



(51) International Patent Classification:

A61K 39/09 (2006.01) A61P 31/04 (2006.01)

A61K 47/64 (2017.01)

(21) International Application Number:

PCT/IB2024/052957

(22) International Filing Date:

27 March 2024 (27.03.2024)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

63/493,046 30 March 2023 (30.03.2023) US

63/563,499 11 March 2024 (11.03.2024) US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, CV, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SC, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, ME, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))

Published:

- without international search report and to be republished upon receipt of that report (Rule 48.2(g))
- with sequence listing part of description (Rule 5.2(a))
- in black and white; the international application as filed contained color or greyscale and is available for download from PATENTSCOPE

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

(57) Abstract: The present invention relates to new immunogenic compositions comprising conjugated Streptococcus pneumoniae capsular saccharide antigens (glycoconjugates), kits comprising said immunogenic compositions and uses thereof. Immunogenic compositions of the present invention will typically comprise at least one glycoconjugate from a *S. pneumoniae* serotype not found in PREVNAR®, SYNFLORIX® and/or PREVNAR 13®. The invention also relates to vaccination of human subjects, in particular infants and elderly, against pneumococcal infections using said novel immunogenic compositions.



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 38 saccharides, glycoconjugates thereof, methods for making *Streptococcus pneumoniae* serotype 38 glycoconjugates and immunogenic composition comprising a *Streptococcus pneumoniae* serotype 38 glycoconjugate.

10 The invention also relates to analytical methods to analyze isolated *S. pneumoniae* serotype 38 polysaccharide, reduced serotype 38 polysaccharide or *Streptococcus pneumoniae* serotype 38 glycoconjugates.

The *Streptococcus pneumoniae* serotype 38 saccharide and glycoconjugates of the invention can be used as a vaccine.

Background of the Invention

20 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).

30 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.

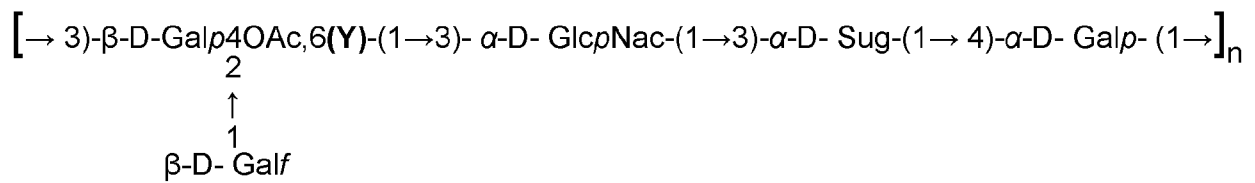
Pneumococcal polysaccharides, in particular capsular polysaccharides, are important immunogens found on the surface of the bacteria. This has led to them being an important component in the design of pneumococcal vaccines. They have proved useful in eliciting immune responses especially when linked to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

Thus, there is a need for antigens which are able to generate a robust immune response to *Streptococcus pneumoniae* serotype 38.

The present invention provides in particular *Streptococcus pneumoniae* serotype 38 glycoconjugates.

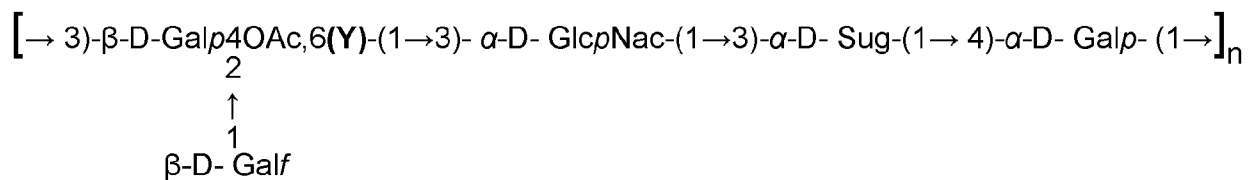
Summary of the Invention

To meet these and other needs, the present invention relates an isolated *S. pneumoniae* serotype 38 saccharide with the following repeating unit:



where n represents the number of repeating units, wherein Y represents either a Serine or a Glycine residue and wherein all the repeating units comprise the same Y residue.

In an aspect the present invention pertains to an isolated *S. pneumoniae* serotype 38 saccharide with the following repeating unit:



where n represents the number of repeating units, wherein Y represents either a Serine or a Glycine residue, wherein all the repeating units comprise the same Y residue and wherein the O-acetyl group at position 4 of β -D-Galp4OAc,6(Y) is present in about 0% to about 100% of the repeating units. In an embodiment said O-acetyl group is present in about 50% to about 100% of the repeating units. Preferably, said O-acetyl group is present in about 80% to about 100% of the repeating units. Even more preferably, said O-acetyl group is present in about 90% to about 100% of the repeating units.

In certain embodiments, the isolated *S. pneumoniae* serotype 38 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 above disclosed repeating unit conjugated to a carrier protein.

The invention further provides *Streptococcus pneumoniae* serotype 38 glycoconjugates bearing reduced α -D-2-acetamido-2,6-dideoxy-xylo-hexos-4-ulose (α -D-Sug) residues.

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 38 saccharide of the invention and/or a *Streptococcus pneumoniae* serotype 38 glycoconjugate of the invention.

The invention further pertains to analytical methods of *S. pneumoniae* serotype 38 saccharides and conjugates.

Detailed description of the drawings

Figures 1A and 1B. Schematic of pneumococcal polysaccharide serotype 38 repeat unit organization, including the most common form (serine form) (A) and the secondary form (glycine form) (B).

Figure 2. The structure of *Streptococcus pneumoniae* serotype 38-serine form equilibrating between the hydrate and keto state. The two states are shown by black circle.

Figure 3. Expanded anomeric and methyl region 1D ^1H spectra of O-acetylated (bottom panel) and de-O-acetylated (top panel) serotype 38 polysaccharides (serine form). The anomeric and the methyl signals are annotated for both spectra. Shaded box denotes the resonances observed due to the O-acetylation of β -D-Galp residue and are absent in the de-O-acetylated serotype 38 polysaccharide.

Figure 4. Expanded carbonyl, anomeric and methyl region 1D ^{13}C spectra of O-acetylated (bottom panel) and de-O-acetylated (top panel) serotype 38 polysaccharides (serine form).

Panels A to D shows the expanded regions of keto, carbonyl, anomeric and methyl resonances, respectively. Shaded box denotes the resonances observed due to the O-acetylation of β -D-Galp residue and are absent in the de-O-acetylated serotype 38 polysaccharide.

Figure 5. 1D ^1H spectrum of reduced serotype 38 polysaccharides. (Inset shows the expanded anomeric region). The anomeric and the methyl signals are annotated.

Figure 6. Schematic of serotype 38 repeat unit (serine form) before and after reduction with NaBH_4 . Reduction forms FucpNAc and QuipNAc sugar.

Figure 7. Pn38 specific opsonophagocytic geomean titers (OPA GMT) of sera from mice vaccinated with serotype 38 polysaccharide conjugated to either CRM₁₉₇ or SCP using either RAC/Aqueous, RAC/DMSO or Click conjugation.

Figure 8. Pn38 specific opsonophagocytic geomean titers (OPA GMT) of sera from mice vaccinated with serotype 38 polysaccharideconjugated to either CRM₁₉₇ or SCP using either RAC/Aqueous or Click conjugation.

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 38.

5 The produced (and purified) polysaccharide was used to generate polysaccharide-protein conjugate (glycoconjugates). *S. pneumoniae* serotype 38 has a unique polysaccharide structure, which results in unique consideration when designing conjugate production process. The *S. pneumoniae* serotype 38 glycoconjugates of the invention also have a unique structure and design.

1. Isolated *Streptococcus pneumoniae* serotype 38 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.

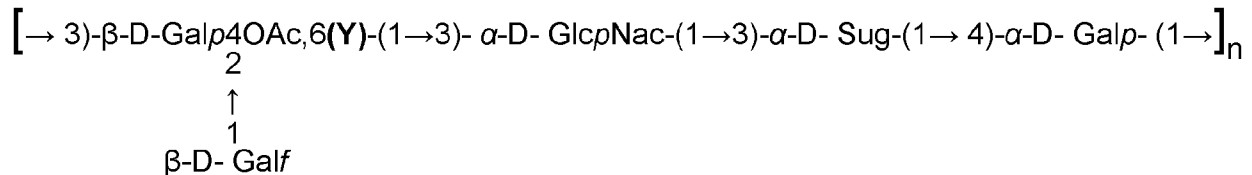
The term "saccharide" throughout this specification may indicate polysaccharide or oligosaccharide and includes both. In preferred embodiments, the saccharide is a polysaccharide, in particular a *S. pneumoniae* serotype 38 capsular polysaccharide.

The term "serotype 38 saccharide", "serotype 38 capsular saccharide", "*S. pneumoniae* serotype 38 saccharide" throughout this specification refers to *S. pneumoniae* serotype 38 capsular saccharide and may be used interchangeably herein.

