the invention is an enzymatically inactive SCP from GBS (SCPB).

5 In another preferred embodiments, the carrier protein of the serotype 21 saccharide glycoconjugate of the invention is an enzymatically inactive SCP from GAS (SCPA).

In an embodiment, the carrier protein of the serotype 21 saccharide glycoconjugate of the invention is a fragment of an SCP. In an embodiment, the carrier protein of the serotype 21 saccharide glycoconjugate of the invention is a fragment of an SCPA. Preferably, the carrier
10 protein of the serotype 21 saccharide glycoconjugate of the invention is a fragment of an SCPB.

In an embodiment, the carrier protein of the serotype 21 saccharide 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.

15 In an embodiment, the carrier protein of the serotype 21 saccharide 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 serotype 21 saccharide glycoconjugate of the
20 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 serotype 21 saccharide glycoconjugate of the
25 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 serotype 21 saccharide glycoconjugate of the
30 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 serotype 21 saccharide
35 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.

5 Therefore, in an embodiment, the carrier protein of the serotype 21 saccharide 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, 10 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.

15 In an embodiment, the carrier protein of the serotype 21 saccharide 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 20 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 serotype 21 saccharide glycoconjugate of the 25 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 30 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 serotype 21 saccharide glycoconjugate of the invention is an enzymatically inactive fragment of an SCP where said inactivation is accomplished 35 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 serotype 21 saccharide 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 serotype 21 saccharide 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 serotype 21 saccharide 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 serotype 21 saccharide 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 serotype 21 saccharide 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.

In an embodiment, the carrier protein of the serotype 21 saccharide 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 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 serotype 21 saccharide glycoconjugate of the invention is an enzymatically inactive fragment of an 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 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 serotype 21 saccharide 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 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 serotype 21 saccharide 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.

20 In an embodiment, the carrier protein of the serotype 21 saccharide 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.

35 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.

5 Therefore, in an embodiment, the carrier protein of the serotype 21 saccharide 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, 10 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 15 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 serotype 21 saccharide 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 20 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. 25 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 serotype 21 saccharide glycoconjugate of the invention is an enzymatically inactive SCPB where said inactivation is accomplished by replacing 30 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. 35 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 serotype 21 saccharide 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 serotype 21 saccharide 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 serotype 21 saccharide 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 serotype 21 saccharide 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 serotype 21 saccharide 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 serotype 21 saccharide 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 serotype 21 saccharide 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 serotype 21 saccharide glycoconjugate of the invention is an enzymatically inactive fragment of an SCP where said inactivation is accomplished
5 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

10 In an embodiment, the carrier protein of the serotype 21 saccharide 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
15 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

20 In an embodiment, the carrier protein of the serotype 21 saccharide 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
25 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

30 In an embodiment, the carrier protein of the serotype 21 saccharide 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
35 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 serotype 21 saccharide glycoconjugate of the invention is an enzymatically inactive fragment of SCP which consists of

5 SEQ ID NO: 1.

In a particular embodiment, the carrier protein of the serotype 21 saccharide glycoconjugate of the invention is an enzymatically inactive fragment of SCP which consists of

SEQ ID NO: 2.

10 SEQ ID NO: 1 :

MAKTADTPATSKATIRDLNDPSQVKTLQEKAGKGAGTVVAVIAAGFDKNH
EAWRLTDKAKARYQSKEDLEKAKKEHGITYGEWWNDKVAYYHDYSKDGTK
AVDQEHGTHVSGILSGNAPSETKEPYRLEGAMPEAQLLLMRVEIVNGLAD
YARNYAQAIRDAINLGAKVINMSFGNAALAYANLPDETKKAFDYAKSKGV
15 SIVTSAGNDSSFSGGKTRLPLADHPDYGVVGTPAAADSTLTVASYSPDKQL
TETVTVKTADQQDKEMPVLSTNRFEPNKAYDYAYANRGTKEDDFKDVKGK
IALIERGDIDFKDKIAKAKKAGAVGVLIYDNQDKGFIELPNVDQMPAAF
ISRKDGLLLLKDNPKQKTITFNATPKVLPTASGKLSRFSSWGLTADGNIKP
DIAAPGQDILSSVANNKYAKLSGTAMSAPLVAGIMGLLQEYETQYPDMT
20 PSERLDLAKKVLMSATALYDEDEKAYFSPRQQGAGAVDAKKASAATMYV
TDKDNNTSSKVHLNNVSDKFEVTVTVHNKSDKPQELYQATVQTDKVDGKH
FALAPKALYETSWQKITIPANSSKQVTPIDASRFSKDLLAQMKNGYFLE
GFVRFKQDPKKEELMSIPYIGFRGDFGNLSALEKPIYDSKDGSSYYHEAN
SDAKDQLDGDGLQFYALKNNFTALTTESNPWTIHKAVKEGVENIEDIESS
25 EITETIFAGTFAKQDDDSHYIHRHANGKPYAAISPNGDGNRDYVQFQGT
FLRNAKNLVAEVLDEKGNVVTSEVTEQVVKYNNNDLASTLGSTRFEKTR
WDGKDKDGKVVANGTYTYRVRYTPISSGAKEQHTDFDVIDNTTPEVATS
ATFSTEDRRLTLASKPKTSQPVYRERIAITYMDEDLPTTEYISPNEGTF
TLPEEAETMEGATVPLKMSDFTYVVEDMAGNITYTPVTKLLEGHSNKPEQ

30

SEQ ID NO: 1 is 950 amino acids long.

SEQ ID NO: 2 :

AKTADTPATSKATIRDLNDPSQVKTLQEKAGKGAGTVVAVIAAGFDKNH
35 EAWRLTDKAKARYQSKEDLEKAKKEHGITYGEWWNDKVAYYHDYSKDGTK
AVDQEHGTHVSGILSGNAPSETKEPYRLEGAMPEAQLLLMRVEIVNGLAD
YARNYAQAIRDAINLGAKVINMSFGNAALAYANLPDETKKAFDYAKSKGV
SIVTSAGNDSSFSGGKTRLPLADHPDYGVVGTPAAADSTLTVASYSPDKQL

TETVTVKTADQQDKEMPVLSTNRFEPNKAYDYAYANRGTKEDDFKDVKGK
IALIERGDIDFKDKIAKAKKAGAVGVLIYDNQDKGFIELPNVDQMPAAF
ISRKDGLLLKDNPKQTITFNATPKVLPTASGTKLSRFSSWGLTADGNIKP
DIAAPGQDILSSVANNKYAKLSGTAMSAPLVAGIMGLLQEQYETQYPDMT
5 PSERLDLAKKVLMSATALYDEDEKAYFSPRQQGAGAVDAKKASAATMYV
TDKDNSTSSKVHLNNSDKFEVTVTVHNKSDKPQELYYQATVQTDKVDGKH
FALAPKALYETSWQKITIPANSSKQVTVPIDASRFSKDLLAQMKNGYFLE
GFVRFKQDPKKEELMSIPYIGFRGDFGNLSALEKPIYDSKDGSSYYHEAN
SDAKDQLDGDGLQFYALKNNFTALTTESNPWTIIKAVKEGVENIEDIESS
10 EITETIFAGTFAKQDDDDSHYYIHRHANGKPYAAISPNGDGNRDYVQFQGT
FLRNAKNLVAEVLDEKGNVVTSEVTEQVVKNNNDLASTLGSTRFEKTR
WDGKDKDGKVVANGTYTYRVRYTPISSGAKEQHTDFDVVDNTTPEVATS
ATFSTEDRRLTLASKPKTSQPVYRERIAITYMDEDLPTTEYISPNEGDTF
TLPEEAETMEGATVPLKMSDFTYVVEDMAGNITYTPVTKLLEGHSNKPEQ

15

SEQ ID NO: 2 is 949 amino acids long.

In a particular embodiment, the carrier protein of the serotype 21 saccharide glycoconjugate of the invention is an enzymatically inactive fragment of SCP consisting of a polypeptide having at least 90% identity with SEQ ID NO: 1.

In a particular embodiment, the carrier protein of the serotype 21 saccharide glycoconjugate of the invention is an enzymatically inactive fragment of SCP consisting of a polypeptide having at least 95% identity with SEQ ID NO: 1.

In a particular embodiment, the carrier protein of the serotype 21 saccharide glycoconjugate of the invention is an enzymatically inactive fragment of SCP consisting of a polypeptide having at least 99% identity with SEQ ID NO: 1.

In a particular embodiment, the carrier protein of the serotype 21 saccharide 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: 1.

In a particular embodiment, the carrier protein of the serotype 21 saccharide 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: 1.

In a particular embodiment, the carrier protein of the serotype 21 saccharide 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: 1.

In a particular embodiment, the carrier protein of the serotype 21 saccharide glycoconjugate of the invention is an enzymatically inactive fragment of SCP consisting of a polypeptide having at least 90% identity with SEQ ID NO: 2.

In a particular embodiment, the carrier protein of the serotype 21 saccharide glycoconjugate of the invention is an enzymatically inactive fragment of SCP consisting of a polypeptide having at least 95% identity with SEQ ID NO: 2.

5 In a particular embodiment, the carrier protein of the serotype 21 saccharide glycoconjugate of the invention is an enzymatically inactive fragment of SCP consisting of a polypeptide having at least 99% identity with SEQ ID NO: 2.

In a particular embodiment, the carrier protein of the serotype 21 saccharide 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: 2.

10 In a particular embodiment, the carrier protein of the serotype 21 saccharide 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: 2.

In a particular embodiment, the carrier protein of the serotype 21 saccharide 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: 2.

3. Immunogenic compositions

In an embodiment the invention relates to an immunogenic composition comprising a *S. pneumoniae* serotype 21 saccharide of the invention.

20 In an embodiment the invention relates to an immunogenic composition comprising a *Streptococcus pneumoniae* serotype 21 glycoconjugate of the invention.

In an embodiment the invention relates to an immunogenic composition comprising a *Streptococcus pneumoniae* serotype 21 glycoconjugate of the invention and comprising from 1 to 45 different glycoconjugates.

25 In an embodiment the invention relates to an immunogenic composition comprising a *Streptococcus pneumoniae* serotype 21 glycoconjugate of the invention and comprising from 1 to 45 glycoconjugates from different serotypes of *S. pneumoniae* (1 to 45 pneumococcal conjugates).

30 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 21, 22, 23, 24 or 25-valent pneumococcal conjugate composition.

35 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 *Streptococcus pneumoniae* serotype 21 glycoconjugate of the invention and comprising from 26
5 to 45 glycoconjugates from different serotypes of *S. pneumoniae* (26 to 45 pneumococcal conjugates). In one embodiment the invention relates to an immunogenic composition comprising glycoconjugates from 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44 or 45 different serotypes of *S. pneumoniae*.

In one embodiment the immunogenic composition comprises glycoconjugates from 35 or
10 45 different serotypes of *S. pneumoniae*. In an embodiment the immunogenic composition is a 35, 36, 37, 38, 39, 40, 41, 42, 43, 44 or 45-valent pneumococcal conjugate compositions. In an embodiment the immunogenic composition is a 40, 41, 42, 43, 44 or 45-valent pneumococcal conjugate compositions. In an embodiment the immunogenic composition is a 40-valent pneumococcal conjugate composition. In an embodiment the immunogenic composition is a 41-
15 valent pneumococcal conjugate composition. In an embodiment the immunogenic composition is a 42-valent pneumococcal conjugate composition. In an embodiment the immunogenic composition is a 43-valent pneumococcal conjugate composition. In an embodiment the immunogenic composition is a 44-valent pneumococcal conjugate composition. In an embodiment the immunogenic composition is a 45-valent pneumococcal conjugate composition.

20 In an embodiment the invention relates to an immunogenic composition comprising a *Streptococcus pneumoniae* serotype 21 glycoconjugate of the invention and further comprising glycoconjugates from *S. pneumoniae* serotypes 4, 6B, 9V, 14, 18C, 19F and 23F.

In an embodiment said immunogenic composition comprises in addition glycoconjugates from *S. pneumoniae* serotypes 1, 5 and 7F.

25 In an embodiment any of the immunogenic compositions above comprises in addition glycoconjugates from *S. pneumoniae* serotype 3.

In an embodiment any of the immunogenic compositions above comprises in addition glycoconjugates from *S. pneumoniae* serotypes 6A and 19A.

In an embodiment any of the immunogenic compositions above comprise in addition a
30 glycoconjugates from *S. pneumoniae* serotype 22F and 33F.

In an embodiment any of the immunogenic compositions above comprise in addition a glycoconjugates from *S. pneumoniae* serotypes 8, 10A, 11A, 12F and 15B.

In an embodiment any of the immunogenic compositions above comprise in addition a glycoconjugates from *S. pneumoniae* serotype 2.

35 In an embodiment any of the immunogenic compositions above comprise in addition a glycoconjugates from *S. pneumoniae* serotype 9N.

In an embodiment any of the immunogenic compositions above comprise in addition a glycoconjugates from *S. pneumoniae* serotype 17F.

In an embodiment any of the immunogenic compositions above comprise in addition a glycoconjugates from *S. pneumoniae* serotype 20.

In an embodiment any of the immunogenic compositions above comprise in addition a glycoconjugates from *S. pneumoniae* serotype 15C.

5 In an embodiment the invention relates to an immunogenic composition comprising a *Streptococcus pneumoniae* serotype 21 glycoconjugate of the invention and further comprising glycoconjugates from *S. pneumoniae* serotypes 4, 6B, 9V, 14, 18C, 19F and 23F. In an embodiment the immunogenic composition is an 8-valent pneumococcal conjugate compositions.

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

15 In an embodiment the invention relates to an immunogenic composition comprising a *S. pneumoniae* serotype 21 glycoconjugate of the invention and further 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 14-valent pneumococcal conjugate compositions. In an embodiment the invention relates to an immunogenic composition comprising a *Streptococcus pneumoniae* serotype 21 glycoconjugate of the invention and further comprising
20 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 16-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a *Streptococcus pneumoniae* serotype 21 glycoconjugate of the invention and further comprising
25 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 21-valent pneumococcal conjugate compositions.

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

In an embodiment the invention relates to an immunogenic composition comprising a *Streptococcus pneumoniae* serotype 21 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, 15C, 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 *Streptococcus pneumoniae* serotype 21 glycoconjugate of the invention and further 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 22-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a *Streptococcus pneumoniae* serotype 21 glycoconjugate of the invention and further comprising glycoconjugates from *S. pneumoniae* serotypes 1, 2, 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 23-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a *Streptococcus pneumoniae* serotype 21 glycoconjugate of the invention and further 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, 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 *Streptococcus pneumoniae* serotype 21 glycoconjugate of the invention and further 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 25-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a *Streptococcus pneumoniae* serotype 21 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, 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 *Streptococcus pneumoniae* serotype 21 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, 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 *Streptococcus pneumoniae* serotype 21 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,

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 *Streptococcus pneumoniae* serotype 21 glycoconjugate of the invention and further comprising
5 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 22-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a *Streptococcus pneumoniae* serotype 21 glycoconjugate of the invention and further comprising
10 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 22-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a *Streptococcus pneumoniae* serotype 21 glycoconjugate of the invention and further comprising
15 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 23-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a *Streptococcus pneumoniae* serotype 21 glycoconjugate of the invention and further comprising
20 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 one embodiment, the *S. pneumoniae* saccharides are conjugated to CRM₁₉₇. In one embodiment, the *S. pneumoniae* saccharides from serotypes 1, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15A, 15B, 18C, 19A, 19F, 22F, 23A, 23B, 23F, 24F, 33F, 35B and 21 are conjugated to CRM₁₉₇ and the *S. pneumoniae*
25 saccharide from serotype 3 is conjugated to SCP. In an embodiment the immunogenic composition is a 26-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a *Streptococcus pneumoniae* serotype 21 glycoconjugate of the invention and further comprising
at least one glycoconjugate selected from the group consisting of glycoconjugates from *S. pneumoniae* serotypes 2, 3, 7C, 9N, 10B, 15A, 16F, 17F, 20, 22A, 23A, 23B, 24B, 24F, 27, 29,
30 31, 33B, 34, 35B, 35F and 38.

In an embodiment the invention relates to an immunogenic composition comprising a *Streptococcus pneumoniae* serotype 21 glycoconjugate of the invention and further comprising
twenty one glycoconjugates selected from the group consisting of glycoconjugates from *S. pneumoniae* serotypes 2, 3, 7C, 9N, 10B, 15A, 16F, 17F, 20, 22A, 23A, 23B, 24B, 24F, 27, 29,
35 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 *Streptococcus pneumoniae* serotype 21 glycoconjugate of the invention and further comprising glycoconjugates from serotypes 2, 3, 7C, 9N, 10B, 15A, 16F, 17F, 20, 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 *Streptococcus pneumoniae* serotype 21 glycoconjugate of the invention and further comprising at least one glycoconjugate selected from the group consisting of glycoconjugates from *S. pneumoniae* serotypes 2, 3, 7C, 9N, 10B, 15A, 16F, 17F, 19A, 19F, 20, 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 *Streptococcus pneumoniae* serotype 21 glycoconjugate of the invention and further comprising twenty two glycoconjugates selected from the group consisting of glycoconjugates from *S. pneumoniae* serotypes 2, 3, 7C, 9N, 10B, 15A, 16F, 17F, 19A, 19F, 20, 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 *Streptococcus pneumoniae* serotype 21 glycoconjugate of the invention and further comprising twenty three glycoconjugates selected from the group consisting of glycoconjugates from *S. pneumoniae* serotypes 2, 3, 7C, 9N, 10B, 15A, 16F, 17F, 19A, 19F, 20, 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.

In an embodiment the invention relates to an immunogenic composition comprising a *Streptococcus pneumoniae* serotype 21 glycoconjugate of the invention and further comprising glycoconjugates from serotypes 2, 3, 7C, 9N, 10B, 15A, 16F, 17F, 20, 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 *Streptococcus pneumoniae* serotype 21 glycoconjugate of the invention and further comprising glycoconjugates from serotypes 2, 3, 7C, 9N, 10B, 15A, 16F, 17F, 19A, 19F, 20, 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.