The structure of *S. pneumoniae* serotype 38 capsular polysaccharide is disclosed for the first time and is shown in Figure 1A and 1B. The inventors found that the serotype 38 polysaccharide is a penta-saccharide: α -D-glucosamine (A), α -D-2-acetamido-2,6-dideoxy-xylo-hexos-4-ulose (α -D-Sug) (B), O-acetylated β -D-galactose (C), α -D-galactose (D), and β -D-galactofuranose (E). Residue C (O-acetylated β -D-galactose) is further linked at the 6 position to serine or glycine amino acid, thus representing the serotype 38 serine and glycine forms of polysaccharide.

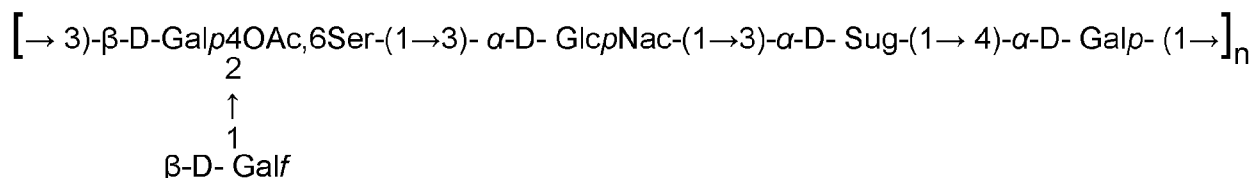
The inventors have found that certain strains of *S. pneumoniae* serotype 38 produce a capsular polysaccharide which has a serine amino acid linked to Galp4OAc sugar (Figure 1A) whereas other strains make polysaccharide where glycine is attached to Galp4OAc sugar (Figure 1B). After characterizing several strains of serotype 38, the serine form has been shown to be the more abundant form.

Accordingly, in one embodiment, the present invention provides an isolated *S. pneumoniae* serotype 38 saccharide with the following repeating unit:



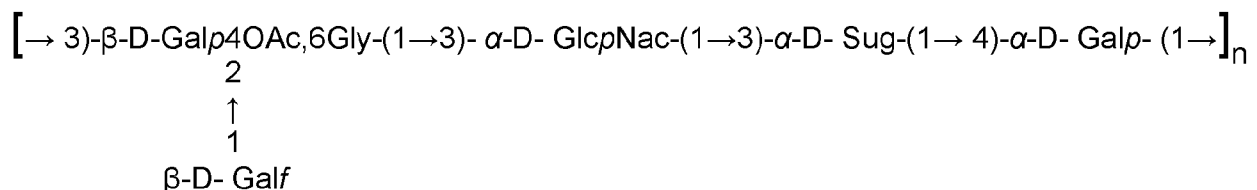
where n represents the number of repeating units, wherein Y represents either a Serine or a Glycine residue and wherein all the repeating units comprise the same Y residue.

Therefore, in one embodiment, the present invention provides an isolated *S. pneumoniae* serotype 38 saccharide with the following repeating unit:



where n represents the number of repeating units.

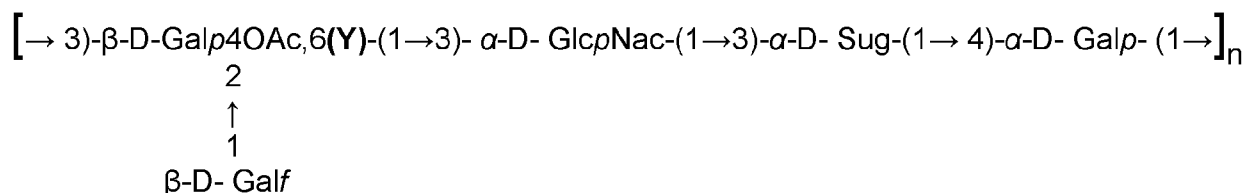
In another embodiment, the present invention provides an isolated *S. pneumoniae* serotype 38 saccharide with the following repeating unit:



where n represents the number of repeating units.

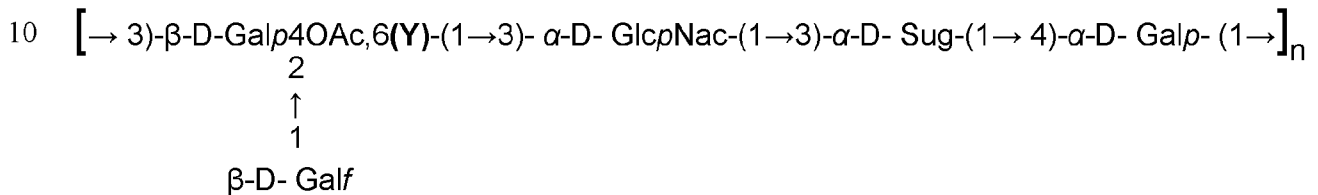
As shown at figure 1A and 1B, $\beta\text{-D-Galp}4\text{OAc},6\text{Ser(or Gly)}$ (residue C) of serotype 38 polysaccharide is O-acetylated at carbon 4 position. O-acetylation level may vary from one strain to another. Furthermore, native *S. pneumoniae* serotype 38 saccharide can be deacetylated, for example by treatment with a base (alkaline pH). As shown in the example section, native serotype 38 polysaccharide can be completely deacetylated after incubating with 0.25N NH_4OH at room temperature for 24 hours.

Accordingly, in one embodiment, the present invention provides an isolated *S. pneumoniae* serotype 38 saccharide with the following repeating unit:



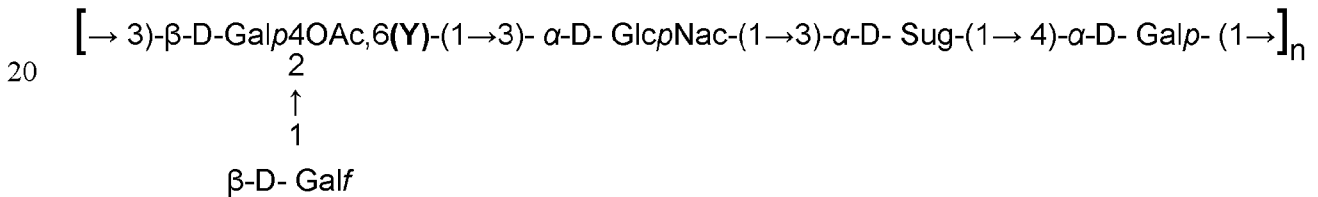
where n represents the number of repeating units, wherein Y represents either a Serine or a Glycine residue, wherein all the repeating units comprise the same Y residue and wherein the O-acetyl group at position 4 of β -D-Galp4OAc,6(Y) is present in about 0% to about 100% of the repeating units. In an embodiment said O-acetyl group is present in about 50% to about 100% of the repeating units. Preferably, said O-acetyl group is present in about 80% to about 100% of the repeating units. Even more preferably, said O-acetyl group is present in about 90% to about 100% of the repeating units.

In one embodiment, the present invention provides an isolated *S. pneumoniae* serotype 38 saccharide with the following repeating unit:



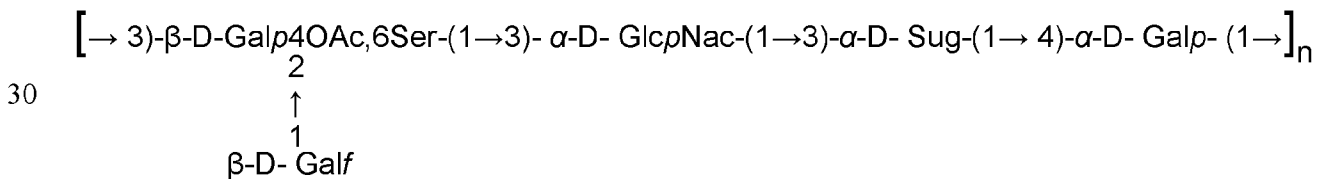
where n represents the number of repeating units, wherein Y represents either a Serine or a Glycine residue, wherein all the repeating units comprise the same Y residue and wherein the O-acetyl group at position 4 of β -D-Galp4OAc,6(Y) is present in about 100% of the repeating units.

In one embodiment, the present invention provides an isolated *S. pneumoniae* serotype 38 saccharide with the following repeating unit:



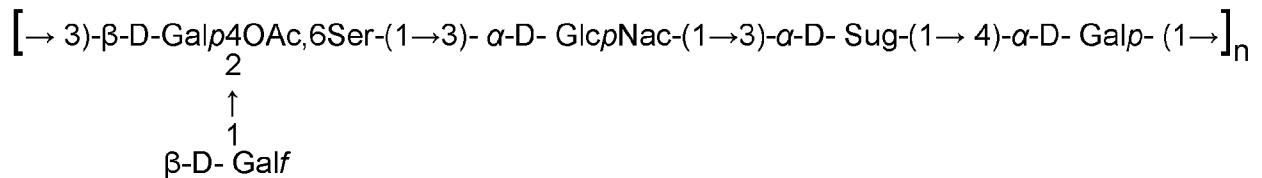
where n represents the number of repeating units, wherein Y represents either a Serine or a Glycine residue, wherein all the repeating units comprise the same Y residue and wherein the O-acetyl group at position 4 of β -D-Galp4OAc,6(Y) is present in about 95% of the repeating units.