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.

4 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.

5 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.

10 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.

20 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 an even 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 thr-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-isoglutaminyl-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.

5 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,

TRITON™ 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,
5 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
10 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
15 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,
20 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
25 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
30 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,
35 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).

5 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
10 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,
15 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
20 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
25 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%,
30 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
35 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, aluminium, etc.) and/or polymers (e.g., thermoplastics, elastomers, thermoplastic-elastomers). In an embodiment, the container of the present disclosure is made of glass.

5 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
10 has a volume of 0.2 mL to 1 mL, even more preferably a volume of about 0.5 mL.

6 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
15 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
20 a human, non-human primate, cat, sheep, pig, horse, bovine or dog. Preferably, 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. In particular, immunogenic compositions described herein may be used to
25 prevent, treat or ameliorate a *S. pneumoniae* infection, disease or condition in a subject. Preferably, the immunogenic compositions described herein may be used to prevent, treat or ameliorate *S. pneumoniae* infection, disease or condition by the serotypes contained in the composition in a subject.

Thus, in one aspect, the disclosure provides a method of preventing, treating or
30 ameliorating an infection, disease or condition associated with *S. pneumoniae* serotype 21 in a subject, comprising administering to the subject an immunologically effective amount of an immunogenic composition of the disclosure.

In some such embodiments, the infection, disease or condition is selected from the group consisting of pneumonia, sinusitis, otitis media, acute otitis media, meningitis, bacteraemia,
35 sepsis, pleural empyema, conjunctivitis, osteomyelitis, septic arthritis, endocarditis, peritonitis, pericarditis, mastoiditis, cellulitis, soft tissue infection and brain abscess.

In an embodiment, the disclosure provides a method of inducing an immune response to *S. pneumoniae* serotype 21 in a subject comprising administering to the subject an

immunologically effective amount of an immunogenic composition of the invention. In one aspect, the subject is a mammal, such as a human, cat, sheep, pig, horse, bovine or dog. Preferably the subject is a human.

In an embodiment, the immunogenic compositions disclosed herein are for use as a vaccine. In such embodiments the immunogenic compositions described herein may be used to prevent *S. pneumoniae* serotype 21 infection in a subject. Thus, in one aspect, the invention provides a method of preventing an infection by *S. pneumoniae* serotype 21 in a subject comprising administering to the subject an immunologically effective amount of an immunogenic composition of the disclosure. In some such embodiments, the infection is selected from the group consisting of pneumonia, sinusitis, otitis media, acute otitis media, meningitis, bacteraemia, sepsis, pleural empyema, conjunctivitis, osteomyelitis, septic arthritis, endocarditis, peritonitis, pericarditis, mastoiditis, cellulitis, soft tissue infection and brain abscess. In one aspect, the subject is a mammal, such as a human, cat, sheep, pig, horse, bovine or dog. Preferably the subject is a human.

The immunogenic composition of the present disclosure can be used to protect or treat a human susceptible to a *S. pneumoniae* serotype 21 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.

7 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
5 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
10 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
15 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),
25 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
35 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 $1 \times$

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 mm^3 . 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 or a human female.

8 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.

5 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.

10 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.

15 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 20 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.

25 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, 30 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.

35 In an embodiment, a prime dose is given at day 0 and a boost is given about 3 months later.

9. Analytical methods

Native serotype 21 polysaccharide is O-acetylated and the total amount of O-acetylation has been found to be approximately one O-acetyl group per polysaccharide repeating unit. The degree of O-acetylation of the polysaccharide can be determined by any method known in the art, for example, by proton NMR or by ion-HPLC analysis (see for example Lemercinier et al. (1996) Carbohydrate Research 296:83-96; Jones et al. (2002) J. Pharmaceutical and Biomedical Analysis 30:1233-1247; Hestrin, S. (1949) J. Biol. Chem. 180:249-261).

In an embodiment the invention relates to a method of detecting the presence of O-acetyl groups in an isolated *S. pneumoniae* serotype 21 polysaccharide, said method comprising the step of: a) isolating an *S. pneumoniae* serotype 21 polysaccharide and b) detecting the presence of O-acetyl groups in said polysaccharide.

In an embodiment the presence of O-acetyl groups is detected by NMR, Mass Spectrometry (MS) or High-performance liquid chromatography (HPLC).

In an embodiment the presence of O-acetyl groups is detected by NMR.

In an embodiment the presence of O-acetyl groups is detected by Mass Spectrometry (MS).

In a preferred embodiment the presence of O-acetyl groups is detected by High-performance liquid chromatography (HPLC).

In an embodiment, the invention relates to a method of detecting the presence of O-acetyl groups in a reduced serotype 21 polysaccharide, said method comprising the step of: a) reacting an isolated *S. pneumoniae* serotype 21 polysaccharide with a reducing agent and b) detecting the presence of O-acetyl groups in said reduced polysaccharide.

In an embodiment the presence of O-acetyl groups is detected by NMR, Mass Spectrometry (MS) or High-performance liquid chromatography (HPLC).

In an embodiment the presence of O-acetyl groups is detected by NMR.

In an embodiment the presence of O-acetyl groups is detected by Mass Spectrometry (MS).

In a preferred embodiment the presence of O-acetyl groups is detected by High-performance liquid chromatography (HPLC).

In an embodiment, said reducing agent is sodium borohydride (NaBH_4).

In an embodiment, said isolated *S. pneumoniae* serotype 21 polysaccharide has been previously treated with an oxidizing agent. In an embodiment, the oxidizing agent is any oxidizing agent which oxidizes a terminal hydroxyl group to an aldehyde. In an embodiment, the oxidizing agent is periodate. In an embodiment, the oxidizing agent is orthoperiodate. In a preferred embodiment, the oxidizing agent is sodium periodate. In an embodiment, the oxidizing agent is metaperiodate. In a preferred embodiment the oxidizing agent is sodium metaperiodate.

In an embodiment, said isolated *S. pneumoniae* serotype 21 polysaccharide has been previously treated with a stable nitroxyl radical compound and an oxidant. In an aspect, said stable

nitroxyl radical compound is a molecule bearing a TEMPO or a PROXYL (2,2,5,5-tetramethyl-1-pyrrolidinyloxy) moiety. Preferably said molecule has the ability to selectively oxidize primary alcohol in the presence of an oxidant, to generate aldehyde groups, without affecting secondary hydroxyl groups. More preferably said molecule has the ability to selectively oxidize primary alcohol in the presence of an oxidant, to generate aldehyde groups, without over oxidation to carboxyl groups. In an aspect, said stable nitroxyl radical compound is TEMPO, 2,2,6,6-Tetramethyl-4-(methylsulfonyloxy)-1-piperidinoxy, 4-Phosphonooxy-TEMPO, 4-Oxo-TEMPO, 4-Methoxy-TEMPO, 4-Isothiocyanato-TEMPO, 4-(2-Iodoacetamido)-TEMPO free radical, 4-Hydroxy-TEMPO, 4-Cyano-TEMPO, 4-Carboxy-TEMPO, 4-(2-Bromoacetamido)-TEMPO, 4-Amino-TEMPO or 4-Acetamido-2,2,6,6-tetramethylpiperidine 1-oxyl. In an aspect, said stable nitroxyl radical compound is selected from the groups consisting of TEMPO, 2,2,6,6-Tetramethyl-4-(methylsulfonyloxy)-1-piperidinoxy, 4-Phosphonooxy-TEMPO, 4-Oxo-TEMPO, 4-Methoxy-TEMPO, 4-Isothiocyanato-TEMPO, 4-(2-Iodoacetamido)-TEMPO free radical, 4-Hydroxy-TEMPO, 4-Cyano-TEMPO, 4-Carboxy-TEMPO, 4-(2-Bromoacetamido)-TEMPO, 4-Amino-TEMPO, 4-Acetamido-2,2,6,6-tetramethylpiperidine 1-oxyl. Preferably said stable nitroxyl radical compound is TEMPO. In a further aspect, said stable nitroxyl radical compound is 3 β -DOXYL-5 α -cholestane, 5-DOXYL-stearic acid, 16-DOXYL-stearic acid, Methyl 5-DOXYL-stearate, 3-(Aminomethyl)-PROXYL, 3-Carbamoyl-PROXYL, 3-Carbamoyl-2,2,5,5-tetramethyl-3-pyrrolin-1-oxyl, 3-Carboxy-PROXYL or 3-Cyano-PROXYL. In a further aspect, said stable nitroxyl radical compound is selected from the groups consisting of 3 β -DOXYL-5 α -cholestane, 5-DOXYL-stearic acid, 16-DOXYL-stearic acid, Methyl 5-DOXYL-stearate, 3-(Aminomethyl)-PROXYL, 3-Carbamoyl-PROXYL, 3-Carbamoyl-2,2,5,5-tetramethyl-3-pyrrolin-1-oxyl, 3-Carboxy-PROXYL, 3-Cyano-PROXYL. In an aspect, the oxidant is a molecule bearing a N-halo moiety. Preferably said molecule has the ability to selectively oxidize primary alcohol in the presence of a nitroxyl radical compound. In an aspect, said oxidant is N-Chlorosuccinimide, N-Bromosuccinimide, N-Iodosuccinimide, Dichloroisocyanuric acid, 1,3,5-trichloro-1,3,5-triazinane-2,4,6-trione, Dibromoisocyanuric acid, 1,3,5-tribromo-1,3,5-triazinane-2,4,6-trione, Diiodoisocyanuric acid or 1,3,5-triiodo-1,3,5-triazinane-2,4,6-trione. In an aspect, said oxidant is selected from the group consisting of N-Chlorosuccinimide, N-Bromosuccinimide, N-Iodosuccinimide, Dichloroisocyanuric acid, 1,3,5-trichloro-1,3,5-triazinane-2,4,6-trione, Dibromoisocyanuric acid, 1,3,5-tribromo-1,3,5-triazinane-2,4,6-trione, Diiodoisocyanuric acid and 1,3,5-triiodo-1,3,5-triazinane-2,4,6-trione. Preferably said oxidant is N-Chlorosuccinimide.

In an aspect, said stable nitroxyl radical compound is 2,2,6,6-Tetramethyl-1-piperidinyloxy free radical (TEMPO) and said oxidant is N-Chlorosuccinimide (NCS).

In an embodiment the invention relates to a method of detecting the presence of O-acetyl groups in *S. pneumoniae* serotype 21 glycoconjugate, said method comprising the step of: a) preparing a *S. pneumoniae* serotype 21 glycoconjugate and b) detecting the presence of O-acetyl groups in said glycoconjugate.

In an embodiment the presence of O-acetyl groups is detected by NMR, Mass Spectrometry (MS) or High-performance liquid chromatography (HPLC).

In an embodiment the presence of O-acetyl groups is detected by NMR.

5 In an embodiment the presence of O-acetyl groups is detected by Mass Spectrometry (MS).

In a preferred embodiment the presence of O-acetyl groups is detected by High-performance liquid chromatography (HPLC).

10 In an embodiment the invention relates to a method of measuring the degree of O-acetylation of an isolated *S. pneumoniae* serotype 21 polysaccharide, said method comprising the step of: a) isolating an *S. pneumoniae* serotype 21 polysaccharide and b) measuring the amount of O-acetyl groups in said polysaccharide.

In an embodiment the amount of O-acetyl groups is measured by NMR, Mass Spectrometry (MS) or High-performance liquid chromatography (HPLC).

In an embodiment the amount of O-acetyl groups is measured by NMR.

15 In an embodiment the amount of O-acetyl groups is measured by Mass Spectrometry (MS).

In a preferred embodiment the amount of O-acetyl groups is measured by High-performance liquid chromatography (HPLC).

20 In an embodiment the invention relates to a method of measuring the degree of O-acetylation of an isolated *S. pneumoniae* serotype 21 polysaccharide, said method comprising the step of: a) isolating an *S. pneumoniae* serotype 21 polysaccharide b) treating said polysaccharide with a base and c) measuring the amount of O-acetyl groups in said polysaccharide.

25 In an embodiment the amount of O-acetyl groups is measured by NMR, Mass Spectrometry (MS) or High-performance liquid chromatography (HPLC).

In an embodiment the amount of O-acetyl groups is measured by NMR.

In an embodiment the amount of O-acetyl groups is measured by Mass Spectrometry (MS).

30 In a preferred embodiment the amount of O-acetyl groups is measured by High-performance liquid chromatography (HPLC).

In an embodiment, the invention relates to a method of measuring the degree of O-acetylation in a reduced serotype 21 polysaccharide, said method comprising the step of: a) reacting an isolated *S. pneumoniae* serotype 21 polysaccharide with a reducing agent and b) measuring the amount of O-acetyl groups in said reduced polysaccharide.

35 In an embodiment the amount of O-acetyl groups is measured by NMR, Mass Spectrometry (MS) or High-performance liquid chromatography (HPLC).

In an embodiment the amount of O-acetyl groups is measured by NMR.

In an embodiment the amount of O-acetyl groups is measured by Mass Spectrometry (MS).

In a preferred embodiment the amount of O-acetyl groups is measured by High-performance liquid chromatography (HPLC).

5 In an embodiment, said reducing agent is sodium borohydride (NaBH₄).

In an embodiment, said isolated *S. pneumoniae* serotype 21 polysaccharide has been previously treated with an oxidizing agent. In an embodiment, the oxidizing agent is any oxidizing agent which oxidizes a terminal hydroxyl group to an aldehyde. In an embodiment, the oxidizing agent is periodate. In an embodiment, the oxidizing agent is orthoperiodate. In a preferred
10 embodiment, the oxidizing agent is sodium periodate. In an embodiment, the oxidizing agent is metaperiodate. In a preferred embodiment the oxidizing agent is sodium metaperiodate.

In an embodiment, said isolated *S. pneumoniae* serotype 21 polysaccharide has been previously treated with a stable nitroxyl radical compound and an oxidant. In an aspect, said stable nitroxyl radical compound is a molecule bearing a TEMPO or a PROXYL (2,2,5,5-tetramethyl-1-pyrrolidinyloxy) moiety. Preferably said molecule has the ability to selectively oxidize primary
15 alcohol in the presence of an oxidant, to generate aldehyde groups, without affecting secondary hydroxyl groups. More preferably said molecule has the ability to selectively oxidize primary alcohol in the presence of an oxidant, to generate aldehyde groups, without over oxidation to carboxyl groups. In an aspect, said stable nitroxyl radical compound is TEMPO, 2,2,6,6-Tetramethyl-4-(methylsulfonyloxy)-1-piperidinoxy, 4-Phosphonoxy-TEMPO, 4-Oxo-TEMPO,
20 4-Methoxy-TEMPO, 4-Isothiocyanato-TEMPO, 4-(2-Iodoacetamido)-TEMPO free radical, 4-Hydroxy-TEMPO, 4-Cyano-TEMPO, 4-Carboxy-TEMPO, 4-(2-Bromoacetamido)-TEMPO, 4-Amino-TEMPO or 4-Acetamido-2,2,6,6-tetramethylpiperidine 1-oxyl. In an aspect, said stable nitroxyl radical compound is selected from the groups consisting of TEMPO, 2,2,6,6-Tetramethyl-4-(methylsulfonyloxy)-1-piperidinoxy, 4-Phosphonoxy-TEMPO, 4-Oxo-TEMPO, 4-Methoxy-
25 TEMPO, 4-Isothiocyanato-TEMPO, 4-(2-Iodoacetamido)-TEMPO free radical, 4-Hydroxy-TEMPO, 4-Cyano-TEMPO, 4-Carboxy-TEMPO, 4-(2-Bromoacetamido)-TEMPO, 4-Amino-TEMPO, 4-Acetamido-2,2,6,6-tetramethylpiperidine 1-oxyl. Preferably said stable nitroxyl radical compound is TEMPO. In a further aspect, said stable nitroxyl radical compound is 3 β -DOXYL-5 α -cholestane, 5-DOXYL-stearic acid, 16-DOXYL-stearic acid, Methyl 5-DOXYL-stearate, 3-(Aminomethyl)-PROXYL, 3-Carbamoyl-PROXYL, 3-Carbamoyl-2,2,5,5-tetramethyl-3-pyrrolin-1-oxyl, 3-Carboxy-PROXYL or 3-Cyano-PROXYL. In a further aspect, said stable nitroxyl radical compound is selected from the groups consisting of 3 β -DOXYL-5 α -cholestane, 5-DOXYL-stearic acid, 16-DOXYL-stearic acid, Methyl 5-DOXYL-stearate, 3-(Aminomethyl)-PROXYL, 3-Carbamoyl-PROXYL, 3-Carbamoyl-2,2,5,5-tetramethyl-3-pyrrolin-1-oxyl, 3-Carboxy-PROXYL, 3-Cyano-PROXYL. In an aspect, the oxidant is a molecule bearing a N-halo moiety. Preferably said molecule has the ability to selectively oxidize primary alcohol in the presence of a nitroxyl radical compound. In an aspect, said oxidant is N-Chlorosuccinimide, N-Bromosuccinimide, N-

Iodosuccinimide, Dichloroisocyanuric acid, 1,3,5-trichloro-1,3,5-triazinane-2,4,6-trione, Dibromoisocyanuric acid, 1,3,5-tribromo-1,3,5-triazinane-2,4,6-trione, Diiodoisocyanuric acid or 1,3,5-triiodo-1,3,5-triazinane-2,4,6-trione. In an aspect, said oxidant is selected from the group consisting of N-Chlorosuccinimide, N-Bromosuccinimide, N-Iodosuccinimide, Dichloroisocyanuric acid, 1,3,5-trichloro-1,3,5-triazinane-2,4,6-trione, Dibromoisocyanuric acid, 1,3,5-tribromo-1,3,5-triazinane-2,4,6-trione, Diiodoisocyanuric acid and 1,3,5-triiodo-1,3,5-triazinane-2,4,6-trione. Preferably said oxidant is N-Chlorosuccinimide.

In an aspect, said stable nitroxyl radical compound is 2,2,6,6-Tetramethyl-1-piperidinyloxy free radical (TEMPO) and said oxidant is N-Chlorosuccinimide (NCS).

In an embodiment the invention relates to a method of measuring the degree of O-acetylation in *S. pneumoniae* serotype 21 glycoconjugate, said method comprising the step of: a) preparing a *S. pneumoniae* serotype 21 glycoconjugate and b) measuring the amount of O-acetyl groups in said glycoconjugate.

In an embodiment the amount of O-acetyl groups is measured by NMR, Mass Spectrometry (MS) or High-performance liquid chromatography (HPLC).

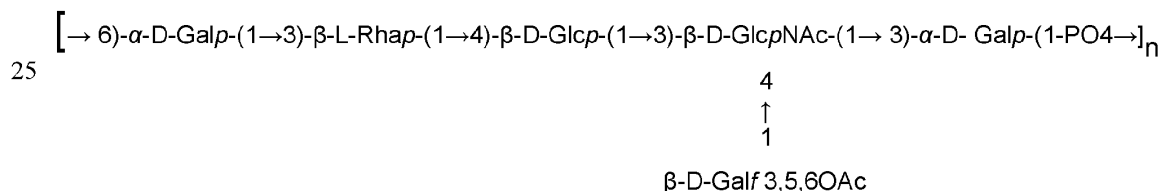
In an embodiment the amount of O-acetyl groups is measured by NMR.

In an embodiment the amount of O-acetyl groups is measured by Mass Spectrometry (MS).

In a preferred embodiment the amount of O-acetyl groups is measured by High-performance liquid chromatography (HPLC).

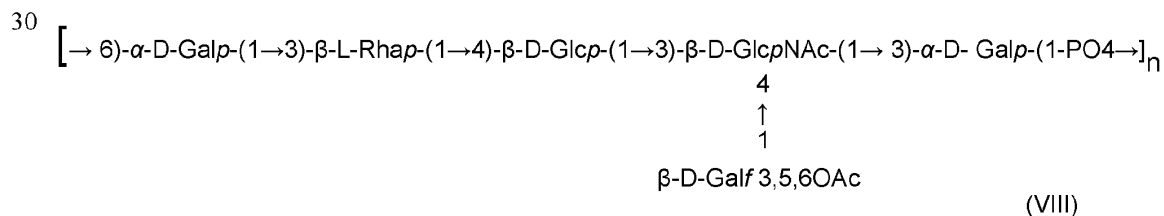
10 Particular embodiments of the invention are set forth in the following numbered paragraphs 1 to 324:

1. An isolated *S. pneumoniae* serotype 21 saccharide with the following repeating unit:



where n represents the number of repeating units.

2. An isolated *S. pneumoniae* serotype 21 saccharide with the following repeating unit:



where n represents the number of repeating units, wherein the O-acetyl group at position 3 of $\beta\text{-D-Galf}$ is present in about 50% to about 80% of the repeating units, the O-acetyl group at position

6 of β -D-Galf is present in about 20% to about 40% of the repeating units, wherein the O-acetyl group at position 5 of β -D-Galf is present in about 1% to about 10% of the repeating units.

3. The isolated *S. pneumoniae* serotype 21 saccharide of paragraph 2, wherein all the repeating unit bears one O-acetyl group on the β -D-Galf residue.

5 4. An isolated *S. pneumoniae* serotype 21 saccharide with the repeating unit of formula (VIII), where n represents the number of repeating units, wherein the O-acetyl group at position 3 of β -D-Galf is present in about 60% of the repeating units, the O-acetyl group at position 6 of β -D-Galf is present in about 30% of the repeating units, wherein the O-acetyl group at position 5 of β -D-Galf is present in about 10% of the repeating units.

10 5. The isolated *S. pneumoniae* serotype 21 saccharide of paragraph 4, wherein all the repeating unit bears one O-acetyl group on the β -D-Galf residue.

6. An isolated *S. pneumoniae* serotype 21 saccharide with the repeating unit of formula (VIII), where n represents the number of repeating units, wherein the O-acetyl group at position 3 of β -D-Galf is present in about 64% of the repeating units, the O-acetyl group at position 6 of β -D-Galf is present in about 30% of the repeating units, wherein the O-acetyl group at position 5 of β -D-Galf is present in about 6% of the repeating units.

7. The isolated *S. pneumoniae* serotype 21 saccharide of paragraph 6, wherein all the repeating unit bears one O-acetyl group on the β -D-Galf residue.

8. An isolated *S. pneumoniae* serotype 21 saccharide with the repeating unit of formula (VIII),
20 where n represents the number of repeating units, wherein the O-acetyl group at position 3 of β -D-Galf is present in about 25% to about 60% of the repeating units, the O-acetyl group at position 6 of β -D-Galf is present in about 30% to about 65% of the repeating units, wherein the O-acetyl group at position 5 of β -D-Galf is present in about 5% to about 15% of the repeating units.

9. The isolated *S. pneumoniae* serotype 21 saccharide of paragraph 8, wherein all the repeating
25 unit bears one O-acetyl group on the β -D-Galf residue.

10. An isolated *S. pneumoniae* serotype 21 saccharide with the repeating unit of formula (VIII), where n represents the number of repeating units, wherein the O-acetyl group at position 3 of β -D-Galf is present in about 50% of the repeating units, the O-acetyl group at position 6 of β -D-Galf is present in about 40% of the repeating units, wherein the O-acetyl group at position 5 of β -D-Galf is present in about 10% of the repeating units.

11. The isolated *S. pneumoniae* serotype 21 saccharide of paragraph 10, wherein all the repeating unit bears one O-acetyl group on the β -D-Galf residue.

12. An isolated *S. pneumoniae* serotype 21 saccharide with the repeating unit of formula (VIII), where n represents the number of repeating units, wherein the O-acetyl group at position 3 of β -D-Galf is present in about 47% of the repeating units, the O-acetyl group at position 6 of β -D-Galf
35

is present in about 42% of the repeating units, wherein the O-acetyl group at position 5 of β -D-Galp is present in about 11% of the repeating units.

13. The isolated *S. pneumoniae* serotype 21 saccharide of paragraph 12, wherein all the repeating unit bears one O-acetyl group on the β -D-Galp residue.

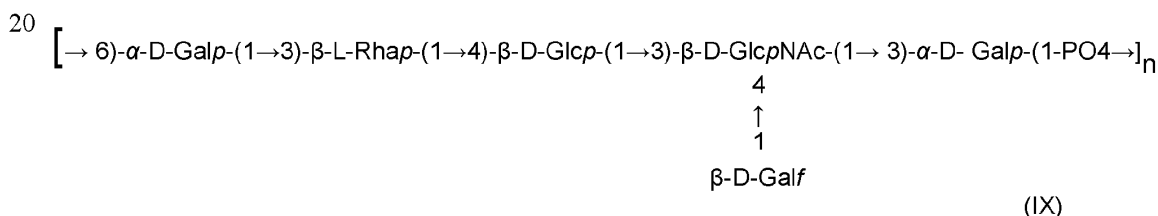
5 14. An isolated *S. pneumoniae* serotype 21 saccharide with the repeating unit of formula (VIII), where n represents the number of repeating units, wherein the O-acetyl group at position 3 of β -D-Galp is present in about 30% of the repeating units, the O-acetyl group at position 6 of β -D-Galp is present in about 60% of the repeating units, wherein the O-acetyl group at position 5 of β -D-Galp is present in about 10% of the repeating units.

10 15. The isolated *S. pneumoniae* serotype 21 saccharide of paragraph 14, wherein all the repeating unit bears one O-acetyl group on the β -D-Galp residue.

16. An isolated *S. pneumoniae* serotype 21 saccharide with the repeating unit of formula (VIII), where n represents the number of repeating units, wherein the O-acetyl group at position 3 of β -D-Galp is present in about 31% of the repeating units, the O-acetyl group at position 6 of β -D-Galp is present in about 61% of the repeating units, wherein the O-acetyl group at position 5 of β -D-Galp is present in about 8% of the repeating units.

17. The isolated *S. pneumoniae* serotype 21 saccharide of paragraph 16, wherein all the repeating unit bears one O-acetyl group on the β -D-Galp residue.

18. An isolated *S. pneumoniae* serotype 21 saccharide with the following repeating unit:



25 where n represents the number of repeating units.

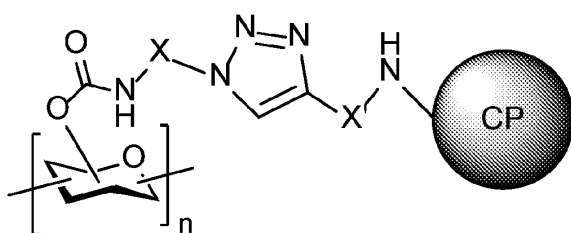
19. An isolated *S. pneumoniae* serotype 21 saccharide with the repeating unit of formula (VIII), where n represents the number of repeating units, and wherein the O-acetyl group at position 3, 5 or 6 of β -D-Galp is present in about 0% to about 95% of the repeating units.

20. An isolated *S. pneumoniae* serotype 21 saccharide with the repeating unit of formula (VIII),
30 where n represents the number of repeating units, and wherein the O-acetyl group at position 3, 5 or 6 of β -D-Galp is present in about 10% to about 90% of the repeating units.

21. An isolated *S. pneumoniae* serotype 21 saccharide with the repeating unit of formula (VIII), where n represents the number of repeating units, and wherein the O-acetyl group at position 3, 5 or 6 of β -D-Galp is present in about 30% to about 60% of the repeating units.

22. The isolated *S. pneumoniae* serotype 21 saccharide of any one of paragraphs 1-21 having between 10 and 5,000 repeating units.
23. The isolated *S. pneumoniae* serotype 21 saccharide of any one of paragraphs 1-21 having between 50 and 4,500 repeating units.
- 5 24. The isolated *S. pneumoniae* serotype 21 saccharide of any one of paragraphs 1-21 having between 100 and 4,500 repeating units.
25. The isolated *S. pneumoniae* serotype 21 saccharide of any one of paragraphs 1-21 having between 150 and 2,000 repeating units.
26. The isolated *S. pneumoniae* serotype 21 saccharide of any one of paragraphs 1-21 having a
10 weight average molecular weight between 5 kDa and 5000 kDa.
27. The isolated *S. pneumoniae* serotype 21 saccharide of any one of paragraphs 1-21 having a weight average molecular weight between 5 kDa and 2000 kDa.
28. The isolated *S. pneumoniae* serotype 21 saccharide of any one of paragraphs 1-21 having a weight average molecular weight between 50 kDa and 5000 kDa.
- 15 29. The isolated *S. pneumoniae* serotype 21 saccharide of any one of paragraphs 1-21 having a weight average molecular weight between 50 kDa and 1000 kDa.
30. The isolated *S. pneumoniae* serotype 21 saccharide of any one of paragraphs 1-21 having a weight average molecular weight between 100 kDa and 5000 kDa.
31. The isolated *S. pneumoniae* serotype 21 saccharide of any one of paragraphs 1-21 having a
20 weight average molecular weight between 100 kDa and 1000 kDa.
32. The isolated *S. pneumoniae* serotype 21 saccharide of any one of paragraphs 1-21 having a weight average molecular weight between 100 kDa and 500 kDa.
33. The isolated *S. pneumoniae* serotype 21 saccharide of any one of paragraphs 1-21 having a weight average molecular weight between 300 kDa and 5000 kDa.
- 25 34. The isolated *S. pneumoniae* serotype 21 saccharide of any one of paragraphs 1-21 having a weight average molecular weight between 300 kDa and 1000 kDa.
35. The isolated *S. pneumoniae* serotype 21 saccharide of any one of paragraphs 1-21 having a weight average molecular weight between 500 kDa and 3000 kDa.
36. The isolated *S. pneumoniae* serotype 21 saccharide of any one of paragraphs 1-21 having a
30 weight average molecular weight between 500 kDa and 2000 kDa.
37. The isolated *S. pneumoniae* serotype 21 saccharide of any one of paragraphs 1-21 having a weight average molecular weight between 500 kDa and 1000 kDa.

38. The isolated *S. pneumoniae* serotype 21 saccharide of any one of paragraphs 1-21 sized to a weight average molecular weight between 10 kDa and 1000 kDa.
39. The isolated *S. pneumoniae* serotype 21 saccharide of any one of paragraphs 1-21 sized to a weight average molecular weight between 50 kDa and 500 kDa.
- 5 40. The isolated *S. pneumoniae* serotype 21 saccharide of any one of paragraphs 1-21 sized to a weight average molecular weight between 100 kDa and 400 kDa.
41. A glycoconjugate comprising an isolated *S. pneumoniae* serotype 21 saccharide of any one of paragraphs 1-40 conjugated to a carrier protein.
42. A glycoconjugate consisting of an isolated *S. pneumoniae* serotype 21 saccharide of any one of paragraphs 1-40 conjugated to a carrier protein.
- 10 43. The serotype 21 glycoconjugate of paragraph 41 or 42 wherein said serotype 21 glycoconjugate comprises a serotype 21 saccharide covalently conjugated to a carrier protein through a (2-((2-oxoethyl)thio)ethyl)carbamate (eTEC) spacer.
44. The serotype 21 glycoconjugate of paragraph 41 or 42 wherein said serotype 21 glycoconjugate is prepared by click chemistry.
- 15 45. The serotype 21 glycoconjugate of paragraph 41 or 42 wherein said serotype 21 glycoconjugate is prepared by direct reductive amination.
- 45a. The serotype 21 glycoconjugate of paragraph 41 or 42 wherein said serotype 21 glycoconjugate is prepared by CDI or CDT chemistry.
- 20 46. A serotype 21 glycoconjugate comprising a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (IV):

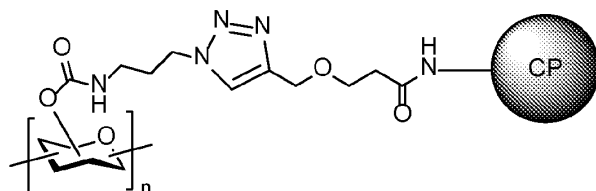


(IV),

- 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 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,
- wherein the structure in square bracket represents a repeat unit of the serotype 21 saccharide and
- 30 wherein n represents the number of repeating units.

47. A serotype 21 glycoconjugate comprising a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (IV), 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.

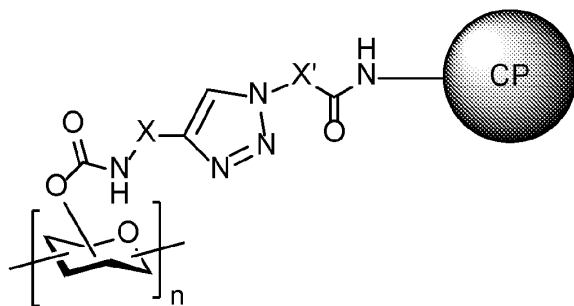
48. A serotype 21 glycoconjugate comprising a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (V),



(V),

wherein the structure in square bracket represents a repeat unit of the serotype 21 saccharide and wherein n represents the number of repeating units.

49. A serotype 21 glycoconjugate comprising a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (XVI):



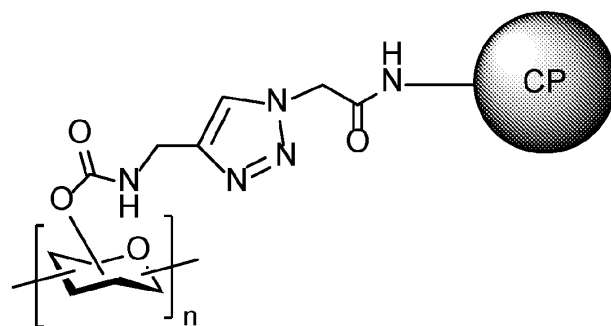
(XVI),

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 0 to 10 and m is selected from 1 to 4,

15 wherein X' is selected from the group consisting of $\text{CH}_2(\text{CH}_2)_{n''}$, $\text{CH}_2\text{O}(\text{CH}_2)_{n''}\text{CH}_2$, $\text{CH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_{m'}(\text{CH}_2)_{n''}\text{CH}_2$, where n'' is selected from 0 to 10 and m' is selected from 0 to 4 and wherein the structure in square bracket represents a repeat unit of the serotype 21 saccharide and wherein n represents the number of repeating units.

50. A serotype 21 glycoconjugate comprising a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (XVI), wherein X is $\text{CH}_2(\text{CH}_2)_{n'}$, where n' is 0 and wherein X' is $\text{CH}_2(\text{CH}_2)_{n''}$ where n'' is 0.

51. A serotype 21 glycoconjugate comprising a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (XVII),



(XVII),

wherein the structure in square bracket represents a repeat unit of the serotype 21 saccharide and wherein n represents the number of repeating units.

52. The glycoconjugate of any one of paragraphs 46 to 51 wherein said a serotype 21 saccharide is an isolated *S. pneumoniae* serotype 21 saccharide of any one of paragraphs 1-40.

53. The glycoconjugate of any one of paragraphs 41 to 52 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 21 saccharide before conjugation is between 50 kDa and 1,000 kDa.

54. The glycoconjugate of any one of paragraphs 41 to 52 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 21 saccharide before conjugation is between 100 kDa and 600 kDa.

55. The glycoconjugate of any one of paragraphs 41 to 52 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 21 saccharide before conjugation is between 100 kDa and 400 kDa.

55a. The glycoconjugate of paragraph 45 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 21 saccharide before conjugation is between 100 kDa and 400 kDa.

56. The glycoconjugate of any one of paragraphs 41 to 52 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 21 saccharide before conjugation is between 150 kDa and 300 kDa.

56a. The glycoconjugate of any one of paragraphs 41 to 52 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 21 saccharide before conjugation is between 150 kDa and 400 kDa.

56b. The glycoconjugate of paragraph 44 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 21 saccharide before conjugation is between 150 kDa and 400 kDa.

57. The glycoconjugate of any one of paragraphs 41 to 56b wherein said serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 250 kDa and 20,000 kDa.