Accordingly, in one embodiment, the present invention provides an isolated *S. pneumoniae* serotype 38 saccharide with the following repeating unit:



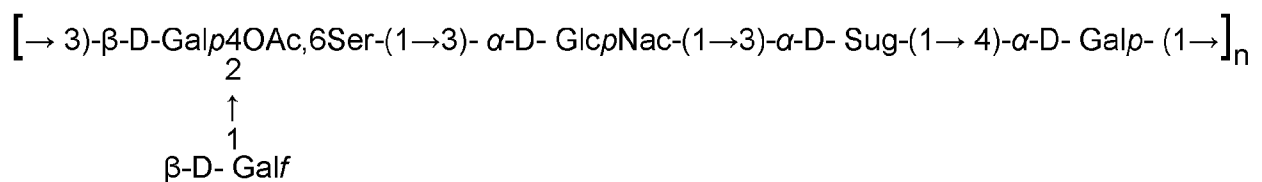
where n represents the number of repeating units and wherein the O-acetyl group at position 4 of β -D-Galp4OAc,6Ser is present in about 0% to about 100% of the repeating units. In an embodiment said O-acetyl group is present in about 50% to about 100% of the repeating units. Preferably, said O-acetyl group is present in about 80% to about 100% of the repeating units. Even more preferably, said O-acetyl group is present in about 90% to about 100% of the repeating units.

In one embodiment, the present invention provides an isolated *S. pneumoniae* serotype 38 saccharide with the following repeating unit:



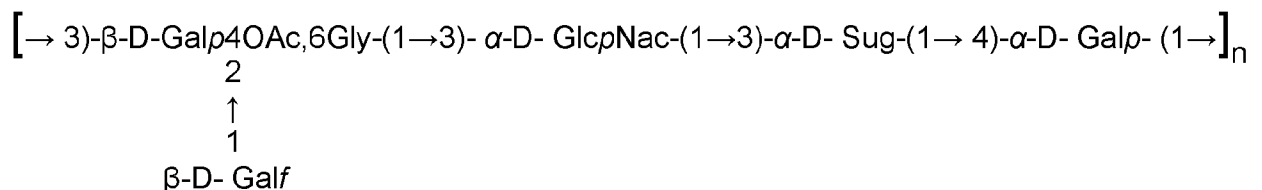
where n represents the number of repeating units and wherein the O-acetyl group at position 4 of $\beta\text{-D-Galp4OAc,6Ser}$ is present in about 100% of the repeating units.

In one particular embodiment, the present invention provides an isolated *S. pneumoniae* serotype 38 saccharide with the following repeating unit:



where n represents the number of repeating units and wherein the O-acetyl group at position 4 of $\beta\text{-D-Galp4OAc,6Ser}$ is present in about 95% of the repeating units.

In another embodiment, the present invention provides an isolated *S. pneumoniae* serotype 38 saccharide with the following repeating unit:



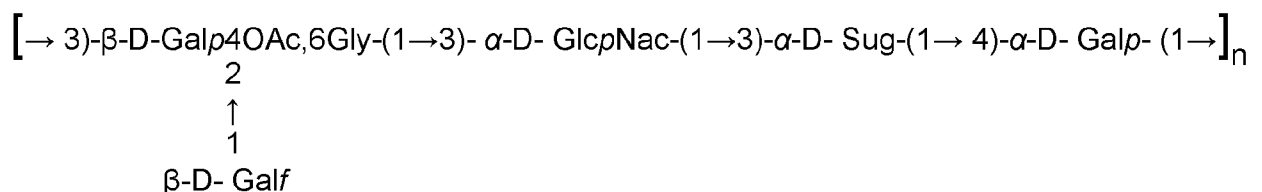
where n represents the number of repeating units and wherein the O-acetyl group at position 4 of $\beta\text{-D-Galp4OAc,6Gly}$ is present in about 0% to about 100% of the repeating units. In an

embodiment said O-acetyl group is present in about 50% to about 100% of the repeating units.

Preferably, said O-acetyl group is present in about 80% to about 100% of the repeating units.

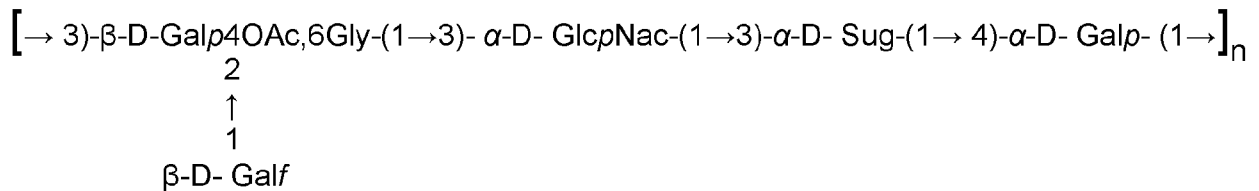
Even more preferably, said O-acetyl group is present in about 90% to about 100% of the repeating units.

In another embodiment, the present invention provides an isolated *S. pneumoniae* serotype 38 saccharide with the following repeating unit:



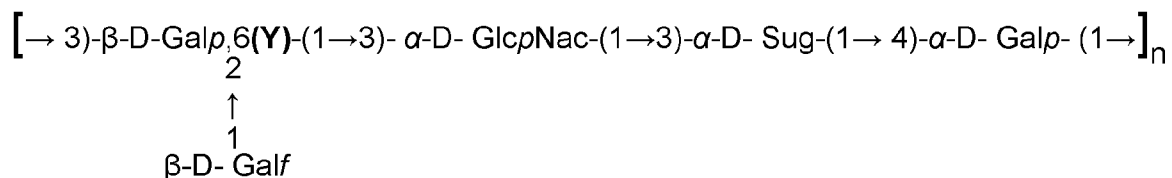
where n represents the number of repeating units and wherein the O-acetyl group at position 4 of $\beta\text{-D-Galp4OAc,6Gly}$ is present in about 100% of the repeating units.

In a particular embodiment, the present invention provides an isolated *S. pneumoniae* serotype 38 saccharide with the following repeating unit:



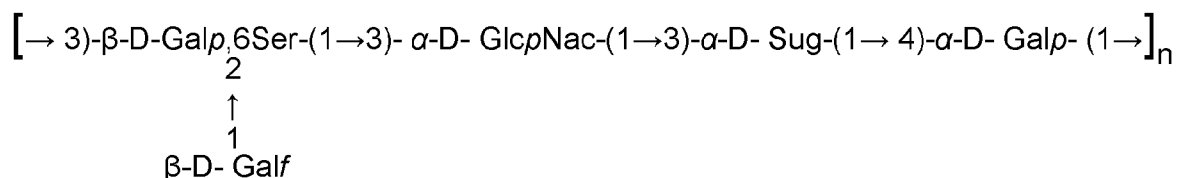
where n represents the number of repeating units and wherein the O-acetyl group at position 4 of $\beta\text{-D-Galp}4\text{OAc},6\text{Gly}$ is present in 95% of the repeating units.

In an embodiment, the *S. pneumoniae* serotype 38 saccharide of the invention does not bear an O-acetyl group at carbon 4 position of the $\beta\text{-D-Galp}4\text{OAc},6\text{Ser}(\text{or Gly})$ residue. Therefore, in one embodiment, the present invention provides an isolated *S. pneumoniae* serotype 38 saccharide with the following repeating unit:



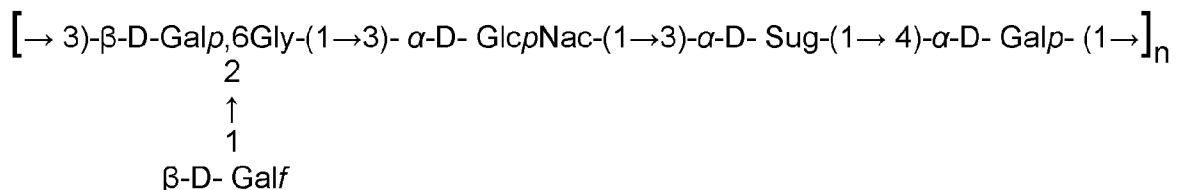
where n represents the number of repeating units, wherein Y represents either a Serine or a Glycine residue and wherein all the repeating units comprise the same Y residue.

In one embodiment, the present invention provides an isolated *S. pneumoniae* serotype 38 saccharide with the following repeating unit:



where n represents the number of repeating units.

In another embodiment, the present invention provides an isolated *S. pneumoniae* serotype 38 saccharide with the following repeating unit:



where n represents the number of repeating units.

As mentioned above, the $\beta\text{-D-Galp}4\text{OAc},6\text{Ser}(\text{or Gly})$ residues (residue C) of serotype 38 saccharide maybe O-acetylated at carbon 4 position. O-acetylation level may not be 100% in the saccharide and when the structure of a saccharide is provided with a repeating unit represented with an O-acetyl group at this position, it should not be understood that such a saccharide always bears an O-acetyl group at every position 4 of $\beta\text{-D-Galp}4\text{OAc},6\text{Ser}(\text{or Gly})$ residue of the

saccharide. Rather, this indicates that a majority of these residues are O-acetylated, preferably, said O-acetyl group is present in about 80% to about 100% of the repeating units. Even more preferably, said O-acetyl group is present in about 90% to about 100% of the repeating units.

In certain embodiments, the isolated *S. pneumoniae* serotype 38 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.