58. The glycoconjugate of any one of paragraphs 41 to 56b wherein said serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 500 kDa and 15,000 kDa.
59. The glycoconjugate of any one of paragraphs 41 to 56b wherein said serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 500 kDa and 10,000 kDa.
60. The glycoconjugate of any one of paragraphs 41 to 56b wherein said serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 750 kDa and 7,500 kDa.
- 60a. The glycoconjugate of paragraph 44 wherein said serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 800 kDa and 7,000 kDa.
- 60b. The glycoconjugate of paragraph 44 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 21 saccharide before conjugation is between 150 kDa and 400 kDa and wherein said serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 800 kDa and 7,000 kDa.
- 60c. The glycoconjugate of paragraph 48 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 21 saccharide before conjugation is between 150 kDa and 400 kDa and wherein said serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 800 kDa and 7,000 kDa.
61. The glycoconjugate of any one of paragraphs 41 to 56b wherein said serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 1,000 kDa and 5,000 kDa.
- 61a. The glycoconjugate of paragraph 45 wherein said serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 2,000 kDa and 10,500 kDa.
- 61b. The glycoconjugate of paragraph 45 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 21 saccharide before conjugation is between 100 kDa and 400 kDa and wherein said serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 2000 kDa and 10,500 kDa.
62. The glycoconjugate of any one of paragraphs 41 to 61b wherein said conjugate comprises a serotype 21 saccharide comprising on average at least 0.5 O-acetyl group per saccharide repeating unit.
63. The glycoconjugate of any one of paragraphs 41 to 61b wherein said conjugate comprises a serotype 21 saccharide comprising on average at least 0.8 O-acetyl group per saccharide repeating unit.

64. The glycoconjugate of any one of paragraphs 41 to 61b wherein said conjugate comprises a serotype 21 saccharide comprising on average between about 0.8 and about 1 O-acetyl group per saccharide repeating unit.
65. The glycoconjugate of any one of paragraphs 41 to 61b wherein the ratio of O-acetyl group per saccharide repeating unit in the glycoconjugate to O-acetyl group per saccharide repeating unit in the isolated polysaccharide is at least 0.6.
66. The glycoconjugate of any one of paragraphs 41 to 61b wherein the ratio of O-acetyl group per saccharide repeating unit in the glycoconjugate to O-acetyl group per saccharide repeating unit in the isolated polysaccharide is at least 0.9.
67. The glycoconjugate of any one of paragraphs 41 to 66 wherein the degree of conjugation of said serotype 21 glycoconjugate is between 2 and 15.
68. The glycoconjugate of any one of paragraphs 41 to 66 wherein the degree of conjugation of said serotype 21 glycoconjugate is between 2 and 10.
69. The glycoconjugate of any one of paragraphs 41 to 66 wherein the degree of conjugation of said serotype 21 glycoconjugate is between 3 and 5.
70. The glycoconjugate of any one of paragraphs 41 to 66 wherein the degree of conjugation of said serotype 21 glycoconjugate is between 2 and 6.
71. The glycoconjugate of any one of paragraphs 41 to 66 wherein the degree of conjugation of said serotype 21 glycoconjugate is between 4 and 10.
- 71a. The glycoconjugate of paragraph 44 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 21 saccharide before conjugation is between 150 kDa and 400 kDa and wherein said serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 800 kDa and 7,000 kDa and wherein the degree of conjugation of said serotype 21 glycoconjugate is between 2 and 10.
- 71b. The glycoconjugate of paragraph 45 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 21 saccharide before conjugation is between 100 kDa and 400 kDa and wherein said serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 2000 kDa and 10,500 kDa and wherein the degree of conjugation of said serotype 21 glycoconjugate is between 2 and 10.
- 71c. The glycoconjugate of paragraph 48 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 21 saccharide before conjugation is between 150 kDa and 400 kDa and wherein said serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 800 kDa and 7,000 kDa and wherein the degree of conjugation of said serotype 21 glycoconjugate is between 2 and 10.

72. The glycoconjugate of any one of paragraphs 41 to 71c wherein the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 3.0.
73. The glycoconjugate of any one of paragraphs 41 to 71c wherein the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 2.0.
- 5 74. The glycoconjugate of any one of paragraphs 41 to 71c wherein the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.5.
75. The glycoconjugate of any one of paragraphs 41 to 71c wherein the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.8 and 1.2.
76. The glycoconjugate of any one of paragraphs 41 to 71c wherein the ratio of serotype 21
10 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.0.
77. The glycoconjugate of any one of paragraphs 41 to 71c wherein the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 1.0 and 1.5.
78. The glycoconjugate of any one of paragraphs 41 to 71c wherein the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 1.0 and 2.0.
- 15 79. The glycoconjugate of any one of paragraphs 41 to 71c wherein the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.8 and 1.2.
80. The glycoconjugate of any one of paragraphs 41 to 71c wherein the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.7 and 1.1.
- 80a. The glycoconjugate of paragraph 44 wherein the weight average molecular weight (Mw) of
20 said *S. pneumoniae* serotype 21 saccharide before conjugation is between 150 kDa and 400 kDa and wherein said serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 800 kDa and 7,000 kDa and the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.1.
- 80b. The glycoconjugate of paragraph 45 wherein the weight average molecular weight (Mw) of
25 said *S. pneumoniae* serotype 21 saccharide before conjugation is between 100 kDa and 400 kDa and wherein said serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 2000 kDa and 10,500 kDa and the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.3.
- 80c. The glycoconjugate of paragraph 44 wherein the weight average molecular weight (Mw) of
30 said *S. pneumoniae* serotype 21 saccharide before conjugation is between 150 kDa and 400 kDa and wherein said serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 800 kDa and 7,000 kDa, wherein the degree of conjugation of said serotype 21 glycoconjugate is between 2 and 10 and the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.1.

- 80d. The glycoconjugate of paragraph 45 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 21 saccharide before conjugation is between 100 kDa and 400 kDa and wherein said serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 2000 kDa and 10,500 kDa, wherein the degree of conjugation of said serotype 21 glycoconjugate is between 2 and 10 and the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.3.
- 80e. The glycoconjugate of paragraph 44 wherein the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.1.
- 80f. The glycoconjugate of paragraph 45 wherein the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.3.
- 80g. The glycoconjugate of paragraph 48 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 21 saccharide before conjugation is between 150 kDa and 400 kDa and wherein said serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 800 kDa and 7,000 kDa and the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.1.
- 80h. The glycoconjugate of paragraph 48 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 21 saccharide before conjugation is between 150 kDa and 400 kDa and wherein said serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 800 kDa and 7,000 kDa, wherein the degree of conjugation of said serotype 21 glycoconjugate is between 2 and 10 and the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.1.
81. The glycoconjugate of any one of paragraphs 41 to 80h wherein said serotype 21 glycoconjugate comprises less than about 50% of free serotype 21 saccharide compared to the total amount of serotype 21 saccharide.
82. The glycoconjugate of any one of paragraphs 41 to 80h wherein said serotype 21 glycoconjugate comprises less than about 25% of free serotype 21 saccharide compared to the total amount of serotype 21 saccharide.
- 82a. The glycoconjugate of paragraph 44 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 21 saccharide before conjugation is between 150 kDa and 400 kDa and wherein said serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 800 kDa and 7,000 kDa, wherein the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.1 and wherein said serotype 21 glycoconjugate comprises less than about 25% of free serotype 21 saccharide compared to the total amount of serotype 21 saccharide.
- 82b. The glycoconjugate of paragraph 45 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 21 saccharide before conjugation is between 100 kDa and 400 kDa

and wherein said serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 2000 kDa and 10,500 kDa, wherein the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.3 and wherein said serotype 21 glycoconjugate comprises less than about 25% of free serotype 21 saccharide compared to the total amount of serotype 21 saccharide.

82c. The glycoconjugate of paragraph 44 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 21 saccharide before conjugation is between 150 kDa and 400 kDa and wherein said serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 800 kDa and 7,000 kDa, wherein the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.1, wherein the degree of conjugation of said serotype 21 glycoconjugate is between 2 and 10 and wherein said serotype 21 glycoconjugate comprises less than about 25% of free serotype 21 saccharide compared to the total amount of serotype 21 saccharide.

82d. The glycoconjugate of paragraph 45 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 21 saccharide before conjugation is between 100 kDa and 400 kDa and wherein said serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 2000 kDa and 10,500 kDa, wherein the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.3, wherein the degree of conjugation of said serotype 21 glycoconjugate is between 2 and 10 and wherein said serotype 21 glycoconjugate comprises less than about 25% of free serotype 21 saccharide compared to the total amount of serotype 21 saccharide.

82e. The glycoconjugate of paragraph 48 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 21 saccharide before conjugation is between 150 kDa and 400 kDa and wherein said serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 800 kDa and 7,000 kDa, wherein the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.1 and wherein said serotype 21 glycoconjugate comprises less than about 25% of free serotype 21 saccharide compared to the total amount of serotype 21 saccharide.

82f. The glycoconjugate of paragraph 48 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 21 saccharide before conjugation is between 150 kDa and 400 kDa and wherein said serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 800 kDa and 7,000 kDa, wherein the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.1, wherein the degree of conjugation of said serotype 21 glycoconjugate is between 2 and 10 and wherein said serotype 21 glycoconjugate comprises less than about 25% of free serotype 21 saccharide compared to the total amount of serotype 21 saccharide.

83. The glycoconjugate of any one of paragraphs 41 to 80h wherein said serotype 21 glycoconjugate comprises less than about 20% of free serotype 21 saccharide compared to the total amount of serotype 21 saccharide.
84. The glycoconjugate of any one of paragraphs 41 to 80h wherein said serotype 21 glycoconjugate comprises less than about 15% of free serotype 21 saccharide compared to the total amount of serotype 21 saccharide.
85. The glycoconjugate of any one of paragraphs 41 to 84 wherein at least 30% of the serotype 21 glycoconjugate has a K_d below or equal to 0.3 in a CL-4B column.
86. The glycoconjugate of any one of paragraphs 41 to 84 wherein at least 40% of the glycoconjugate has a K_d below or equal to 0.3 in a CL-4B column.
87. The glycoconjugate of any one of paragraphs 41 to 84 wherein at least 60% of the serotype 21 glycoconjugate has a K_d below or equal to 0.3 in a CL-4B column.
88. The glycoconjugate of any one of paragraphs 41 to 84 wherein between 50% and 80% of the serotype 21 glycoconjugate has a K_d below or equal to 0.3 in a CL-4B column.
89. The glycoconjugate of any one of paragraphs 41 to 84 wherein between 65% and 80% of the serotype 21 glycoconjugate has a K_d below or equal to 0.3 in a CL-4B column.
- 89a. The glycoconjugate of paragraph 44 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 21 saccharide before conjugation is between 150 kDa and 400 kDa and wherein said serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 800 kDa and 7,000 kDa, wherein the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.1, wherein said serotype 21 glycoconjugate comprises less than about 25% of free serotype 21 saccharide compared to the total amount of serotype 21 saccharide and wherein between 65% and 80% of the serotype 21 glycoconjugate has a K_d below or equal to 0.3 in a CL-4B column.
- 89b. The glycoconjugate of paragraph 45 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 21 saccharide before conjugation is between 100 kDa and 400 kDa and wherein said serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 2000 kDa and 10,500 kDa, wherein the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.3, wherein said serotype 21 glycoconjugate comprises less than about 25% of free serotype 21 saccharide compared to the total amount of serotype 21 saccharide and wherein between 65% and 80% of the serotype 21 glycoconjugate has a K_d below or equal to 0.3 in a CL-4B column.
- 89c. The glycoconjugate of paragraph 44 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 21 saccharide before conjugation is between 150 kDa and 400 kDa and wherein said serotype 21 glycoconjugate has a weight average molecular weight (Mw) of

between 800 kDa and 7,000 kDa, wherein the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.1, wherein the degree of conjugation of said serotype 21 glycoconjugate is between 2 and 10, wherein said serotype 21 glycoconjugate comprises less than about 25% of free serotype 21 saccharide compared to the total amount of serotype 21 saccharide and wherein between 65% and 80% of the serotype 21 glycoconjugate has a K_d below or equal to 0.3 in a CL-4B column.

89d. The glycoconjugate of paragraph 45 wherein the weight average molecular weight (M_w) of said *S. pneumoniae* serotype 21 saccharide before conjugation is between 100 kDa and 400 kDa and wherein said serotype 21 glycoconjugate has a weight average molecular weight (M_w) of between 2000 kDa and 10,500 kDa, wherein the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.3, wherein the degree of conjugation of said serotype 21 glycoconjugate is between 2 and 10, wherein said serotype 21 glycoconjugate comprises less than about 25% of free serotype 21 saccharide compared to the total amount of serotype 21 saccharide and wherein between 65% and 80% of the serotype 21 glycoconjugate has a K_d below or equal to 0.3 in a CL-4B column.

89e. The glycoconjugate of paragraph 48 wherein the weight average molecular weight (M_w) of said *S. pneumoniae* serotype 21 saccharide before conjugation is between 150 kDa and 400 kDa and wherein said serotype 21 glycoconjugate has a weight average molecular weight (M_w) of between 800 kDa and 7,000 kDa, wherein the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.1, wherein said serotype 21 glycoconjugate comprises less than about 25% of free serotype 21 saccharide compared to the total amount of serotype 21 saccharide and wherein between 65% and 80% of the serotype 21 glycoconjugate has a K_d below or equal to 0.3 in a CL-4B column.

89f. The glycoconjugate of paragraph 48 wherein the weight average molecular weight (M_w) of said *S. pneumoniae* serotype 21 saccharide before conjugation is between 150 kDa and 400 kDa and wherein said serotype 21 glycoconjugate has a weight average molecular weight (M_w) of between 800 kDa and 7,000 kDa, wherein the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.1, wherein the degree of conjugation of said serotype 21 glycoconjugate is between 2 and 10, wherein said serotype 21 glycoconjugate comprises less than about 25% of free serotype 21 saccharide compared to the total amount of serotype 21 saccharide and wherein between 65% and 80% of the serotype 21 glycoconjugate has a K_d below or equal to 0.3 in a CL-4B column.

90. The glycoconjugate of any one of paragraphs 41 to 89f wherein the carrier protein of the serotype 21 saccharide glycoconjugate is selected in the group consisting of: TT, DT, DT mutants and a C5a peptidase from *Streptococcus* (SCP).

91. The glycoconjugate of any one of paragraphs 41 to 89f wherein the carrier protein of the serotype 21 saccharide glycoconjugate is rhizavidin [aa 45-179J-GGGGSSS-SP1500- AAA-SP0785] (CP1).
- 91a. The glycoconjugate of any one of paragraphs 41 to 89f wherein the carrier protein of the serotype 21 saccharide glycoconjugate is Rhavi-linker-PdT(G294P)-linker-SP0435 [aa 62-185] fusion protein (SPP2).
92. The glycoconjugate of any one of paragraphs 41 to 89f wherein the carrier protein of the serotype 21 saccharide glycoconjugate is DT.
93. The glycoconjugate of any one of paragraphs 41 to 89f wherein the carrier protein of the serotype 21 saccharide glycoconjugate is TT.
94. The glycoconjugate of any one of paragraphs 41 to 89f wherein the carrier protein of the serotype 21 saccharide glycoconjugate is PD.
95. The glycoconjugate of any one of paragraphs 41 to 89f wherein the carrier protein of the serotype 21 saccharide glycoconjugate is CRM₁₉₇ or a C5a peptidase from *Streptococcus* (SCP).
96. The glycoconjugate of any one of paragraphs 41 to 89f wherein the carrier protein of the serotype 21 saccharide glycoconjugate is CRM₁₉₇.
97. The glycoconjugate of any one of paragraphs 41 to 89f wherein the carrier protein of the serotype 21 saccharide glycoconjugate is SCP.
98. The glycoconjugate of any one of paragraphs 41 to 89f wherein the carrier protein of the serotype 21 saccharide glycoconjugate is an enzymatically inactive SCP.
99. The glycoconjugate of any one of paragraphs 41 to 89f wherein the carrier protein of the serotype 21 saccharide glycoconjugate is an enzymatically inactive SCP from GBS (SCPB).
100. The glycoconjugate of any one of paragraphs 41 to 89f wherein the carrier protein of the serotype 21 saccharide glycoconjugate is an enzymatically inactive SCP from GAS (SCPA).
101. The glycoconjugate of any one of paragraphs 41 to 89f wherein the carrier protein of the serotype 21 saccharide glycoconjugate is a fragment of an SCPB.
102. The glycoconjugate of any one of paragraphs 41 to 89f wherein the carrier protein of the serotype 21 saccharide glycoconjugate is an enzymatically inactive fragment of SCP which consists of SEQ ID NO: 1.
103. The glycoconjugate of any one of paragraphs 41 to 89f wherein the carrier protein of the serotype 21 saccharide glycoconjugate is an enzymatically inactive fragment of SCP which consists of SEQ ID NO: 2.
104. An immunogenic composition comprising a *S. pneumoniae* serotype 21 saccharide according to any one of paragraphs 1-40.

105. An immunogenic composition comprising a *S. pneumoniae* serotype 21 saccharide glycoconjugate according to any one of paragraphs 41 to 103.
106. The immunogenic composition of paragraph 105 comprising from 1 to 45 different glycoconjugates.
- 5 107. The immunogenic composition of paragraph 105 comprising from 1 to 45 glycoconjugates from different serotypes of *S. pneumoniae*.
108. The immunogenic composition of paragraph 105 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*.
109. The immunogenic composition of paragraph 105 comprising glycoconjugates from 16 or 20
10 different serotypes of *S. pneumoniae*.
110. The immunogenic composition of paragraph 105 which is a 21, 22, 23, 24 or 25-valent pneumococcal conjugate composition.
111. The immunogenic composition of paragraph 105 which is a 21-valent pneumococcal conjugate composition.
- 15 112. The immunogenic composition of paragraph 105 which is a 22-valent pneumococcal conjugate composition.
113. The immunogenic composition of paragraph 105 which is a 23-valent pneumococcal conjugate composition.
114. The immunogenic composition of paragraph 105 which is a 24-valent pneumococcal
20 conjugate composition.
115. The immunogenic composition of paragraph 105 which is a 25-valent pneumococcal conjugate composition.
116. The immunogenic composition of paragraph 105 comprising glycoconjugates from 26 to 45 glycoconjugates from different serotypes of *S. pneumoniae*.
- 25 117. The immunogenic composition of paragraph 105 comprising glycoconjugates from 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44 or 45 different serotypes of *S. pneumoniae*.
118. The immunogenic composition of paragraph 105 comprising glycoconjugates from 35 or 45 different serotypes of *S. pneumoniae*.
- 30 118a. The immunogenic composition of paragraph 105 which is a 35-valent pneumococcal conjugate composition
119. The immunogenic composition of paragraph 105 which is a 35, 36, 37, 38, 39, 40, 41, 42, 43, 44 or 45-valent pneumococcal conjugate composition.

120. The immunogenic composition of paragraph 105 which is a 40, 41, 42, 43, 44 or 45-valent pneumococcal conjugate composition.
121. The immunogenic composition of paragraph 105 which is a 40-valent pneumococcal conjugate composition.
- 5 122. The immunogenic composition of paragraph 105 which is a 41-valent pneumococcal conjugate composition.
123. The immunogenic composition of paragraph 105 which is a 42-valent pneumococcal conjugate composition.
124. The immunogenic composition of paragraph 105 which is a 43-valent pneumococcal
10 conjugate composition.
125. The immunogenic composition of paragraph 105 which is a 44-valent pneumococcal conjugate composition.
126. The immunogenic composition of paragraph 105 which is a 45-valent pneumococcal conjugate composition.
- 15 127. The immunogenic composition of any one of paragraphs 105-126 further comprising glycoconjugates from *S. pneumoniae* serotypes 4, 6B, 9V, 14, 18C, 19F and 23F.
128. The immunogenic composition of paragraph 127 further comprising glycoconjugates from *S. pneumoniae* serotypes 1, 5 and 7F.
129. The immunogenic composition of paragraph 128 further comprising a glycoconjugate from
20 *S. pneumoniae* serotype 3.
130. The immunogenic composition of paragraph 129 further comprising glycoconjugates from *S. pneumoniae* serotypes 6A and 19A.
131. The immunogenic composition of paragraph 130 further comprising glycoconjugates from *S. pneumoniae* serotype 22F and 33F.
- 25 132. The immunogenic composition of paragraph 1131 further comprising glycoconjugates from *S. pneumoniae* serotypes 8, 10A, 11A, 12F and 15B.
133. The immunogenic composition of paragraph 132 further comprising a glycoconjugate from *S. pneumoniae* serotype 2.
134. The immunogenic composition of paragraph 133 further comprising a glycoconjugate from
30 *S. pneumoniae* serotype 9N.
135. The immunogenic composition of paragraph 134 further comprising a glycoconjugate from *S. pneumoniae* serotype 17F.

136. The immunogenic composition of paragraph 135 further comprising a glycoconjugate from *S. pneumoniae* serotype 20.
137. The immunogenic composition of paragraph 136 further comprising a glycoconjugate from *S. pneumoniae* serotype 2.
- 5 138. The immunogenic composition of paragraph 137 further comprising a glycoconjugate from *S. pneumoniae* serotype 15C.
139. The immunogenic composition of any one of paragraphs 105-108 further comprising glycoconjugates from *S. pneumoniae* serotypes 4, 6B, 9V, 14, 18C, 19F and 23F and wherein said immunogenic composition is an 8-valent pneumococcal conjugate composition.
- 10 140. The immunogenic composition of any one of paragraphs 105-108 further comprising glycoconjugates from *S. pneumoniae* serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F and wherein said immunogenic composition is an 11-valent pneumococcal conjugate composition.
141. The immunogenic composition of any one of paragraphs 105-108 further comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and
15 23F and wherein said immunogenic composition is a 14-valent pneumococcal conjugate composition.
142. The immunogenic composition of any one of paragraphs 105-108 further comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F and wherein said immunogenic composition is a 16-valent pneumococcal
20 conjugate composition.
143. The immunogenic composition of any one of paragraphs 105-108 further 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 wherein said immunogenic composition is a 21-valent pneumococcal conjugate composition.
- 25 143. The immunogenic composition of any one of paragraphs 105-108 further comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15C, 18C, 19A, 19F, 22F, 23F and 33F.
144. The immunogenic composition of paragraph 168 which is a 21-valent pneumococcal conjugate composition.
- 30 145. The immunogenic composition of any one of paragraphs 105-108 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.
146. The immunogenic composition of paragraph 145 which is a 22-valent pneumococcal conjugate composition.

147. The immunogenic composition of any one of paragraphs 105-108 further comprising glycoconjugates from *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15C, 18C, 19A, 19F, 22F, 23F and 33F.
148. The immunogenic composition of paragraph 147 which is a 22-valent pneumococcal conjugate composition.
149. The immunogenic composition of any one of paragraphs 105-108 further 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.
150. The immunogenic composition of paragraph 149 which is a 22-valent pneumococcal conjugate composition.
151. The immunogenic composition of any one of paragraphs 105-108 further comprising glycoconjugates from *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 9N, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F and 33F.
152. The immunogenic composition of paragraph 151 which is a 23-valent pneumococcal conjugate composition.
153. The immunogenic composition of any one of paragraphs 105-108 further 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, 22F, 23F and 33F.
154. The immunogenic composition of paragraph 153 which is a 24-valent pneumococcal conjugate composition.
155. The immunogenic composition of any one of paragraphs 105-108 further 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.
156. The immunogenic composition of paragraph 155 which is a 25-valent pneumococcal conjugate composition.
157. The immunogenic composition of any one of paragraphs 105-108 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, 23F and 33F.
158. The immunogenic composition of paragraph 157 which is a 22-valent pneumococcal conjugate composition.
159. The immunogenic composition of any one of paragraphs 105-108 further 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.

160. The immunogenic composition of paragraph 159 which is a 22-valent pneumococcal conjugate composition.

161. The immunogenic composition of any one of paragraphs 105-108 further comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14,
5 15B, 18C, 19A, 19F, 22F, 23B, 23F and 33F.

162. The immunogenic composition of paragraph 186 which is a 22-valent pneumococcal conjugate composition.

163. The immunogenic composition of any one of paragraphs 105-108 further comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14,
10 15B, 18C, 19A, 19F, 22F, 23F, 24F and 33F.

164. The immunogenic composition of paragraph 163 which is a 22-valent pneumococcal conjugate composition.

165. The immunogenic composition of any one of paragraphs 105-108 further comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14,
15 15B, 18C, 19A, 19F, 22F, 23F, 33F and 35B.

166. The immunogenic composition of paragraph 165 which is a 22-valent pneumococcal conjugate composition.

167. The immunogenic composition of any one of paragraphs 105-108 further comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14,
20 15A, 15B, 18C, 19A, 19F, 22F, 23A, 23F and 33F.

168. The immunogenic composition of paragraph 167 which is a 23-valent pneumococcal conjugate composition.

169. The immunogenic composition of any one of paragraphs 105-108 further comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14,
25 15A, 15B, 18C, 19A, 19F, 22F, 23A, 23B, 23F, 24F, 33F and 35B.

170. The immunogenic composition of paragraph 169 wherein the *S. pneumoniae* saccharides are conjugated to CRM₁₉₇.

171. The immunogenic composition of paragraph 169 wherein the *S. pneumoniae* saccharides from serotypes 1, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15A, 15B, 18C, 19A, 19F, 21, 22F,
30 23A, 23B, 23F, 24F, 33F and 35B are conjugated to CRM₁₉₇ and the *S. pneumoniae* saccharide serotype 3 is conjugated to SCP.

172. The immunogenic composition of paragraph 171 which is a 26-valent pneumococcal conjugate composition.

173. The immunogenic composition of any one of paragraphs 105-108 further comprising at least one glycoconjugate selected from the group consisting of glycoconjugates from *S. pneumoniae* serotypes 2, 3, 7C, 9N, 10B, 15A, 16F, 17F, 20, 22A, 23A, 23B, 24B, 24F, 27, 29, 31, 33B, 34, 35B, 35F and 38.
- 5 174. The immunogenic composition of any one of paragraphs 105-108 further comprising twenty-one glycoconjugates selected from the group consisting of glycoconjugates from *S. pneumoniae* serotypes 2, 3, 7C, 9N, 10B, 15A, 16F, 17F, 20, 22A, 23A, 23B, 24B, 24F, 27, 29, 31, 33B, 34, 35B, 35F and 38.
- 10 175. The immunogenic composition of paragraph 174 which is a 22-valent pneumococcal conjugate composition.
176. The immunogenic composition of any one of paragraphs 105-108 further comprising glycoconjugates from serotypes 2, 3, 7C, 9N, 10B, 15A, 16F, 17F, 20, 22A, 23A, 23B, 24B, 24F, 27, 29, 31, 33B, 34, 35B, 35F and 38.
- 15 177. The immunogenic composition of paragraph 176 which is a 23-valent pneumococcal conjugate composition.
178. The immunogenic composition of any one of paragraphs 105-108 further comprising at least one glycoconjugate selected from the group consisting of glycoconjugates from *S. pneumoniae* serotypes 2, 3, 7C, 9N, 10B, 15A, 16F, 17F, 19A, 19F, 20, 22A, 23A, 23B, 24B, 24F, 27, 29, 31, 33B, 34, 35B, 35F and 38.
- 20 179. The immunogenic composition of any one of paragraphs 105-108 further comprising twenty-two glycoconjugates selected from the group consisting of glycoconjugates from *S. pneumoniae* serotypes 2, 3, 7C, 9N, 10B, 15A, 16F, 17F, 19A, 19F, 20, 22A, 23A, 23B, 24B, 24F, 27, 29, 31, 33B, 34, 35B, 35F and 38.
- 25 180. The immunogenic composition of paragraph 179 which is a 23-valent pneumococcal conjugate composition.
181. The immunogenic composition of any one of paragraphs 105-108 further comprising twenty-three glycoconjugates selected from the group consisting of glycoconjugates from *S. pneumoniae* serotypes 2, 3, 7C, 9N, 10B, 15A, 16F, 17F, 19A, 19F, 20, 22A, 23A, 23B, 24B, 24F, 27, 29, 31, 33B, 34, 35B, 35F and 38.
- 30 182. The immunogenic composition of paragraph 181 which is a 24-valent pneumococcal conjugate composition.
183. The immunogenic composition of any one of paragraphs 105-150 further comprising glycoconjugates from serotypes 2, 3, 7C, 9N, 10B, 15A, 16F, 17F, 20, 22A, 23A, 23B, 24B, 24F, 27, 29, 31, 33B, 34, 35B, 35F and 38.

184. The immunogenic composition of paragraph 183 which is a 23-valent pneumococcal conjugate composition.
185. The immunogenic composition of any one of paragraphs 105-108 further comprising glycoconjugates from serotypes 2, 3, 7C, 9N, 10B, 15A, 16F, 17F, 19A, 19F, 20, 22A, 23A, 23B, 24B, 24F, 27, 29, 31, 33B, 34, 35B, 35F and 38.
186. The immunogenic composition of paragraph 185 which is a 23-valent pneumococcal conjugate composition.
187. The immunogenic composition of any one of paragraphs 104-186 further comprising at least one, two or three adjuvants.
188. The immunogenic composition of any one of paragraphs 104-186 further comprising one adjuvant.
189. The immunogenic composition of any one of paragraphs 104-186 further comprising two adjuvants.
190. The immunogenic composition of any one of paragraphs 104-186 further comprising aluminum salts (alum) as adjuvant.
191. The immunogenic composition of any one of paragraphs 104-186 further comprising aluminum phosphate as adjuvant.
192. The immunogenic composition of any one of paragraphs 104-186 further comprising a saponin based adjuvant.
193. The immunogenic composition of any one of paragraphs 104-186 further comprising a QS21 based adjuvant.
194. The immunogenic composition of any one of paragraphs 104-186 further comprising a CpG Oligonucleotide as adjuvant.
195. The immunogenic composition of any one of paragraphs 104-194 for use as a vaccine.
196. The immunogenic composition of any one of paragraphs 104-194 for use in a method of preventing, treating or ameliorating an infection, disease or condition associated with *S. pneumoniae* serotype 21 in a subject.
197. The immunogenic composition of any one of paragraphs 104-194 for use in a method of inducing an immune response to *S. pneumoniae* serotype 21 in a subject said method comprising administering to the subject an immunologically effective amount of said immunogenic composition.
198. The immunogenic composition of any one of paragraphs 104-194 for use in a method of preventing an infection by *S. pneumoniae* 21 in a subject said method comprising administering to the subject an immunologically effective amount of said immunogenic composition.

199. A method of detecting the presence of O-acetyl groups in an isolated *S. pneumoniae* serotype 21 polysaccharide, said method comprising the step of: a) isolating an *S. pneumoniae* serotype 21 polysaccharide and b) detecting the presence of O-acetyl groups in said polysaccharide.
- 5 200. The method of paragraph 199, wherein the presence of O-acetyl groups is detected by NMR, Mass Spectrometry (MS) or High-performance liquid chromatography (HPLC).
201. The method of paragraph 199, wherein the presence of O-acetyl groups is detected by NMR.
202. The method of paragraph 199, wherein the presence of O-acetyl groups is detected by Mass Spectrometry (MS).
- 10 203. The method of paragraph 199, wherein the presence of O-acetyl groups is detected by High-performance liquid chromatography (HPLC).
204. The method of paragraph 199, wherein the presence of O-acetyl groups is detected by High-performance liquid chromatography (HPLC).
205. A method of detecting the presence of O-acetyl groups in a reduced serotype 21
15 polysaccharide, said method comprising the step of: a) reacting an isolated *S. pneumoniae* serotype 21 polysaccharide with a reducing agent and b) detecting the presence of O-acetyl groups in said reduced polysaccharide.
206. The method of paragraph 205, wherein the presence of O-acetyl groups is detected by NMR, Mass Spectrometry (MS) or High-performance liquid chromatography (HPLC).
- 20 207. The method of paragraph 205, wherein the presence of O-acetyl groups is detected by NMR.
208. The method of paragraph 205, wherein the presence of O-acetyl groups is detected by Mass Spectrometry (MS).
209. The method of paragraph 205, wherein the presence of O-acetyl groups is detected by High-performance liquid chromatography (HPLC).
- 25 210. The method of paragraph 205, wherein the presence of O-acetyl groups is detected by High-performance liquid chromatography (HPLC).
211. The method of any one of paragraphs 205 to 210, wherein said reducing agent is sodium borohydride (NaBH_4).
212. The method of any one of paragraphs 205 to 211, wherein said isolated *S. pneumoniae*
30 serotype 21 polysaccharide has been previously treated with an oxidizing agent.
213. The method of paragraph 212, wherein said oxidizing agent is any oxidizing agent which oxidizes a terminal hydroxyl group to an aldehyde.
214. The method of paragraph 212, wherein said oxidizing agent is periodate.

215. The method of paragraph 212, wherein said oxidizing agent is sodium metaperiodate.

216. The method of any one of paragraphs 205 to 211, wherein said isolated *S. pneumoniae* serotype 21 polysaccharide has been previously treated with a stable nitroxyl radical compound and an oxidant.

5 217. The method of paragraph 216, wherein said stable nitroxyl radical compound is a molecule bearing a TEMPO or a PROXYL (2,2,5,5-tetramethyl-1-pyrrolidinyloxy) moiety.

218. The method of paragraph 216 or 217, wherein said oxidant is a molecule bearing a N-halo moiety.

219. The method of paragraph 216 or 217, wherein said oxidant is N-Chlorosuccinimide, N-Bromosuccinimide, N-Iodosuccinimide, Dichloroisocyanuric acid, 1,3,5-trichloro-1,3,5-triazinane-2,4,6-trione, Dibromoisocyanuric acid, 1,3,5-tribromo-1,3,5-triazinane-2,4,6-trione, Diiodoisocyanuric acid or 1,3,5-triiodo-1,3,5-triazinane-2,4,6-trione. In an aspect, said oxidant is selected from the group consisting of N-Chlorosuccinimide, N-Bromosuccinimide, N-Iodosuccinimide, Dichloroisocyanuric acid, 1,3,5-trichloro-1,3,5-triazinane-2,4,6-trione, 10 Dibromoisocyanuric acid, 1,3,5-tribromo-1,3,5-triazinane-2,4,6-trione, Diiodoisocyanuric acid and 1,3,5-triiodo-1,3,5-triazinane-2,4,6-trione.

220. The method of paragraph 216 or 217, wherein said oxidant is N-Chlorosuccinimide.

221. The method of paragraph 216, wherein said stable nitroxyl radical compound is 2,2,6,6-Tetramethyl-1-piperidinyloxy free radical (TEMPO) and said oxidant is N-Chlorosuccinimide (NCS). 20

222. A method of detecting the presence of O-acetyl groups in *S. pneumoniae* serotype 21 glycoconjugate, said method comprising the step of: a) preparing a *S. pneumoniae* serotype 21 glycoconjugate and b) detecting the presence of O-acetyl groups in said glycoconjugate.

223. The method of paragraph 222, wherein the presence of O-acetyl groups is detected by NMR, Mass Spectrometry (MS) or High-performance liquid chromatography (HPLC). 25

224. The method of paragraph 222, wherein the presence of O-acetyl groups is detected by NMR.

225. The method of paragraph 222, wherein the presence of O-acetyl groups is detected by Mass Spectrometry (MS).

226. The method of paragraph 222, wherein the presence of O-acetyl groups is detected by High-performance liquid chromatography (HPLC). 30

227. The method of paragraph 222, wherein the presence of O-acetyl groups is detected by High-performance liquid chromatography (HPLC).