Isolated capsular saccharides from *S. pneumoniae* serotype 38 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 38 strain in a medium (e.g., in a soy-based medium), the polysaccharides are then prepared from the bacteria culture. Serotype 38 *Streptococcus pneumoniae* strains may be obtained from established culture collections (such as 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 38) 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 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).

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 38 saccharide of the invention has a weight average molecular weight between 5 kDa and 5000 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 38 saccharide has a weight average molecular weight between 5 kDa and 2000 kDa.

In an embodiment, the isolated *S. pneumoniae* serotype 38 polysaccharide has a weight average molecular weight between 50 kDa and 5000 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 38 polysaccharide has a weight average molecular weight between 50 kDa and 2000 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 38 polysaccharide has a weight average molecular weight between 50 kDa and 1000 kDa.

In an embodiment, the isolated *S. pneumoniae* serotype 38 polysaccharide has a weight average molecular weight between 100 kDa and 5000 kDa. In an embodiment, the isolated *S.*

pneumoniae serotype 38 polysaccharide has a weight average molecular weight between 100 kDa and 2000 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 38 polysaccharide has a weight average molecular weight between 100 kDa and 1000 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 38 polysaccharide has a weight average molecular weight between 100 kDa and 500 kDa.

In an embodiment, the isolated *S. pneumoniae* serotype 38 polysaccharide has a weight average molecular weight between 300 kDa and 5000 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 38 polysaccharide has a weight average molecular weight between 300 kDa and 2000 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 38 polysaccharide has a weight average molecular weight between 300 kDa and 1000 kDa.

In an embodiment, the isolated *S. pneumoniae* serotype 38 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.

Advantageously, the size of the purified capsular *S. pneumoniae* serotype 38 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 38 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 38 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.

5 In a preferred embodiment, the isolated *S. pneumoniae* serotype 38 capsular saccharide is sized to a weight average molecular weight between 10 kDa and 1000 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 38 capsular saccharide is sized to a weight average molecular weight between 50 kDa and 500 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 38 capsular saccharide is sized to a weight average molecular weight between 50 kDa
10 and 400 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 38 capsular saccharide is sized to a weight average molecular weight between 50 kDa and 250 kDa.

In a preferred embodiment, the isolated *S. pneumoniae* serotype 38 capsular saccharide is sized to a weight average molecular weight between 100 kDa and 1000 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 38 capsular saccharide is sized to a weight average
15 molecular weight between 100 kDa and 500 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 38 capsular saccharide is sized to a weight average molecular weight between 100 kDa and 400 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 38 capsular saccharide is sized to a weight average molecular weight between 100 kDa and 250 kDa.

In an embodiment, the isolated *S. pneumoniae* serotype 38 capsular saccharide is sized
20 to a weight average molecular weight between 250 kDa and 1000 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 38 capsular saccharide is sized to a weight average molecular weight between 250 kDa and 500 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 38 capsular saccharide is sized to a weight average molecular weight between 250 kDa and 400 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 38 capsular saccharide is sized to
25 a weight average molecular weight between 200 kDa and 800 kDa.

In a preferred embodiment, the isolated *S. pneumoniae* serotype 38 capsular saccharide is sized to a weight average molecular weight between 150 kDa and 300 kDa.

In an embodiment, the isolated *S. pneumoniae* serotype 38 capsular saccharide capsular
saccharide is sized to a weight average molecular weight of about 250 kDa. In an embodiment,
30 the isolated *S. pneumoniae* serotype 38 capsular saccharide is sized to a weight average molecular weight of about 300 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 38 capsular saccharide is sized to a weight average molecular weight of about 350 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 38 capsular saccharide is sized to a weight average molecular weight of about 400 kDa. In an embodiment, the isolated *S. pneumoniae*
35 serotype 38 capsular saccharide is sized to a weight average molecular weight of about 450 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 38 capsular saccharide is sized to a weight average molecular weight of about 500 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 38 capsular saccharide is sized to a weight average molecular weight of

about 550 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 38 capsular saccharide is sized to a weight average molecular weight of about 600 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 38 capsular saccharide is sized to a weight average molecular weight of about 700 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 38 capsular saccharide is sized to a weight average molecular weight of about 800 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 38 capsular saccharide is sized to a weight average molecular weight of about 900 kDa. In an embodiment, the isolated *S. pneumoniae* serotype 38 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 38 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 38 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.

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 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 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 38 glycoconjugate of the present invention comprises a serotype 38 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 38 glycoconjugate of the present invention comprises a serotype 38 polysaccharide wherein the weight average molecular weight (Mw) of said polysaccharide before conjugation is between 100 kDa and 600 kDa.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 polysaccharide wherein the weight average molecular weight (Mw) of said polysaccharide before conjugation is between 100 kDa and 400 kDa.

In a preferred embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 polysaccharide wherein the weight average molecular weight (Mw) of said polysaccharide before conjugation is between 150 kDa and 300 kDa.

In some embodiments, the serotype 38 glycoconjugate of the invention has a weight average molecular weight (Mw) of between 250 kDa and 20,000 kDa.

In other embodiments, the serotype 38 glycoconjugate has a weight average molecular weight (Mw) of between 500 kDa and 15,000 kDa.

In other embodiments, the serotype 38 glycoconjugate has a weight average molecular weight (Mw) of between 500 kDa and 10,000 kDa.

In still other embodiments, the serotype 38 glycoconjugate has a weight average molecular weight (Mw) of between 750 kDa and 7,500 kDa.

In other embodiments, the serotype 38 glycoconjugate has a weight average molecular weight (Mw) of between 1,000 kDa and 5,000 kDa.

In preferred embodiments, the serotype 38 glycoconjugate has a weight average molecular weight (Mw) of between 1,000 kDa and 10,000 kDa.

Another way to characterize the serotype 38 glycoconjugates of the invention is by the 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 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 38 glycoconjugate of the invention is between 2 and 15. In an embodiment, the degree of conjugation of the serotype 38 glycoconjugate of the invention is between 2 and 10. In an embodiment, the degree of conjugation of the serotype 38 glycoconjugate of the invention is between 3 and 5. In an embodiment, the degree of conjugation of the serotype 38 glycoconjugate of the invention is between 2 and 6. In a preferred embodiment, the degree of conjugation of the serotype 38 glycoconjugate of the invention is between 4 and 10.

The serotype 38 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 38 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 3.0. In other embodiments, the saccharide to carrier protein ratio (w/w) is between 0.5 and 2.0. In other embodiments, the saccharide to carrier protein ratio (w/w) is between 0.5 and 1.5. In other embodiments, the saccharide to carrier protein ratio (w/w) is between 0.8 and 1.2. In other embodiments, the saccharide to carrier protein ratio (w/w) is between 0.5 and 1.0. In other embodiments, the saccharide to carrier protein ratio (w/w) is between 1.0 and 1.5. In other embodiments, the saccharide to carrier protein ratio (w/w) is between 1.0 and 2.0. In further embodiments, the saccharide to carrier protein ratio (w/w) is between 0.8 and 1.2. In further embodiments, the ratio of serotype 38 saccharide to carrier protein in the conjugate is between 0.7 and 1.1. In preferred embodiments, the ratio of serotype 38 saccharide to carrier protein in the conjugate is between 0.5 and 1.5. In some such embodiments, the carrier protein is CRM₁₉₇. In some preferred embodiments, the carrier protein is SCP.

The serotype 38 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 38 glycoconjugate comprises less than about 50% of free serotype 38 saccharide compared to the total amount of serotype 38 saccharide. In a preferred embodiment, the serotype 38 glycoconjugate comprises less than about 25% of free serotype 38 saccharide compared to the total amount of serotype 38 saccharide. In an even preferred embodiment, the serotype 38 glycoconjugate comprises less than about 20% of free serotype 38 saccharide compared to the total amount of serotype 38 saccharide. In a yet preferred embodiment, the serotype 38 glycoconjugate comprises less than about 15% of free serotype 38 saccharide compared to the total amount of serotype 38 saccharide.

The serotype 38 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 38 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 38 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 38 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 38 glycoconjugate has a K_d below or equal to 0.3 in a CL-4B column.

2.2 *Streptococcus pneumoniae* serotype 38 glycoconjugates of the invention bearing reduced α -D-2-acetamido-2,6-dideoxy-xylo-hexos-4-ulose (α -D-Sug) residues

The process to prepare the serotype 38 glycoconjugate of the invention may comprise the use of reducing agent. In particular, unreacted aldehyde groups following oxidation (in particular when reductive amination is used, see below) may be capped using a suitable capping agent (reducing agent). In one embodiment this capping agent is sodium borohydride (NaBH_4).