228. A method of measuring the degree of O-acetylation of an isolated *S. pneumoniae* serotype 21 polysaccharide, said method comprising the step of: a) isolating an *S. pneumoniae* serotype 21 polysaccharide and b) measuring the amount of O-acetyl groups in said polysaccharide.
229. The method of paragraph 228, wherein the amount of O-acetyl groups is measured by NMR,
5 Mass Spectrometry (MS) or High-performance liquid chromatography (HPLC).
230. The method of paragraph 228, wherein the amount of O-acetyl groups is measured by NMR.
231. The method of paragraph 228, wherein the amount of O-acetyl groups is measured by Mass Spectrometry (MS).
232. The method of paragraph 228, wherein the amount of O-acetyl groups is measured by High-
10 performance liquid chromatography (HPLC).
233. A method of measuring the degree of O-acetylation of an isolated *S. pneumoniae* serotype 21 polysaccharide, said method comprising the step of: a) isolating an *S. pneumoniae* serotype 21 polysaccharide b) treating said polysaccharide with a base and c) measuring the amount of O-acetyl groups in said polysaccharide.
- 15 234. The method of paragraph 233, wherein the amount of O-acetyl groups is measured by NMR, Mass Spectrometry (MS) or High-performance liquid chromatography (HPLC).
235. The method of paragraph 233, wherein the amount of O-acetyl groups is measured by NMR.
236. The method of paragraph 233, wherein the amount of O-acetyl groups is measured by Mass Spectrometry (MS).
- 20 237. The method of paragraph 233, wherein the amount of O-acetyl groups is measured by High-performance liquid chromatography (HPLC).
238. A method of measuring the degree of O-acetylation in a reduced serotype 21 polysaccharide, said method comprising the step of: a) reacting an isolated *S. pneumoniae* serotype 21 polysaccharide with a reducing agent and b) measuring the amount of O-acetyl groups in said
25 reduced polysaccharide.
239. The method of paragraph 238, wherein the amount of O-acetyl groups is measured by NMR, Mass Spectrometry (MS) or High-performance liquid chromatography (HPLC).
240. The method of paragraph 238, wherein the amount of O-acetyl groups is measured by NMR.
241. The method of paragraph 238, wherein the amount of O-acetyl groups is measured by Mass
30 Spectrometry (MS).
242. The method of paragraph 238, wherein the amount of O-acetyl groups is measured by High-performance liquid chromatography (HPLC).

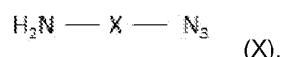
243. A method of measuring the degree of O-acetylation in *S. pneumoniae* serotype 21 glycoconjugate, said method comprising the step of: a) preparing a *S. pneumoniae* serotype 21 glycoconjugate and b) measuring the amount of O-acetyl groups in said glycoconjugate.
244. The method of paragraph 243, wherein the amount of O-acetyl groups is measured by NMR,
5 Mass Spectrometry (MS) or High-performance liquid chromatography (HPLC).
245. The method of paragraph 243, wherein the amount of O-acetyl groups is measured by NMR.
246. The method of paragraph 243, wherein the amount of O-acetyl groups is measured by Mass Spectrometry (MS).
247. The method of paragraph 243, wherein the amount of O-acetyl groups is measured by High-
10 performance liquid chromatography (HPLC).
248. The glycoconjugate of any one of paragraphs 41-103 for use as an antigen.
249. The glycoconjugate of any one of paragraphs 41-103 for use as a medicament.
250. The glycoconjugate of any one of paragraphs 41-103 for use as a vaccine.
251. The glycoconjugate of any one of paragraphs 41-103 for use in generating an immune
15 response in a subject.
252. The glycoconjugate for use of paragraph 251 wherein said subject is a human.
253. The immunogenic composition of any one of paragraphs 104-194 for use as a medicament.
254. The immunogenic composition of any one of paragraphs 104-194 for use in generating an
20 immune response in a subject.
255. The immunogenic composition for use of paragraph 254 wherein said subject is a human.
256. The immunogenic composition of any one of paragraphs 104-194 for use is a method of preventing, treating or ameliorating a bacterial infection, disease or condition in a subject.
257. The immunogenic composition of any one of paragraphs 104-194 for use is a method of
25 preventing, treating or ameliorating a *S. pneumoniae* serotype 21 infection, disease or condition in a subject.
258. The immunogenic composition of any one of paragraphs 104-194 for use is a method of inducing an immune response to *S. pneumoniae* serotype 21 in a subject.
259. The immunogenic composition of any one of paragraphs 104-194 for use is a method of
30 preventing an infection by *S. pneumoniae* serotype 21 in a subject.
260. The immunogenic composition of any one of paragraphs 104-194 for use is a method of protecting a human susceptible to a *S. pneumoniae* serotype 21 infection.

261. A method of making a *Streptococcus pneumoniae* serotype 21 glycoconjugate, using click chemistry said method comprising the steps of (a) reacting an isolated serotype 21 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.
262. The method of paragraph 261 wherein before the activation (a), sizing of the saccharide to a target molecular weight (MW) range has been performed.
263. The method of paragraph 261 wherein the isolated serotype 21 saccharide is sized before activation with a carbonic acid derivative and an azido linker.
264. The method of paragraph 263 wherein the isolated serotype 21 capsular polysaccharide is sized to a weight average molecular weight between 50 kDa and 500 kDa.
265. The method of paragraph 263 wherein the isolated serotype 21 capsular polysaccharide is sized to a weight average molecular weight between 50 kDa and 400 kDa.
266. The method of paragraph 263 wherein the isolated serotype 21 capsular polysaccharide is sized to a weight average molecular weight between 100 kDa and 500 kDa.
267. The method of paragraph 263 wherein the isolated serotype 21 capsular polysaccharide is sized to a weight average molecular weight between about 100 kDa and about 400 kDa.
268. The method of paragraph 263 wherein the isolated serotype 21 capsular polysaccharide is sized to a weight average molecular weight between about 150 kDa and about 300 kDa.
- 268a. The method of paragraph 263 wherein the isolated serotype 21 capsular polysaccharide is sized to a weight average molecular weight between about 150 kDa and about 400 kDa.
269. The method of paragraph 261 wherein the isolated serotype 21 capsular polysaccharide is not sized before activation with a carbonic acid derivative and an azido linker.
270. The method of any one of paragraphs 261 to 269 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), N,N'-Disuccinimidyl carbonate (DSC) and N-hydroxysuccinimidyl chloroformate.
271. The method of any one of paragraphs 261 to 269 wherein said carbonic acid derivative is 1,1'-carbonyldiimidazole (CDI).
272. The method of any one of paragraphs 261 to 269 wherein said carbonic acid derivative is 1,1'-Carbonyl-di-(1,2,4-triazole) (CDT).

273. The method of any one of paragraphs 261 to 269 wherein said carbonic acid derivative is N,N'-Disuccinimidyl carbonate (DSC).

274. The method of any one of paragraphs 261 to 269 wherein said carbonic acid derivative is N-hydroxysuccinimidyl chloroformate.

5 275. The method of any one of paragraphs 261 to 274 wherein said azido linker is a compound of formula (X),



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.

276. The method of any one of paragraphs 261 to 274 wherein said azido linker is a compound of formula (X), wherein X is $\text{CH}_2(\text{CH}_2)_n$, and n is selected from 1 to 10.

277. The method of any one of paragraphs 261 to 274 wherein said azido linker is a compound of formula (X), 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.

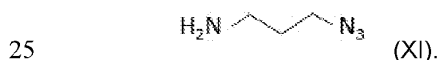
15 278. The method of any one of paragraphs 261 to 274 wherein said azido linker is a compound of formula (X), wherein X is $\text{NHCO}(\text{CH}_2)_n$, and n is selected from 1 to 10.

279. The method of any one of paragraphs 261 to 274 wherein said azido linker is a compound of formula (X), 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.

20 280. The method of any one of paragraphs 261 to 274 wherein said azido linker is a compound of formula (X), wherein X is $\text{OCH}_2(\text{CH}_2)_n$, and n is selected from 1 to 10.

281. The method of any one of paragraphs 261 to 274 wherein said azido linker is a compound of formula (X), wherein X is $\text{O}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$, where m is selected from 1 to 4.

282. The method of any one of paragraphs 261 to 274 wherein said azido linker is a compound of formula (XI),



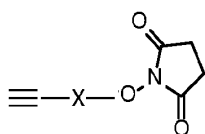
283. The method of any one of paragraphs 261 to 274 wherein said azido linker is 3-azido-propylamine.

284. The method of any one of paragraphs 261 to 283 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.

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285. The method of any one of paragraphs 261 to 283 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.

286. The method of any one of paragraphs 261 to 283 wherein said agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group is a compound of formula (XII),



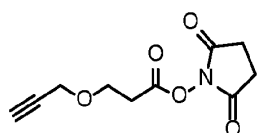
(XII),

10 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.

287. The method of any one of paragraphs 261 to 283 wherein said agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group is a compound of formula (XII), wherein
15 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.

288. The method of any one of paragraphs 261 to 283 wherein said agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group is a compound of formula (XII), 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.

20 289. The method of any one of paragraphs 261 to 283 wherein said agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group is a compound of formula (XIII):



(XIII).

290. The method of any one of paragraphs 261 to 289 wherein, step a) comprises reacting the
25 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.

291. The method of any one of paragraphs 261 to 289 wherein 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.

30 292. The method of any one of paragraphs 261 to 289 wherein at step a) the isolated saccharide is reacted with a carbonic acid derivative in an aprotic solvent.

293. The method of any one of paragraphs 261 to 289 wherein the isolated saccharide is reacted with CDI in an aprotic solvent comprising 0.1% to 1% (v/v) water.
294. The method of any one of paragraphs 261 to 293 wherein step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is
5 between 0.01-10 molar equivalent to the amount of polysaccharide Repeat Unit of the activated saccharide (molar equivalent of RU).
295. The method of any one of paragraphs 261 to 294 wherein the degree of activation of the activated saccharide following step a) is between 1.0 to 100%.
296. The method of any one of paragraphs 261 to 294 wherein the degree of activation of the
10 activated saccharide following step a) is between 5 to 70%.
297. The method of any one of paragraphs 261 to 294 wherein the degree of activation of the activated saccharide following step a) is between 15 to 50%.
298. The method of any one of paragraphs 261 to 294 wherein the degree of activation of the activated saccharide following step a) is between 10 to 40%.
- 15 299. The method of any one of paragraphs 261 to 294 wherein the degree of activation of the activated saccharide following step a) is about 25%.
300. The method of any one of paragraphs 261 to 299 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.
- 20 301. The method of any one of paragraphs 261 to 299 wherein the degree of activation of the activated carrier following step b) is between 1 and 50.
302. The method of any one of paragraphs 261 to 299 wherein the degree of activation of the activated carrier following step b) may be between 5 to 50.
303. The method of any one of paragraphs 261 to 299 wherein the degree of activation of the
25 activated carrier following step b) is between 10 to 40.
304. The method of any one of paragraphs 261 to 299 wherein the degree of activation of the activated carrier following step b) is between 15 to 25.
305. The method of any one of paragraphs 261 to 304 wherein the conjugation reaction c) is carried out in aqueous buffer.
- 30 306. The method of any one of paragraphs 261 to 304 wherein the conjugation reaction c) is carried out in aqueous buffer in the presence of copper (I) as catalyst.
307. The method of any one of paragraphs 261 to 304 wherein the conjugation reaction c) is carried out in aqueous buffer in the presence an oxidant and of copper (I) as catalyst.

308. The method of any one of paragraphs 261 to 307 wherein the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at step c) is between 0.8 and 1.2.
309. The method of any one of paragraphs 261 to 308 wherein following step c), unreacted azido groups in the conjugates, are capped using a suitable azido group capping agent.
- 5 310. The method of any one of paragraphs 261 to 309 wherein following step c) unreacted alkyne groups are capped using a suitable alkyne group capping agent.
311. The method of any one of paragraphs 261 to 310 wherein said *Streptococcus pneumoniae* serotype 21 glycoconjugate is according to any one of paragraphs 1-40.
312. A method of making a *Streptococcus pneumoniae* serotype 21 glycoconjugate, using
10 reductive amination said method comprising the steps of (1) oxidation (activation) of a serotype 21 purified saccharide, (2) reduction of the activated saccharide and the carrier protein to form a glycoconjugate.
313. The method of paragraph 312 wherein the isolated serotype 21 polysaccharide is sized before oxidation.
- 15 314. A method of making a *Streptococcus pneumoniae* serotype 21 glycoconjugate, comprising the step of: (a) reacting an isolated serotype 21 saccharide with an oxidizing agent; (b) compounding the activated saccharide of step (a) with a carrier protein; and (c) reacting the compounded activated saccharide and carrier protein with a reducing agent to form a glycoconjugate.
- 20 315. The method of paragraph 314 wherein the oxidizing agent is periodate.
316. The method of paragraph 315 wherein the degree of oxidation of the activated serotype 21 saccharide is between 2 and 30.
317. The method of paragraph 315 wherein the degree of oxidation (DO) of the activated serotype 21 saccharide is between 10 and 21.
- 25 318. The method of paragraph 315 wherein the degree of oxidation (DO) of the activated serotype 21 saccharide is between 10 and 21 and wherein the initial input ratio (weight by weight) of activated serotype 21 saccharide to carrier protein at step b) is between 1.5:1 and 0.5:1.
319. The method of paragraph 315 wherein the degree of oxidation (DO) of the activated serotype 21 saccharide is between 10 and 21 and wherein the initial input ratio (weight by weight)
30 of activated serotype 21 saccharide to carrier protein at step b) is between 1.5:1 and 0.5:1, wherein the reduction reaction (c) is carried out in an aprotic solvent.
320. The method of paragraph 315 wherein the degree of oxidation (DO) of the activated serotype 21 saccharide is between 10 and 21 and wherein the initial input ratio (weight by weight) of activated serotype 21 saccharide to carrier protein at step b) is between 1.5:1 and 0.5:1,

wherein the reduction reaction (c) is carried out in a solution consisting essentially of dimethylsulphoxide (DMSO).

321. The method of paragraph 315 wherein the degree of oxidation (DO) of the activated serotype 21 saccharide is between 10 and 21 and wherein the initial input ratio (weight by weight) of activated serotype 21 saccharide to carrier protein at step b) is between 1.5:1 and 0.5:1, wherein the reduction reaction (c) is carried out in DMSO (dimethylsulfoxide) solvent.

322. The method of paragraph 315 wherein the degree of oxidation (DO) of the activated serotype 21 saccharide is between 10 and 21 and wherein the initial input ratio (weight by weight) of activated serotype 21 saccharide to carrier protein at step b) is between 1.5:1 and 0.5:1, wherein the reduction reaction (c) is carried out in DMSO (dimethylsulfoxide) solvent and wherein the reducing agent is sodium cyanoborohydride.

323. The method of any one of paragraphs 312 to 322 wherein said *Streptococcus pneumoniae* serotype 21 glycoconjugate is according to paragraph 41 or 42.

324. The method of any one of paragraphs 312 to 322 wherein said *Streptococcus pneumoniae* serotype 21 glycoconjugate is according to any one of paragraphs 41-103.

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. 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 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.

5 Examples

Example 1: Serotype 21 capsular saccharides, samples preparation

Streptococcus pneumoniae serotype 21 polysaccharide :

25mg of lyophilized native *Streptococcus pneumoniae* serotype 21 was weighed and dissolved
10 in 1000 μ L of D₂O. The sample was bath sonicated for 20 minutes at 50°C. The polysaccharide solution was sized using sonication for 2 minutes with 20% amplitude power for 15 second ON and 15 second OFF cycles. Final concentration of the native and sized samples was 25 mg/mL. The sample was transferred into the NMR tube for NMR data collection and analysis.

Deacetylated *Streptococcus pneumoniae* serotype 21 polysaccharide:

15 25mg of native serotype 21 polysaccharide was completely deacetylated after incubating with 0.25N NH₄OH at room temperature for 24 hours with constant stirring. The sample was then dialyzed against water and lyophilized. The lyophilized polysaccharide was then resuspended in 1000 μ L of D₂O. The sample was transferred into the NMR tube for NMR data collection and analysis.

20 Example 2: Structural elucidation of *Streptococcus pneumoniae* serotype 21 polysaccharide by NMR experiments

Both one-dimensional (1D) and two-dimensional (2D) NMR experiments were conducted. 1D ¹H spectrum was collected to identify the chemical shift fingerprint for each proton in the molecule. 2D homo- and hetero-nuclear spectra are collected to identify the chemical bond pattern between
25 protons and carbons in the molecule, to map the backbone of the representative sugars in the serotype 21 polymer repeat unit, the inter-sugar glycosidic linkages, and to identify the location of the different functional groups on the sugar backbones.

All 1D and 2D data was collected at 75°C using Bruker 600 MHz spectrometer equipped with BBO cryoprobe. The NMR data was processed using NvX and analyzed using MestraNova and
30 NMRViewJ (NvJ) software (*Methods Mol. Biol.* 278, 313, 2004). The proton signals were referenced using the TMS signal set to 0 ppm, whereas the carbon signals were indirectly referenced against the TMS signal (0 ppm).

1D proton data was collected with 32 scans, with recycle delay of 10s. The following 2D NMR experiments were recorded: ¹H-¹H homonuclear COSY (Correlation Spectroscopy, 2048 x 512:
35 total number of point in the ¹H and ¹³C dimensions, respectively, with 2 scans, recycle delay 1s),

^1H - ^{13}C HSQC (Heteronuclear Single Quantum Coherence, 2048 x 256: total number of point in the ^1H and ^{13}C dimensions, respectively, with 4 scans, recycle delay 1s), ^1H - ^{13}C HSQC-TOCSY (Heteronuclear Single Quantum Coherence - Total Correlation Spectroscopy, 2048 x 256: total number of point in the ^1H and ^{13}C dimensions, respectively, with 48 scans, recycle delay 1s mixing time of 120 ms), ^1H - ^{13}C HMBC (Heteronuclear Multiple Bond Correlation, 2048 x 256: total number of point in the ^1H and ^{13}C dimensions, respectively, long range J ~ 5-10 Hz, with 48 scans, recycle delay 1s) and ^1H - ^{13}C HSQC-COSY (Heteronuclear Single Quantum Coherence – Correlation Spectroscopy, 2048 x 256: total number of point in the ^1H and ^{13}C dimensions, respectively, with 48 scans, recycle delay 1s), ^1H - ^{13}C CLIP-HSQC (^1H decoupled Heteronuclear Single Quantum Coherence, 2048 x 256: total number of point in the ^1H and ^{13}C dimensions, respectively, with 8 scans, recycle delay 1s). 1D ^{31}P data was collected with 256 scans, with recycle delay of 5s. ^1H - ^{31}P HMBC (Heteronuclear Multiple Bond Correlation, 2048 x 32: total number of points in the ^1H and ^{31}P dimensions, respectively, long range J ~ 5-10 Hz).

Structural elucidation of the polysaccharides entails assigning all the resonances of the polymer repeat unit (RU). We achieve this by analyzing both one-dimensional (1D) and two-dimensional (2D) NMR experiments. 1D ^1H spectrum was collected to identify the chemical shift fingerprint for each proton in the molecule. 2D homo- and hetero-nuclear spectra are collected to identify the chemical bond pattern between protons and carbons in the molecule, to map the backbone of the representative sugars in the serotype 21 polymer repeat unit, the inter-sugar glycosidic linkages, and to identify the location of the different functional groups on the sugar backbones.

The chemical structure of *Streptococcus pneumoniae* serotype 21 is shown in Figure 1. The serotype 21 polysaccharide is a hexasaccharide: α -D-galactopyranose (A), β -L-rhamnose (B), β -D-glucose (C), β -D-glucosamine (D), α -D-galactopyranose (E) and O-acetylated β -D-galactofuranose (F). The O-acetylation of β -D-galactofuranose is at 3, 5 and 6 position at different levels. The main chain consists of five sugars and one branched sugar (β -D-galactofuranose) linked to 4 position of β -D-glucosamine (D). The backbone repeating unit is linked via phosphate group at the reducing end of the residue E (α -D-galactopyranose) and links the 6 position of residue A (α -D-galactopyranose).

Three different populations of serotype 21 repeat unit have been found: ~64% O-acetylated at the C3 position of β -D-Galp_{3,64}OAc; ~30% O-acetylated at β -D-Galp_{6,30}OAc and ~6% O-acetylated at β -D-Galp_{5,06}OAc.

1D ^1H NMR analysis of the serotype 21 polysaccharide

The 1D ^1H NMR spectrum of native polysaccharide shown in Figure 2 can be correlated to the polysaccharide backbone containing the 6 sugar residues. The anomeric protons of the α -D-Galp (Residue A), β -L-Rhap (Residue B), β -D-Glcp (Residue C), β -D-GlcpNAc (Residue D), α -D-Galp (Residue E) and β -D-Galp_{3,64,5,06,6,30}OAc (Residue F) are at δ 5.13 ppm, δ 4.86 ppm, δ 4.53 ppm, δ 4.80 ppm, δ 5.51 ppm and δ 5.22 ppm (β -D-Galp_{3,64}OAc); δ 5.19 (β -D-Galp_{6,30}OAc),

respectively. The inset table in Figure 2 shows the excellent agreement between the normalized area under the anomeric proton peaks and the expected theoretical area. The area under these peaks were deconvoluted using the MestraNova software. The Galf (residue F) of serotype 21 polysaccharide is O-acetylated at different carbon position. The level of O-acetylation at position 3, 5 and 6 of Galf (residue F) is around 64%, 6% and 30%, respectively. The amount of O-acetylation was determined using the F1 anomeric signals and the O-acetylation signals (see inset showing the O-acetyl resonances). There is good agreement between the level of O-acetylation calculated using different resonance signals. The expanded 1D ^1H spectrum of the anomeric region of serotype 21 polysaccharide is also shown in Figure 2.

10 The anomers α or β of each sugar units were determined using the three-bond ^1H - ^1H coupling constant (3J) measured from anomeric proton signals and the ^1H - ^{13}C coupling constant ($^1J_{\text{CH}}$) of the anomeric carbon and proton. Figure 3 and Table 1 show the $^1J_{\text{CH}}$ and $^3J_{\text{HH}}$ coupling constants for the six anomeric resonances for serotype 21, which were used to determine the structure of serotype 21.

15 Table 1: $^1J_{\text{CH}}$ and $^3J_{\text{HH}}$ coupling constants for the six anomeric resonances for serotype 21.

	$^1J_{\text{CH}}$ (Hz)	$^3J_{\text{HH}}$ (Hz)
α -D-Galp (A)	174	3.7
β -L-Rhap (B)	161	-
β -D-Glcp (C)	161	7.9
β -D-GlcpNAc (D)	163	8.3
α -D-Galp (E)	174	3.3
β -D-Galf3,5,6 OAc (F)	175	-

2D NMR analysis of the serotype 21 polysaccharide

The complete structural elucidation of sized serotype 21 polysaccharide was achieved by doing the complete assignment of all the resonances of each sugar unit that are present in the repeat unit. Each of the sugar units were assigned using 2D heteronuclear NMR experiments that use short range ^1H - ^1H (HSQC-COSY), long range (multiple bonds) ^1H - ^1H (HSQC-TOCSY) or ^{13}C - ^{13}C (HMBC) correlation to map the resonances within the sugar ring.

The 2D HSQC experiment provides the correlation between the ^1H and ^{13}C signals that are directly bonded and is very sensitive for analyzing the polysaccharides. 2D HSQC-COSY yields a ^1H - ^1H short range and HSQC-TOCSY yields a ^1H - ^1H long range (mixing time ~ 120 ms) proton correlation and is very useful in assigning the resonances within the sugar ring. Similarly, the inverse detected 2D ^1H - ^{13}C HMBC experiment gives rise to cross-peaks between proton and carbon atoms that are long range scalar coupled through multiple carbon bonds. The intensities

of these cross peaks are reflected in the $^2J_{C,H}$ or $^3J_{C,H}$ values. Generally, three bond correlations from the anomeric carbon to the carbon at 3 and 5 positions are seen. Also quite prominent are the correlations to the neighbouring carbon through the glycosidic bond, which are helpful in sequential assignment of the sugar units and their connectivity.

- 5 All the sugar backbone resonances were assigned by comparing the resonances from the short range (HSQC-COSY) and long range (HSQC-TOCSY), 1H - ^{13}C HMBC NMR experiments.

The linkage between the phosphodiester and residue A and residue E was determined comparing the ^{31}P - 1H HMBC and ^{13}C - 1H HSQC spectra.

- 10 The complete set of the resonances of all sugars of serotype 21 is tabulated in Table 2 for β -D-Galf_{30.64}OAc population (left), β -D-Galf_{60.32}OAc population (middle), along with resonances of deacetylated serotype 21 (right).

Table 2. Chemical shift assignment of serotype 21 for the population β -D-Galf_{30.64}OAc (left), for the population β -D-Galf_{60.32}OAc (middle) and de-O-acetylated serotype 21 (right).

		Acetylated Pn-21 (Galf ₃₀ OAc)		Acetylated Pn-21 (Galf ₆₀ OAc)		O-Deacetylated Pn-21	
		1H (ppm)	^{13}C (ppm)	1H (ppm)	^{13}C (ppm)	1H (ppm)	^{13}C (ppm)
α -D-Galp (A)	A1	5.13	96.3	5.13	96.3	5.13	96.3
	A2	3.86	68.7	3.86	68.7	3.86	68.7
	A3	3.95	69.7	3.95	69.7	3.95	69.7
	A4	4.08	69.3	4.08	69.3	4.07	69.3
	A5	4.33	70.1	4.33	70.1	4.32	70.1
	A6-1	4.05	64.7	4.05	64.7	4.04	64.7
	A6-2	4.02	64.7	4.02	64.7	4.02	64.7
β -L-Rhap (B)	B1	4.86	100.8	4.86	100.8	4.86	100.8
	B2	4.27	67.8	4.27	67.8	4.27	67.8
	B3	3.68	78.5	3.68	78.5	3.68	78.5
	B4	3.47	71.0	3.47	71.0	3.45	71.0
	B5	3.42	72.5	3.42	72.5	3.45	72.5
	B6	1.33	17.3	1.33	17.3	1.34	17.3
β -D-Glcp (C)	C1 _{3OAc}	4.53	101.7	4.52	102.2	4.52	102.0
	C2 _{3OAc}	3.38	74.1	3.35	74.0	3.36	74.0
	C3	3.66	76.1	3.66	76.1	3.65	76.0
	C4 _{3OAc}	3.52	77.8	3.60	77.4	3.59	77.5
	C5 _{3OAc}	3.52	75.4	3.50	75.4	3.49	75.3
	C6 _{3OAc} -1	4.00	62.1	3.96	61.4	3.94	61.4
	C6 _{3OAc} -2	3.75	62.1	3.81	61.4	3.80	61.4
β -D-GlcpNAc (D)	D1	4.80	102.5	4.80	102.5	4.80	102.5
	D2	3.91	56.1	3.91	56.1	3.91	56.2
	D3 _{3OAc}	3.92	74.1	3.87	74.7	3.87	74.5
	D4 _{3OAc}	3.86	78.7	3.91	77.0	3.90	76.7

	D5	3.59	75.7	3.59	75.7	3.59	75.7
	D6-1	3.99	60.4	3.99	60.4	3.96	60.3
	D6-2	3.88	60.3	3.88	60.3	3.88	60.3
	D-NAC	2.05	22.7	2.05	22.7	2.05	22.8
	CO		175.0		175.0		175.1
α -D-Galp (E)	E1	5.51	96.0	5.51	96.0	5.51	96.0
	E2	3.92	67.6	3.92	67.6	3.92	67.6
	E3	3.94	79.5	3.94	79.5	3.93	79.5
	E4	4.24	69.3	4.24	69.3	4.23	69.3
	E5	4.11	71.8	4.11	71.8	4.10	71.8
	E6	3.73	61.4	3.73	61.4	3.73	61.4
β -Gal _f 3OAc (F)	F1 _{3OAc}	5.22	109.1			5.18	108.0
	F2 _{3OAc}	4.15	80.6			4.05	81.7
	F3 _{3OAc}	4.98	80.4			4.00	77.9
	F4 _{3OAc}	4.46	82.1			4.24	84.1
	F5 _{3OAc}	3.86	71.6			3.83	71.7
	F6 _{3OAc} -1	3.67	63.4			3.71	63.5
	F6 _{3OAc} -2	3.62	63.4			3.66	63.5
	F-3OAc	2.18	20.7				
	CO		174.4				
β -Gal _f 6OAc (F)	F1 _{6OAc}			5.19	108.2		
	F2 _{6OAc}			4.06	81.8		
	F3 _{6OAc}			4.02	77.7		
	F4 _{6OAc}			4.25	83.9		
	F5 _{6OAc}			4.03	69.0		
	F6 _{6OAc} -1			4.28	66.5		
	F6 _{6OAc} -2			4.17	66.5		
	F-6OAc			2.16	20.7		
	CO				173.9		

Example 3: Acetyl migration in serotype 21 polysaccharide by NMR experiments

Acetylation migration in serotype 21 polysaccharide has been measured at room and elevated temperature. The migration of the O-Acetyl group from 3 position to 6 position of β -D-Galp sugar has been observed after comparing the 1D ^1H spectra of serotype 21 polysaccharide at 0, 9 and 15 hours recorded at 75°C. The methyl signal ($-\text{COOCH}_3$) from 3 position of β -D-Galp decreased whereas the methyl signal at 6 position of β -D-Galp increased (Figure 4). This clearly suggests that at elevated temperature O-acetyl migration from 3 to 6 position of β -D-Galp (residue F) is observed. The level of O-acetylation at 6 position doubled after incubation at elevated temperature for 9 hrs. No further migration was observed after 9 hours as no increase in the O-acetyl signal at the 6 position was observed. The acetylation migration was observed at room temperature albeit at slower rate. The level of O-acetylation at the 6 position of β -D-Galp increased

from 29% to 42% after incubating the polysaccharide for 14 days at room temperature (21°C) (Figure 5, Table 3).

Table 3. Acetyl migration in serotype 21 when incubated at room temperature.

Days		Normalized Peak Area (E1)	Expected Peak Area	% Acetylation
0	F-5OAc	0.27	3	9
	F-3OAc	1.82		62
	F-6OAc	0.86		29
9	F-5OAc	0.28	3	10
	F-3OAc	1.54		53
	F-6OAc	1.10		38
14	F-5OAc	0.31	3	11
	F-3OAc	1.39		47
	F-6OAc	1.24		42

Example 4: Structural comparison of O-acetylated and de-O-acetylated serotype 21 polysaccharide by NMR experiments

The comparison of the anomeric and methyl regions of 1D ¹H NMR spectrum of acetylated (bottom panel) and deacetylated (top panel) serotype 21 polysaccharides is shown at Figure 6. All the resonances are annotated. Shaded box denotes the resonances which are observed due to the acetylation of β-D-Galf residue are absent in the completely deacetylated serotype 21 polysaccharide. The structural impact on the polysaccharide upon deacetylation was demonstrated by plotting the chemical shift difference between the carbon and proton resonances of the acetylated and deacetylated serotype 21 polysaccharides using the following equation: $CSD = \sqrt{\delta H^2 + (0.3 \times \delta C^2)}$, where δH and δC are the chemical shift difference of the proton and carbon resonances (Figure 7; Table 2). Left bottom panel of figure 7 shows the structural changes caused by deacetylation at Galf6OAc sugar whereas left top panel shows the structural changes caused by deacetylation at Galf3OAc sugar. Most significant shifts are observed around the deacetylation sites, illustrated using filled circles on the structure of serotype 21 polysaccharide (Figure 7, right bottom and top panel). Deacetylation at the Galf3OAc has greater impact on the structural fold of serotype 21 polysaccharide compared to the deacetylation at the Galf6OAc, which can impact the immunogenic response.

Example 5: Conjugation of *S. pneumoniae* Serotype 21 Capsular Polysaccharides by Reductive Amination

Mechanical sizing of Serotype 21 Capsular Polysaccharide

The native polysaccharide was subjected to mechanical sizing under 15000 psi pressure to
5 reduce the MW size. The sizing study was performed to identify the number of passes required
to achieve the target polysaccharide MW of 120-250 kDa.

Activating Polysaccharide

Polysaccharide oxidation was carried out in 50 mM potassium phosphate buffer (pH 6.0 $\pm$ 0.2) by
sequential addition of calculated amount of 1 M potassium phosphate buffer (pH 5.7) and water
10 for injection (WFI) to give final polysaccharide concentration of 2.0 g/L. If required, the reaction
pH was adjusted to pH 6.0, approximately. After pH adjustment, the reaction temperature was
adjusted to 5 $\pm$ 3 °C. Oxidation was initiated by the addition of approximately 0.10 molar
equivalents of sodium periodate. The oxidation reaction was performed at 5 $\pm$ 3 °C during 20 hrs,
approximately.

15 Concentration and diafiltration of the activated polysaccharide was carried out using 10K MWCO
ultrafiltration cassettes. Diafiltration was performed against 20-fold diavolumes of WFI. The
purified activated polysaccharide was then stored at 5 $\pm$ 3°C. The purified activated saccharide is
characterized, *inter alia*, by (i) saccharide concentration by colorimetric assay; (ii) aldehyde
concentration by colorimetric assay; (iii) degree of oxidation; and (iv) molecular weight by SEC-
20 MALLS.

The degree of oxidation (DO = moles of sugar repeat unit / moles of aldehyde) of the activated
polysaccharide was determined as follows:

The moles of sugar repeat unit are determined by various colorimetric methods, for example, by
using the Anthrone method. By the Anthrone method, the polysaccharide is first broken down to
25 monosaccharides by the action of sulfuric acid and heat. The Anthrone reagent reacts with the
hexoses to form a yellow-green colored complex whose absorbance is read
spectrophotometrically at 625nm. Within the range of the assay, the absorbance is directly
proportional to the amount of hexose present.

The moles of aldehyde are also determined simultaneously, using the MBTH colorimetric method.
30 The MBTH assay involves the formation of an azine compound by reacting aldehyde groups (from
a given sample) with a 3-methyl-2-benzothiazolone hydrazone (MBTH assay reagent). The
excess 3-methyl-2-benzothiazolone hydrazone oxidizes to form a reactive cation. The reactive
cation and the azine react to form a blue chromophore. The formed chromophore is then read
spectroscopically at 650 nm.

35 **Compounding Activated Polysaccharide with Sucrose Excipient, and Lyophilizing**

The activated polysaccharide was compounded with sucrose to a ratio of 25 grams of sucrose
per gram of activated polysaccharide. The bottle of compounded mixture was then lyophilized.
Following lyophilization, bottles containing lyophilized activated polysaccharide were stored at -

20 ± 5°C. Calculated amount of CRM₁₉₇ protein was shell-frozen and lyophilized separately. Lyophilized CRM₁₉₇ was stored at -20 ± 5°C.

Reconstituting Lyophilized Activated Polysaccharide and Carrier Protein

Lyophilized activated polysaccharide was reconstituted in anhydrous dimethyl sulfoxide (DMSO).

- 5 Upon complete dissolution of polysaccharide, an equal amount of anhydrous DMSO was added to lyophilized CRM₁₉₇ for reconstitution.

Conjugating and Capping

- Reconstituted activated polysaccharide was combined with reconstituted CRM₁₉₇ in the reaction vessel, followed by mixing thoroughly to obtain a clear solution before initiating the conjugation with sodium cyanoborohydride. The final polysaccharide concentration in reaction solution was approximately 1 g/L. Conjugation was initiated by adding 1.0 MEq of sodium cyanoborohydride to the reaction mixture and incubating at 23 ± 2 °C for 20-28 hrs. The conjugation reaction was terminated by adding 2 MEq of sodium borohydride (NaBH₄) to cap unreacted aldehydes. This capping reaction continued at 23 ± 2°C for 3 ± 1 hrs.

- 15 Purifying the Conjugate

The conjugate solution was diluted 1:5 with chilled 5 mM succinate/ saline (pH 6.0) in preparation for purification by tangential flow filtration using 100K MWCO membranes.