As shown at Example 4, the α -D-2-acetamido-2,6-dideoxy-xylo-hexos-4-ulose (D-Sug) residue is sensitive to reduction using NaBH_4 . Treatment of serotype 38 polysaccharide with NaBH_4 specifically reduces the position 4 of the D-Sug residue from a ketone/hydrate to an alcohol and transform the D-Sug to a mixture of D-FucNAc and D-QuiNAc, characterized by position 4 hydroxyl at axial and equatorial orientations, respectively as illustrated in Figure 6.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 1 to about 70 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 1 to about 68 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 1 to about 65 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 1 to about 60 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 1 to about 50 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 1 to about 40 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 1 to about 30 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 1 to about 20 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 1 to about 10 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 1 to about 5 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 10 to about 70 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 10 to about 68 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 10 to about 65 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 10 to about 60 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 10 to about 50 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 10 to about 40 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 10 to about 30 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

5 In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 10 to about 20 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 20 to about 70 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

10 In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 20 to about 68 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 20 to about 65 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 20 to about 60 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

20 In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 20 to about 50 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 20 to about 40 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

25 In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 20 to about 30 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 40 to about 70 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 40 to about 68 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

35 In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 40 to about 65 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 40 to about 60 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 40 to about 50 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 50 to about 70 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 50 to about 68 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 50 to about 65 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 50 to about 60 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 60 to about 70 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 60 to about 68 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 60 to about 65 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising about 70 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising about 68 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising about 65 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising about 60 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

5 In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising about 50 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising about 40 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

10 In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising about 30 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising about 20 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

15 In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising about 10 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising about 5 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

20 In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising about 1 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

25 In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 0.5 to about 35 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 0.5 to about 32 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

30 In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 0.5 to about 30 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 0.5 to about 25 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 0.5 to about 20 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 0.5 to about 15 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 0.5 to about 10 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 0.5 to about 5 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 0.5 to about 2.5 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 5 to about 35 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 5 to about 32 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 5 to about 30 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 5 to about 25 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 5 to about 20 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 5 to about 15 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 5 to about 10 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 10 to about 35 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 10 to about 32 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 10 to about 30 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 10 to about 25 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 10 to about 20 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 10 to about 15 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 20 to about 35 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 20 to about 32 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 20 to about 30 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 20 to about 25 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 25 to about 35 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 25 to about 32 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 25 to about 30 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 30 to about 35 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 30 to about 32 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising about 35 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising about 32 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising about 30 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising about 25 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising about 20 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising about 15 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising about 10 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising about 5 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising about 2.5 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising about 0.5 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

5 In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 1 to about 70 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 0.5 to about 30 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

10 In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 1 to about 68 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 0.5 to about 32 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

15 In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 1 to about 65 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 0.5 to about 32.5 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

20 In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 1 to about 60 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 0.5 to about 30 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

25 In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 1 to about 50 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 0.5 to about 25 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

30 In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 1 to about 40 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 0.5 to about 20 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

35 In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 1 to about 30 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 0.5 to about 15 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 1 to about 20 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 0.5 to about 10 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 1 to about 10 N-acetyl-D-fucosamine

(D-FucNAc) residues and between about 0.5 to about 5 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 1 to about 5 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 0.5 to about 2.5 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 1 to about 2 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 0.5 to about 1 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 5 to about 70 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 2.5 to about 35 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 5 to about 68 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 2.5 to about 32 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 5 to about 65 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 2.5 to about 32.5 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 5 to about 60 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 2.5 to about 30 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 5 to about 50 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 2.5 to about 25 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 5 to about 40 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 2.5 to about 20 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 5 to about 30 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 2.5 to about 15 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 5 to about 20 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 2.5 to about 10 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

5 In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 5 to about 10 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 2.5 to about 5 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

10 In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 10 to about 70 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 5 to about 35 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

15 In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 10 to about 68 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 5 to about 32 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

20 In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 10 to about 65 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 5 to about 32.5 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 10 to about 60 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 5 to about 30 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

25 In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 10 to about 50 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 5 to about 25 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

30 In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 10 to about 40 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 5 to about 20 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

35 In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 10 to about 30 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 5 to about 15 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 10 to about 20 N-acetyl-D-

fucosamine (D-FucNAc) residues and between about 5 to about 10 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 20 to about 70 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 10 to about 35 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 20 to about 68 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 10 to about 32 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 20 to about 65 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 10 to about 32.5 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 20 to about 60 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 10 to about 30 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 20 to about 50 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 10 to about 25 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 20 to about 40 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 10 to about 20 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 20 to about 30 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 10 to about 15 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 30 to about 70 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 15 to about 35 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 30 to about 68 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 15 to about 32 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 30 to about 65 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 15 to about 32.5 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

5 In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 30 to about 60 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 15 to about 30 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

10 In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 30 to about 50 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 15 to about 25 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

15 In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 30 to about 40 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 15 to about 20 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

20 In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 40 to about 70 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 20 to about 35 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 40 to about 68 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 20 to about 32 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

25 In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 40 to about 65 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 20 to about 32.5 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

30 In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 40 to about 60 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 20 to about 30 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

35 In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 40 to about 50 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 20 to about 25 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 50 to about 70 N-acetyl-D-

fucosamine (D-FucNAc) residues and between about 25 to about 35 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 50 to about 68 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 25 to about 32 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 50 to about 65 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 25 to about 32.5 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 50 to about 60 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 25 to about 30 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 60 to about 70 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 30 to about 35 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 60 to about 68 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 30 to about 32 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising between about 60 to about 65 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 30 to about 32.5 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising about 1 N-acetyl-D-fucosamine (D-FucNAc) residue and about 0.5 N-acetyl-D-quinovosamine (D-QuiNAc) residue in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising about 2 N-acetyl-D-fucosamine (D-FucNAc) residues and about 1 N-acetyl-D-quinovosamine (D-QuiNAc) residue in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising about 5 N-acetyl-D-fucosamine (D-FucNAc) residues and about 2.5 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising about 10 N-acetyl-D-fucosamine (D-FucNAc) residues and about 5 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

5 In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising about 20 N-acetyl-D-fucosamine (D-FucNAc) residues and about 10 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

10 In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising about 30 N-acetyl-D-fucosamine (D-FucNAc) residues and about 15 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

15 In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising about 40 N-acetyl-D-fucosamine (D-FucNAc) residues and about 20 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

20 In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising about 50 N-acetyl-D-fucosamine (D-FucNAc) residues and about 25 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

25 In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising about 60 N-acetyl-D-fucosamine (D-FucNAc) residues and about 30 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

30 In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising about 68 N-acetyl-D-fucosamine (D-FucNAc) residues and about 32 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

35 In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising about 70 N-acetyl-D-fucosamine (D-FucNAc) residues and about 35 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising N-acetyl-D-fucosamine (D-FucNAc) residues and N-acetyl-D-quinovosamine (D-QuiNAc) residues where preferably the number of D-FucNAc residues is about the double of the number of D-QuiNAc residues.

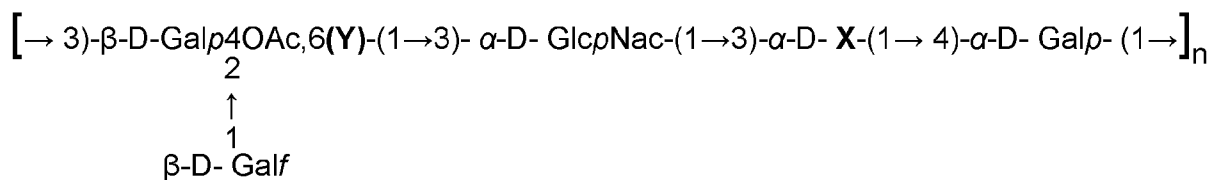
In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising N-acetyl-D-fucosamine (D-FucNAc) residues and N-

acetyl-D-quinovosamine (D-QuiNAc) residues where preferably the number of D-FucNAc residues is about the double of the number of D-QuiNAc residues.

In any of the above embodiments, the remaining sugar residues at the same position in the repeat unit may be D-2-acetamido-2,6-dideoxy-xylo-hexos-4-ulose (D-Sug). Therefore, in an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising N-acetyl-D-fucosamine (D-FucNAc), N-acetyl-D-quinovosamine (D-QuiNAc) and D-2-acetamido-2,6-dideoxy-xylo-hexos-4-ulose (D-Sug) residues.

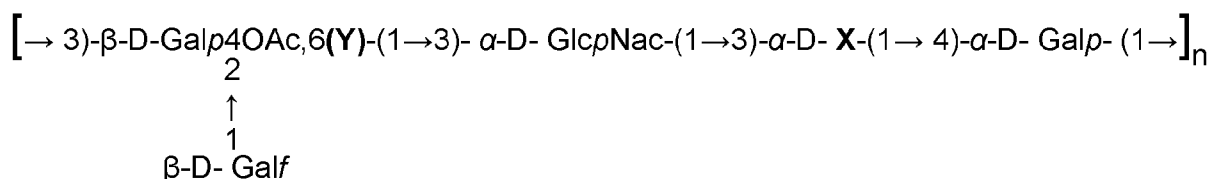
In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide comprising at the same position of the repeat unit either a N-acetyl-D-fucosamine (D-FucNAc) residue, a N-acetyl-D-quinovosamine (D-QuiNAc) residue or a D-2-acetamido-2,6-dideoxy-xylo-hexos-4-ulose (D-Sug) residue. In an embodiment, the number of D-FucNAc residues is about the double of the number of D-QuiNAc residues.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide with the following repeating unit:



where n represents the number of repeating units, wherein Y represents either a Serine or a Glycine residue wherein all the repeating units comprise the same Y residue and where X represents either a N-acetyl-D-fucosamine (D-FucNAc) residue or a N-acetyl-D-quinovosamine (D-QuiNAc) residue. In an embodiment, said serotype 38 capsular saccharide comprises about 70 N-acetyl-D-fucosamine (D-FucNAc) residues and about 30 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide. In an embodiment, said serotype 38 capsular saccharide comprises about 68 N-acetyl-D-fucosamine (D-FucNAc) residues and about 32 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