- The diluted conjugate solution was passed through a 5 µm filter, and diafiltration was performed using 5 mM succinate / saline (pH 6.0) as the medium. After the diafiltration was completed, the conjugate retentate was transferred through a 0.22µm filter. The conjugate was diluted further with 5 mM succinate / saline (pH 6.0), to a target saccharide concentration of approximately 0.5 mg/mL.

Table 4 below comprises characterizing data for serotype 21 polysaccharide conjugates obtained by methods of this invention.

- 25 **Table 4. Pneumo St 21-CRM₁₉₇ Conjugates by Reductive Amination Conjugation in DMSO**

Serotype 21 Conjugates						
Conjugate #	1	2	3	4	5	6
Sized Poly Mw (kDa)	236	236	236	187	152	128
Activation						
NaIO ₄ (MEq)	0.1	0.15	0.2	0.15	0.15	0.15
DO	20	14	11	15	16	16
Act poly Mw (kDa)	232	228	224	198	150	127
Act poly yield (%)	90	88	84	91	92	89
Conjugation						
Poly yield (%)	46	63	63	79	98	87
SPR (Output Ratio)	0.9	0.95	0.9	0.94	1.2	1
Free saccharide (%)	<5	<5	<5	<5	16	31
Conjugate MW (kDa)	2715	2558	2956	4223	3069	3358

Poly: Polysaccharide; Mw: molecular weight; MEq: Molar Equivalent; DO: Degree of Oxidation; Act Poly: Activated Polysaccharide SPR: Saccharide to protein ratio

Example 6: Conjugation of *S. pneumoniae* Serotype 21 Capsular Polysaccharides using click chemistry

1. Mechanical sizing of Serotype 21 Capsular Polysaccharide (optional)

The native polysaccharide was either used without sizing (unsized) or subjected to mechanical
5 sizing under 15000 psi pressure to reduce the MW size. The sizing study was performed to identify the number of passes required to achieve the target polysaccharide MW of 140-200 kDa.

2. Activation of Serotype 21 Capsular Polysaccharide with azido linker

Serotype 21 polysaccharide (either unsized or sized) 400 mg was mixed with 1.2 g imidazole then
frozen and lyophilized. After lyophilization, the lyophilized polysaccharide was reconstituted with
10 anhydrous DMSO 200 mL. The reaction mixture was then warmed to 35 °C, and then CDI (100 mg/mL in DMSO, 1.25 mL, 2 MEq) was added. The reaction mixture was stirred at 35 °C for 3 hrs. After the reaction mixture was cooled to 23 °C, Water For Injection (WFI) 4 mL (2% v/v) was added to quench free CDI and stirred for 30 min at 23 °C.

To the reaction mixture, 3-Azido-1-propylamine 76 µL (2 MEq) and 110 µL of triethylamine (2
15 MEq) was added and then stirred at 23 °C for 20 hrs. After the reaction, the mixture was diluted with chilled 10 mM SPB in saline (pH 7.0) (5X). The diluted reaction mixture was then purified by UF/DF using 10K MWCO PES membrane against 10 mM SPB in saline (pH 7.0) (30X volume).

3. Activation of SCP to alkyne-SCP with Alkyne NHS ester

To the SCP solution (2 g, 116 mL), 284 mL WFI and 100 mL 0.5 M Sodium Phosphate Buffer
20 (SPB) (pH 8.3) were added to achieve final concentration of 4 mg/mL with 0.1 M ionic strength for the reaction. After cooled to 8°C, 3-Propargyloxy-propanoic acid NHS ester (POPS) (20 mg/mL in DMSO) 9.2 mL (0.5 MEq to lysine on SCP) was added to the reaction mixture dropwise maintaining the reaction temperature at 8 ± 3 °C. After reaction mixture was stirred for 30 min at 10 °C, the reaction mixture was purified by UF/DF using 10K MWCO PES membrane against 10
25 mM SPB in saline (pH 7.0) (30X diavolume). The 418 mL retentate was taken and 62.7 gm sucrose (15% w/v) was added and followed by 0.22 µm (Millipak 60) filtration. The retentate was analyzed using Lowry assay for protein quantification and SEC-MALLS for Mw.

4. Click Conjugation

To the mixture of Serotype 21 azido polysaccharide (as obtained at point 2 above) (62.5 mL) and
30 alkyne-SCP (as obtained at point 3 above) (31.2 mL) or alkyne-CRM197, 1 M SPB (pH 7) 11 mL and 5 mL of WFI were added to make a 1-2 g/L reaction mixture with an ionic strength of 100 mM SPB (pH 7). The mixture was stirred at room temperature for 1 hour followed by addition of mixture of copper sulfate (31 µmol, 6.2 mL) and Tris(3-hydroxypropyltriazolymethyl)amine (THPTA) (155 µmol, 6.2 mL), aminoguanidine (1240 µmol, 12.4 mL), and sodium ascorbate (1240 µmol, 12.4
35 mL). The reaction mixture was stirred for 1.5 hours at room temperature.

After 1.5 hours, propargyl alcohol 36 µL (20 MEq per alkyne) was added to the reaction mixture to cap the unreacted azide on polysaccharide. Additional Click reagents (half amount) were

subsequently added to enhance the capping reactions; mixture of 5 mM copper sulfate 3.1 mL and 25 mM THPTA 3.1 mL, 100 mM aminoguanidine (same volume as copper mixture solution), and 100 mM ascorbate (same volume as copper mixture solution). The reaction mixture was stirred for an hour.

- 5 Afterwards, 3-azido-propyl alcohol 115 μ L (40 MEq per alkyne) was added to cap the unreacted alkyne SCP (or CRM197) and stirred for another hour. The reaction mixture was purified by UF/DF using 100K MWCO RC membrane against 10 mM EDTA + 10 mM SPB in saline (pH 7.0) (30X diavolume), followed by 2nd UF/DF against 5 mM succinate in saline (pH 6.0) (20X diavolume). After UF/DF, the retentate was filtered through 0.22 μ m filter (Millipak 40), and subsequently
- 10 analyzed.

Table 5 shows attributes of some of the conjugates obtained.

Table 5

Serotype 21 Conjugates		
Conjugate#	7	8
Poly MW (KDa)	313 (unsized)	162
Activated polysaccharide attributes		
Degree of Activation of Act poly	26%	25%
Act Poly Mw (kDa)	391	222
Activated carrier attributes		
Degree of Activation (DoA)	21	21
Activated carrier MW, kDa	101.4	101.4
Conjugate data		
Carrier protein	SCP	SCP
Conjugation yield (%)	72	72
SPR (Output Ratio)	0.66	1.04
Free Saccharide (%)	<5%	17
Conjugate Mw (kDa)	2312	820

Mw: molecular weight; SPR: Saccharide to protein ratio; NA: Not Available; Act Poly: Activated Polysaccharide

Example 7: Additional *S. pneumoniae* serotype 21 conjugate using Reductive Amination Chemistry in DMSO (RAC-DMSO)

Additional serotype 21 conjugate has been generated using RAC-DMSO (see example 5).
Table 6 below comprises characterizing data for serotype 21 polysaccharide conjugates obtained
5 by the methods of this invention. Native (unsized) polysaccharide was used.

Table 6

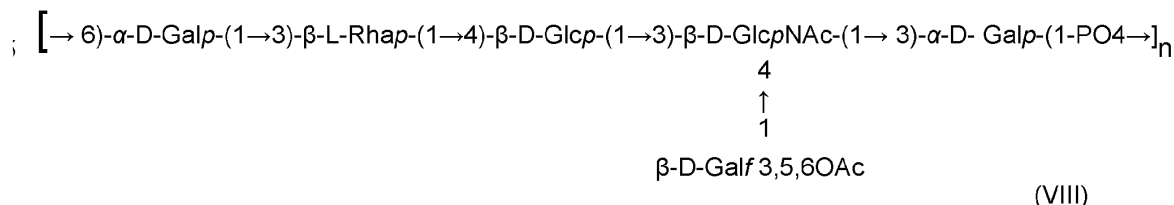
Serotype 21 Conjugates	
Conjugate#	9
Poly MW (KDa)	361
Chemistry	RAC-DMSO
DO	18
Act Poly Mw (kDa)	380
Conjugate data	
Carrier protein	CRM ₁₉₇
Conjugation yield (%)	57
SPR (Output Ratio)	0.9
Free Saccharide (%)	<5
Conjugate Mw (kDa)	6212

Mw: molecular weight; SPR: Saccharide to protein ratio; Act Poly: Activated Polysaccharide.
DO: Degree of Oxidation

10 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
15 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 glycoconjugate comprising an isolated *S. pneumoniae* serotype 21 saccharide with the following repeating unit:



where n represents the number of repeating units, wherein the O-acetyl group at position 3 of β -D-Galf is present in about 50% to about 80% of the repeating units, the O-acetyl group at position 6 of β -D-Galf is present in about 20% to about 40% of the repeating units, wherein the O-acetyl group at position 5 of β -D-Galf is present in about 1% to about 10% of the repeating units.

2. The glycoconjugate of claim 1, wherein the O-acetyl group at position 3 of β -D-Galf is present in about 64% of the repeating units, the O-acetyl group at position 6 of β -D-Galf is present in about 30% of the repeating units, wherein the O-acetyl group at position 5 of β -D-Galf is present in about 6% of the repeating units.

3. A glycoconjugate comprising an isolated *S. pneumoniae* serotype 21 saccharide with the repeating unit of formula (VIII), where n represents the number of repeating units, wherein the O-acetyl group at position 3 of β -D-Galf is present in about 25% to about 60% of the repeating units, the O-acetyl group at position 6 of β -D-Galf is present in about 30% to about 65% of the repeating units, wherein the O-acetyl group at position 5 of β -D-Galf is present in about 5% to about 15% of the repeating units.

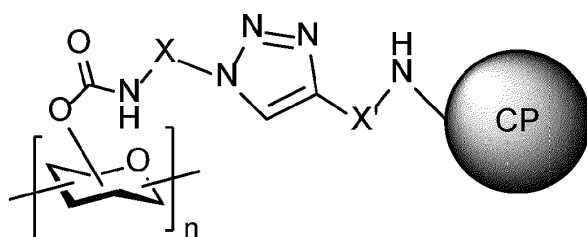
4. The glycoconjugate of any one of claims 1-3, wherein all the repeating unit bears one O-acetyl group on the β -D-Galf residue.

5. A glycoconjugate comprising an isolated *S. pneumoniae* serotype 21 saccharide with the repeating unit of formula (VIII), where n represents the number of repeating units, and wherein the O-acetyl group at position 3, 5 or 6 of β -D-Galf is present in about 0% to about 95% of the repeating units.

6. The serotype 21 glycoconjugate of any one of claims 1-5 wherein said serotype 21 glycoconjugate is prepared by direct reductive amination.

7. The serotype 21 glycoconjugate of any one of claims 1-5 wherein said serotype 21 glycoconjugate is prepared by click chemistry.

8. A serotype 21 glycoconjugate comprising a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (IV):



(IV),

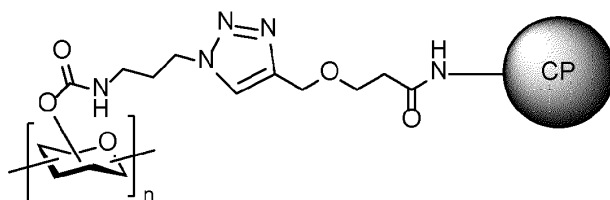
wherein X is selected from the group consisting of $\text{CH}_2(\text{CH}_2)_{n'}$, $(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$, $\text{NHCO}(\text{CH}_2)_{n'}$, $\text{NHCO}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$, $\text{OCH}_2(\text{CH}_2)_{n'}$ and $\text{O}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$;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

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,

wherein the structure in square bracket represents a repeat unit of the serotype 21 saccharide and wherein n represents the number of repeating units.

9. A serotype 21 glycoconjugate comprising a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (IV), 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.

10. A serotype 21 glycoconjugate comprising a serotype 21 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (V),



(V),

wherein the structure in square bracket represents a repeat unit of the serotype 21 saccharide and wherein n represents the number of repeating units.

11. The glycoconjugate of any one of claims 1 to 10 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 21 saccharide before conjugation is between 100 kDa and 400 kDa.

12. The glycoconjugate of any one of claims 1 to 10 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 21 saccharide before conjugation is between 150 kDa and 400 kDa.

13. The glycoconjugate of any one of claims 1 to 12 wherein said serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 500 kDa and 15,000 kDa.

14. The glycoconjugate of any one of claims 1 to 12 wherein said serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 750 kDa and 7,500 kDa.

15. The glycoconjugate of any one of claims 7 to 10 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 21 saccharide before conjugation is between 150 kDa and 400 kDa and wherein said serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 800 kDa and 7,000 kDa.

16. The glycoconjugate of claim 6 wherein said serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 2,000 kDa and 10,500 kDa.

17. The glycoconjugate of claim 6 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 21 saccharide before conjugation is between 100 kDa and 400 kDa and wherein said serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 2000 kDa and 10,500 kDa.

18. The glycoconjugate of any one of claims 1 to 17 wherein the degree of conjugation of said serotype 21 glycoconjugate is between 2 and 15.

19. The glycoconjugate of any one of claims 7 to 10 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 21 saccharide before conjugation is between 150 kDa and 400 kDa and wherein said serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 800 kDa and 7,000 kDa and wherein the degree of conjugation of said serotype 21 glycoconjugate is between 2 and 10.

20. The glycoconjugate of claim 6 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 21 saccharide before conjugation is between 100 kDa and 400 kDa and wherein said serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 2000 kDa and 10,500 kDa and wherein the degree of conjugation of said serotype 21 glycoconjugate is between 2 and 10.

21. The glycoconjugate of any one of claims 1 to 20 wherein the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.5.

22. The glycoconjugate of any one of claims 7 to 10 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 21 saccharide before conjugation is between 150 kDa and 400 kDa and wherein said serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 800 kDa and 7,000 kDa and the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.1.

23. The glycoconjugate of claim 6 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 21 saccharide before conjugation is between 100 kDa and 400 kDa and wherein said serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between

2000 kDa and 10,500 kDa and the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.3.

24. The glycoconjugate of any one of claims 1 to 23 wherein said serotype 21 glycoconjugate comprises less than about 25% of free serotype 21 saccharide compared to the total amount of serotype 21 saccharide.

25. The glycoconjugate of any one of claims 7 to 10 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 21 saccharide before conjugation is between 150 kDa and 400 kDa and wherein said serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 800 kDa and 7,000 kDa, wherein the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.1 and wherein said serotype 21 glycoconjugate comprises less than about 25% of free serotype 21 saccharide compared to the total amount of serotype 21 saccharide.

26. The glycoconjugate of claim 6 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 21 saccharide before conjugation is between 100 kDa and 400 kDa and wherein said serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 2000 kDa and 10,500 kDa, wherein the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.3 and wherein said serotype 21 glycoconjugate comprises less than about 25% of free serotype 21 saccharide compared to the total amount of serotype 21 saccharide.

27. The glycoconjugate of any one of claims 7 to 10 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 21 saccharide before conjugation is between 150 kDa and 400 kDa and wherein said serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 800 kDa and 7,000 kDa, wherein the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.1, wherein said serotype 21 glycoconjugate comprises less than about 25% of free serotype 21 saccharide compared to the total amount of serotype 21 saccharide and wherein between 65% and 80% of the serotype 21 glycoconjugate has a K_d below or equal to 0.3 in a CL-4B column.

28. The glycoconjugate of claim 6 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 21 saccharide before conjugation is between 100 kDa and 400 kDa and wherein said serotype 21 glycoconjugate has a weight average molecular weight (Mw) of between 2000 kDa and 10,500 kDa, wherein the ratio of serotype 21 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.3, wherein said serotype 21 glycoconjugate comprises less than about 25% of free serotype 21 saccharide compared to the total amount of serotype 21 saccharide and wherein between 65% and 80% of the serotype 21 glycoconjugate has a K_d below or equal to 0.3 in a CL-4B column.

29. The glycoconjugate of any one of claims 1 to 28 wherein the carrier protein of the serotype 21 saccharide glycoconjugate is selected in the group consisting of: TT, DT, DT mutants and a C5a peptidase from *Streptococcus* (SCP).
30. The glycoconjugate of any one of claims 1 to 28 wherein the carrier protein of the serotype 21 saccharide glycoconjugate is rhizavidin [aa 45-179J-GGGGSSS-SP1500- AAA-SP0785] (CP1).
31. The glycoconjugate of any one of claims 1 to 28 wherein the carrier protein of the serotype 21 saccharide glycoconjugate is Rhavi-linker-PdT(G294P)-linker-SP0435 [aa 62-185] fusion protein (SPP2).
32. The glycoconjugate of any one of claims 1 to 28 wherein the carrier protein of the serotype 21 saccharide glycoconjugate is CRM₁₉₇.
33. The glycoconjugate of any one of claims 1 to 28 wherein the carrier protein of the serotype 21 saccharide glycoconjugate is SCP.
34. An immunogenic composition comprising a *S. pneumoniae* serotype 21 saccharide according to any one of paragraphs 1-33.
35. The immunogenic composition of claim 34 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.
36. The immunogenic composition of claim 34 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, 23A, 23B, 23F, 24F, 33F and 35B.
37. The immunogenic composition of claim 34 wherein the *S. pneumoniae* saccharides from serotypes 1, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15A, 15B, 18C, 19A, 19F, 22F, 23A, 23B, 23F, 24F, 33F, 35B and 38 are conjugated to CRM₁₉₇ and the *S. pneumoniae* saccharide serotype 3 is conjugated to SCP.
38. The immunogenic composition of any one of claims 34-37 further comprising one adjuvant.
39. The immunogenic composition of any one of claims 34-38 for use as a vaccine.
40. The immunogenic composition of any one of claims 34-38 for use in a method of preventing an infection by *S. pneumoniae* 21 in a subject said method comprising administering to the subject an immunologically effective amount of said immunogenic composition.