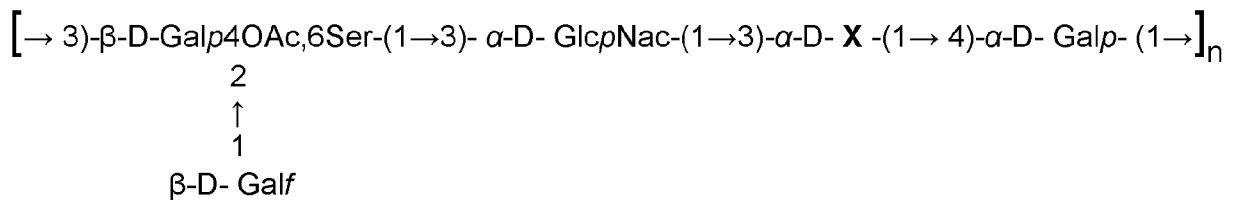
In an embodiment, essentially all the D-Sug residues have been reduced. Therefore, in an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide with the following repeating unit:



where n represents the number of repeating units, wherein Y represents either a Serine or a Glycine residue wherein all the repeating units comprise the same Y residue and where X represents either a N-acetyl-D-fucosamine (D-FucNAc) residue or a N-acetyl-D-quinovosamine (D-QuiNAc) residue. In an embodiment, said serotype 38 capsular saccharide comprises about

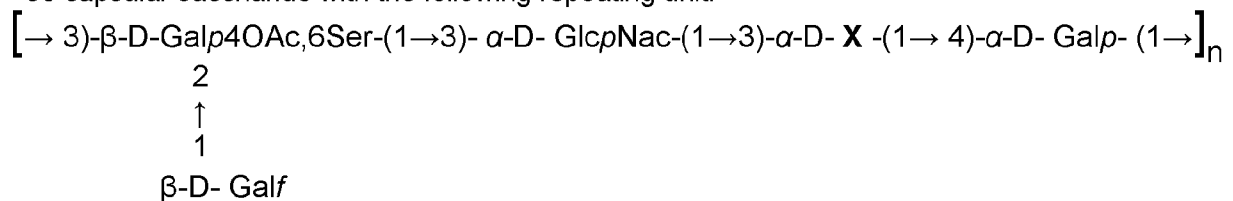
70 N-acetyl-D-fucosamine (D-FucNAc) residues and about 30 N-acetyl-D-quinovosamine (D-
QuiNAc) residues in every 100 saccharide repeat units of the saccharide. In an embodiment, said
serotype 38 capsular saccharide comprises about 68 N-acetyl-D-fucosamine (D-FucNAc)
residues and about 32 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide
5 repeat units of the saccharide.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a
serotype 38 capsular saccharide with the following repeating unit:



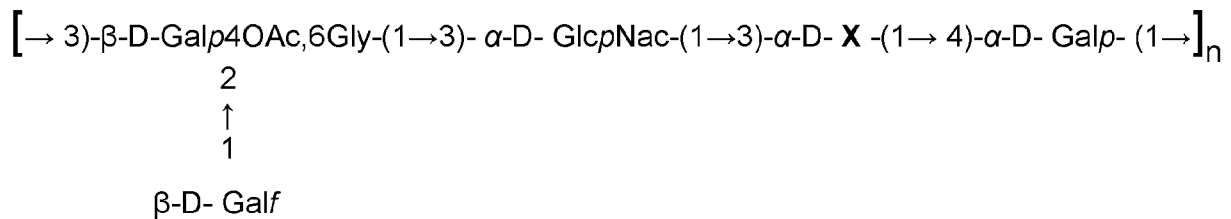
where n represents the number of repeating units and where X represents either a N-acetyl-D-
fucosamine (D-FucNAc) residue, a N-acetyl-D-quinovosamine (D-QuiNAc) residue or a D-2-
acetamido-2,6-dideoxy-xylo-hexos-4-ulose (D-Sug) residue. In an embodiment, said serotype 38
15 capsular saccharide comprises between about 1 to about 70 N-acetyl-D-fucosamine (D-FucNAc)
residues, between about 0.5 to about 30 N-acetyl-D-quinovosamine (D-QuiNAc) residues and
between about 0 to about 98.5 D-2-acetamido-2,6-dideoxy-xylo-hexos-4-ulose (D-Sug) residues
in every 100 saccharide repeat units of the saccharide.

In an embodiment, essentially all the D-Sug residues have been reduced. Therefore, in
20 an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype
38 capsular saccharide with the following repeating unit:



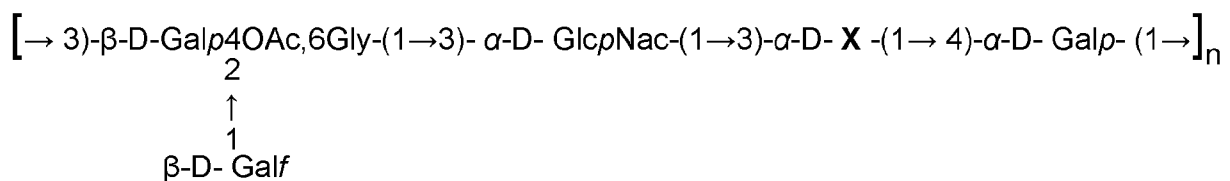
where n represents the number of repeating units and where X represents either a N-acetyl-D-
fucosamine (D-FucNAc) residue or a N-acetyl-D-quinovosamine (D-QuiNAc) residue. In an
embodiment, said serotype 38 capsular saccharide comprises about 70 N-acetyl-D-fucosamine
(D-FucNAc) residues and about 30 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100
30 saccharide repeat units of the saccharide. In an embodiment, said serotype 38 capsular
saccharide comprises about 68 N-acetyl-D-fucosamine (D-FucNAc) residues and about 32 N-
acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the
saccharide.

35 In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype
38 capsular saccharide with the following repeating unit:



where n represents the number of repeating units and where X represents either a N-acetyl-D-fucosamine (D-FucNAc) residue, a N-acetyl-D-quinovosamine (D-QuiNAc) residue or a D-2-acetamido-2,6-dideoxy-xylo-hexos-4-ulose (D-Sug) residue. In an embodiment, said serotype 38 capsular saccharide comprises between about 1 to about 70 N-acetyl-D-fucosamine (D-FucNAc) residues, between about 0.5 to about 30 N-acetyl-D-quinovosamine (D-QuiNAc) residues and between about 0 to about 98.5 D-2-acetamido-2,6-dideoxy-xylo-hexos-4-ulose (D-Sug) residues in every 100 saccharide repeat units of the saccharide.

In an embodiment, essentially all the D-Sug residues have been reduced. Therefore, in an embodiment, essentially all the D-Sug residues have been reduced. Therefore, in an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 capsular saccharide with the following repeating unit:



where n represents the number of repeating units and where X represents either a N-acetyl-D-fucosamine (D-FucNAc) residue or a N-acetyl-D-quinovosamine (D-QuiNAc) residue. In an embodiment, said serotype 38 capsular saccharide comprises about 70 N-acetyl-D-fucosamine (D-FucNAc) residues and about 30 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide. In an embodiment, said serotype 38 capsular saccharide comprises about 68 N-acetyl-D-fucosamine (D-FucNAc) residues and about 32 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

2.3 Mode of preparation of the *Streptococcus pneumoniae* serotype 38 glycoconjugates of the invention

The serotype 38 glycoconjugate of the present invention can be prepared by any coupling technique known to those of ordinary skill in the art.

In an embodiment, the serotype 38 saccharide is coupled to the carrier protein via non-covalent bonds (see e.g. WO2012155007, WO2020056202).

In an embodiment, the serotype 38 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.

In an embodiment, the serotype 38 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.

A possible linker is adipic acid dihydrazide (ADH). Other linkers include B-propionamido (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 38 glycoconjugate of the present invention is conjugated directly to the carrier protein (without a linker).

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 38 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 38 glycoconjugate of the present invention is prepared using CDAP chemistry. In said embodiment, the serotype 38 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 38 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

In an embodiment, the serotype 38 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 (activation) of the serotype 38 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 38 saccharide to a target molecular weight (MW) range can be performed. Therefore, in an embodiment, the isolated polysaccharide is sized before oxidation.

In an embodiment, the serotype 38 saccharide of the invention is conjugated to a carrier protein by a process comprising the step of:

- (a) reacting said serotype 38 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.

In an embodiment, the serotype 38 saccharide of the invention is conjugated to a carrier protein by a process comprising the step of:

- (a) reacting said serotype 38 saccharide with an oxidizing agent;
- (a') quenching the oxidation reaction by addition of a quenching 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.

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

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 (IO_4^-) and orthoperiodate (IO_6^{5-}) and the various salts of periodate (e.g., sodium periodate and potassium periodate).

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

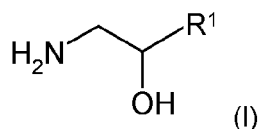
5 TEMPO, 2,2,6,6-Tetramethyl-4-(methylsulfonyloxy)-1-piperidinoxy, 4-Phosphonooxy-TEMPO, 4-Oxo-TEMPO, 4-Methoxy-TEMPO, 4-Isothiocyanato-TEMPO, 4-(2-Iodoacetamido)-TEMPO free radical, 4-Hydroxy-TEMPO, 4-Cyano-TEMPO, 4-Carboxy-TEMPO, 4-(2-Bromoacetamido)-TEMPO or 4-Amino-TEMPO, 4-Acetamido-2,2,6,6-tetramethylpiperidine 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 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 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):



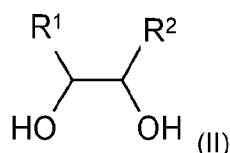
wherein R¹ is selected from H, methyl, ethyl, propyl or isopropyl.

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 (also named "degree of activation" in the present document) of the activated serotype 38 saccharide is between 2 and 30. In an embodiment the degree of oxidation (DO) of the activated serotype 38 polysaccharide is between 10 and 25.

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 38 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 38 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 triacetoxymethylborohydride, 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-BuMeⁱPrN-BH₃, benzylamine-BH₃ or 5-ethyl-2-methylpyridine borane (PEMB). In an embodiment, the reducing agent is sodium triacetoxymethylborohydride. 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.

CDI and/or CDT chemistry

In an embodiment, the serotype 38 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 38 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 38 saccharide to a target molecular weight (MW) range can be performed.

Therefore, in an embodiment, the serotype 38 saccharide is sized before activation with CDI. In an embodiment, the isolated polysaccharide is sized before activation with CDT. In an embodiment, the serotype 38 saccharide is sized to any of the target molecular weight (MW) range defined above.

Therefore, in an embodiment, the serotype 38 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 38 saccharide with CDI.

In one embodiment step a) comprises reacting the serotype 38 saccharide with an amount of CDI that is between 0.5-10 molar equivalent to the amount of serotype 38 saccharide present in the reaction mixture.

In one embodiment step a) comprises reacting the serotype 38 saccharide with CDT.

In one embodiment step a) comprises reacting the serotype 38 saccharide with an amount of CDT that is between 0.5-10 molar equivalent to the amount of serotype 38 saccharide present in the reaction mixture.

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).

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).

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).

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 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 38 glycoconjugate of the present invention is prepared by eTEC chemistry as disclosed WO2014027302

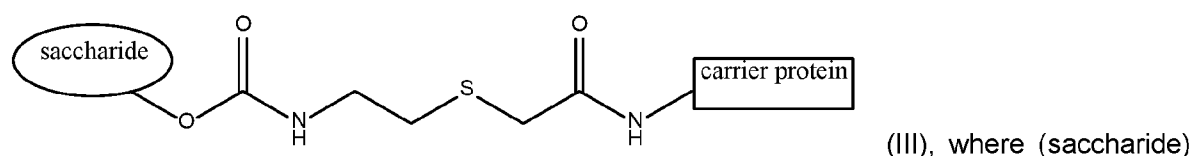
The eTEC spacer includes seven linear atoms (i.e., $-\text{C}(\text{O})\text{NH}(\text{CH}_2)_2\text{SCH}_2\text{C}(\text{O})-$) and provides stable thioether and amide bonds between the saccharide and carrier protein. Synthesis of the eTEC linked glycoconjugate involves reaction of an activated hydroxyl 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 38 glycoconjugate of the present invention comprises a serotype 38 saccharide covalently conjugated to a carrier protein through a (2-((2-oxoethyl)thio)ethyl)carbamate (eTEC) spacer.

In an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 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):



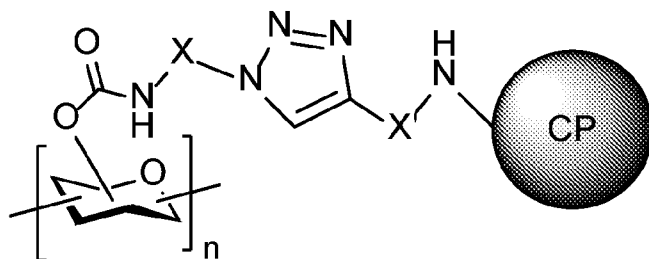
represents the serotype 38 saccharide.

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 38 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 remains unmodified and covalent linkages between the carrier protein and the saccharide is for a minority of the saccharide repeat units.

Click chemistry

In a preferred embodiment, the serotype 38 glycoconjugate of the present invention is prepared by click chemistry (see e.g. PCT/IB2023/050202).

Therefore, in an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (IV):



(IV),

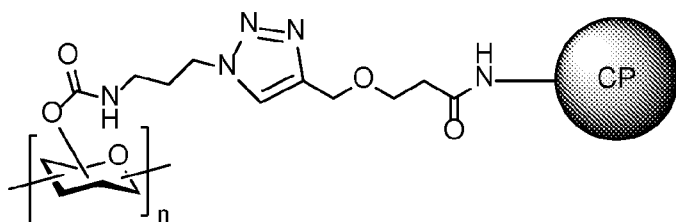
wherein X is selected from the group consisting of $\text{CH}_2(\text{CH}_2)_{n'}$, $(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$, $\text{NHCO}(\text{CH}_2)_{n'}$, $\text{NHCO}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$, $\text{OCH}_2(\text{CH}_2)_{n'}$ and $\text{O}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$; 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 38 saccharide and wherein n represents the number of repeating units.

In a particular aspect, the invention is directed to a serotype 38 glycoconjugate comprising a serotype 38 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.

In a particular aspect, the invention pertains to a serotype 38 glycoconjugate comprising a serotype 38 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 38 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 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 38 saccharide.

In an embodiment, the invention provides a serotype 38 glycoconjugate comprising a serotype 38 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 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 38 glycoconjugate comprising a serotype 38 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.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

In an embodiment, the invention provides a serotype 38 glycoconjugate comprising a serotype 38 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.

In an embodiment, the invention provides a serotype 38 glycoconjugate comprising a serotype 38 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.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

In an embodiment, the invention provides a serotype 38 glycoconjugate comprising a serotype 38 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 38 glycoconjugate comprising a serotype 38 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.

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

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

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

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

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

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

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

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

10 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.

20 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.

25 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.

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

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

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

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

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

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

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

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

In an embodiment, the invention provides a serotype 38 glycoconjugate comprising a serotype 38 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.

5 In an embodiment, the invention provides a serotype 38 glycoconjugate comprising a serotype 38 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.

10 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.

15 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.

20 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.

25 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.

30 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.

35 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 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 38 glycoconjugate comprising a serotype 38 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 38 glycoconjugate comprising a serotype 38 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.

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

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

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

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

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

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

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

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

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

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

In an embodiment, the invention provides a serotype 38 glycoconjugate comprising a serotype 38 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 38 glycoconjugate comprising a serotype 38 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 $CH_2O(CH_2CH_2O)_{m'}(CH_2)_{n''}CH_2C=O$, where n'' is selected from 0 to 10 and m' is selected from 0 to 4.

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

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

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

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

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

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

In another embodiment, m is 2, m' is 0 and n'' is 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 a very preferred embodiment of the present invention, the serotype 38 glycoconjugate of the present invention are prepared using click chemistry. The invention also relates to a method of making serotype 38 glycoconjugate, as disclosed herein above.

In an embodiment, click chemistry may comprise three steps, (a) reacting an isolated serotype 38 saccharide with a carbonic acid derivative and an azido linker in an aprotic solvent to produce an activated azido saccharide (activation of the saccharide), (b) reacting a carrier protein with an agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group where the NHS moiety reacts with the amino groups to form an amide linkage thereby obtaining an alkyne functionalized carrier protein (activation of the carrier protein), (c) reacting the activated azido saccharide of step (a) with the activated alkyne-carrier protein of step (b) by Cu^{+1} mediated azide-alkyne cycloaddition reaction to form a glycoconjugate.

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

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

As mentioned above, before the activation (a), sizing of the saccharide to a target molecular weight (MW) range may be performed. Therefore, in an embodiment, the isolated serotype 38 saccharide is sized before activation with a carbonic acid derivative and an azido

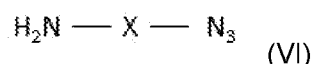
linker. In an embodiment, the isolated serotype 38 saccharide is sized to any of the target molecular weight (MW) range defined above. In an embodiment, the isolated serotype 38 saccharide is not sized before activation with a carbonic acid derivative and an azido linker.

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.

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 (VI),



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 (VI), 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 (VI), 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 (VI), 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 (VI), 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 (VI), 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 (VI), 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 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 (VII),

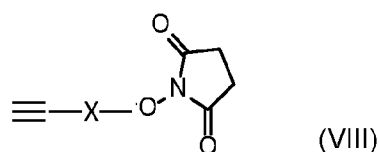


In a preferred embodiment, said azido linker is 3-azido-propylamine.

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 (VIII),



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.

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

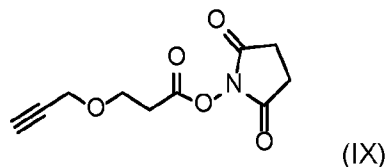
embodiment, n is 5 and m is 1. In an embodiment, n is 6 and m is 1. In an embodiment, n is 7 and m is 1. In an embodiment, n is 8 and m is 1. In an embodiment, n is 9 and m is 1. In an embodiment, n is 10 and m is 1.

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 (IX):



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.

5 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.

10 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.

15 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.

20 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.

25 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.

30 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 equivalent to the amount of saccharide present in the reaction mixture.

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

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

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

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

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

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

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

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

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

It has been surprisingly found that reacting the isolated saccharide with CDI in an environment with a moisture level of about 0.1% to 1% (v/v) allows to avoid side reactions. Therefore, in one embodiment the isolated saccharide is reacted with CDI in an aprotic solvent comprising 0.1% to 1% (v/v) water. In one embodiment the isolated saccharide is reacted with CDI in an aprotic solvent comprising 0.1% to 0.5% (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.

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).

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

10 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
15 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.

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
20 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%.

In another embodiment the degree of activation of the activated saccharide following step
25 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%.

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

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
35 about 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 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.

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 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.

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 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.

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.

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 5 molar equivalents to the lysines on the carrier.

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

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

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

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

In an embodiment, the carrier protein is CRM₁₉₇, which contains 39 lysine residues. In said embodiment the degree of activation of the activated carrier following step b) may be between 1 to 30. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is between 5 to 20. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is between 9 to 18. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is between 8 to 11. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is between 15 to 20. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 5. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 6. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 7. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 8. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 9. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 10. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 11. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 12. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 13. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 14. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 15. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 16. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 17. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 18. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 19. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 20. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 21. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 22. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 23. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 24. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 25.

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

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

In an embodiment, the conjugation reaction c) is carried out in aqueous buffer. In an embodiment, the conjugation reaction c) is carried out in aqueous buffer in the presence of copper (I) as catalyst. In an embodiment, the conjugation reaction c) is carried out in aqueous buffer in the presence an oxidant and of copper (I) as catalyst. In a preferred embodiment, the conjugation reaction c) is carried out in aqueous buffer in the presence of copper (I) as catalyst and ascorbate as oxidant. In an embodiment, THPTA (tris(3-hydroxypropyltriazolylmethyl)amine) and

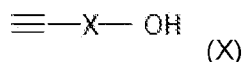
aminoguanidine may be further added to protect the protein from side reactions. Therefore, in a preferred embodiment, the conjugation reaction c) is carried out in aqueous buffer in the presence of copper (I) as catalyst and ascorbate as oxidant, wherein the reaction mixture further comprises THPTA (tris(3-hydroxypropyltriazolylmethyl)amine)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

5 In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at setp c) is between 0.1 and 3. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at setp c) is between 0.5 and 2. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at setp c) is between 0.6 and 1.5. In a preferred embodiment the initial
10 input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at setp c) is between 0.8 and 1. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at setp c) is about 0.5. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at setp c) is about 0.6. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at setp c) is about 0.7. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at setp c) is about 0.8. In an
15 embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at setp c) is about 0.9. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at setp c) is about 1. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at setp c) is about 1.1. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at setp c) is about 1.2. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at setp c) is about 1.3. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at setp c) is about 1.4. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at setp c) is about 1.5. In an
20 embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at setp c) is about 1.6. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at setp c) is about 1.7. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at setp c) is about 1.8. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at setp c) is about 1.9. In an embodiment the initial input ratio (weight by weight) of activated azido saccharide to activated alkyne-carrier at setp c) is about 2.
25

35 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 (X),



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.

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.

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.

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.

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.

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.

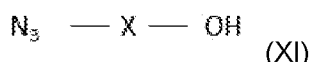
In an embodiment the capping of the unreacted azido groups is performed with an amount of capping agent that is about 1.5 molar equivalent to the amount of polysaccharide repeat unit of the activated saccharide.

5 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.

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.

10 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 (XI),



15 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.

20 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.

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 10 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 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.

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

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

5 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.

10 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.

15 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.

20 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.

25 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.

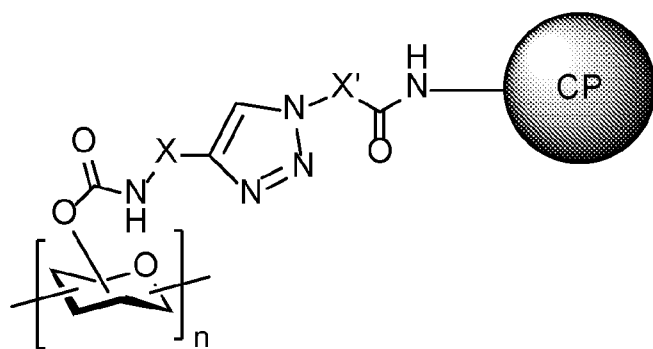
30 Following conjugation to the carrier protein, the glycoconjugate can be purified (enriched with respect to the amount of saccharide-protein conjugate) by a variety of techniques known to the skilled person. These techniques include dialysis, concentration/diafiltration operations, tangential flow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

35 In an aspect, the invention provides a serotype 38 glycoconjugate produced according to any of the methods disclosed herein.

Alternative click chemistry

In an embodiment of the present invention, the serotype 38 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).

- 5 Therefore, in an embodiment, the serotype 38 glycoconjugate of the present invention comprises a serotype 38 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (XII):



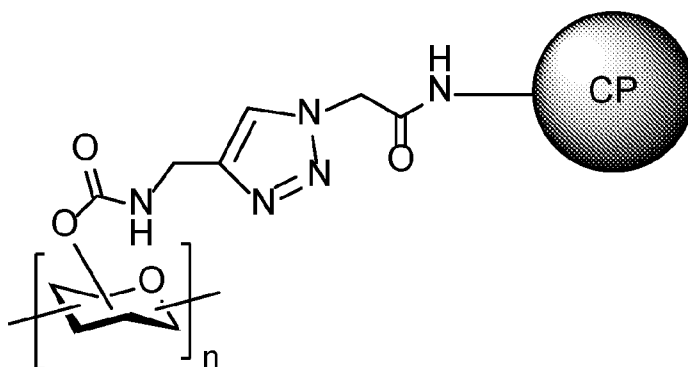
(XII),

- 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 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 38 saccharide and wherein n represents the number of repeating units.

In a particular aspect, the invention is directed to a serotype 38 glycoconjugate comprising a serotype 38 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (XII), 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.

- 20 In a particular aspect, the invention pertains to a serotype 38 glycoconjugate comprising a serotype 38 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (XIII),



(XIII)

wherein the structure in square bracket represents a repeat unit of the serotype 38 saccharide and wherein n represents the number of repeating units.

Formulas (XII) and (XIII) 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 38 saccharide.

In an embodiment, the invention provides a serotype 38 glycoconjugate comprising a serotype 38 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (XII), 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.

5 In an embodiment, the invention provides a serotype 38 glycoconjugate comprising a serotype 38 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (XII), 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 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 38 glycoconjugate comprising a serotype 38 saccharide covalently conjugated to a carrier protein (CP) through a spacer and

having the general formula (XII), 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.

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 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 38 glycoconjugate comprising a serotype 38 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (XII), 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 38 glycoconjugate comprising a serotype 38 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (XII), 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 38 glycoconjugate comprising a serotype 38 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (XII), 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 $\text{CH}_2\text{O}(\text{CH}_2\text{CH}_2\text{O})_{m'}(\text{CH}_2)_{n''}\text{CH}_2$, 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 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 38 glycoconjugate comprising a serotype 38 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (XII), 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 38 glycoconjugate comprising a serotype 38 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (VII), wherein X is $\text{NHCO}(\text{CH}_2)_{n'}$, where n' is selected from 1 to 10 and wherein X' is $\text{CH}_2\text{O}(\text{CH}_2)_{n''}\text{CH}_2$, 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 38 glycoconjugate comprising a serotype 38 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (XII), 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 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 a particular embodiment, n' is 1, m' is 0 and n'' is 4. In a particular embodiment, n' is 1, m' is 1 and n'' is 4. In a particular embodiment, n' is 1, m' is 2 and n'' is 4. In a particular embodiment, n' is 1, m' is 3 and n'' is 4.

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

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

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

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

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

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

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

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

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

In an embodiment, the invention provides a serotype 38 glycoconjugate comprising a serotype 38 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (XII), 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 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 38 glycoconjugate comprising a serotype 38 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (XII), 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 38 glycoconjugate comprising a serotype 38 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (XII), 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.

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 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 38 glycoconjugate comprising a serotype 38 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (XII), 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 38 glycoconjugate comprising a serotype 38 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (XII), 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.

In an embodiment, the invention provides a serotype 38 glycoconjugate comprising a serotype 38 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (XII), 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.

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
5 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.

10 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.

20 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.

25 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.

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

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

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

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

10 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.

20 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.

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

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

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

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

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

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

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

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

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

In an embodiment, the invention provides a serotype 38 glycoconjugate comprising a serotype 38 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (XII), 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 38 glycoconjugate comprising a serotype 38 saccharide covalently conjugated to a carrier protein (CP) through a spacer and

having the general formula (XII), wherein X is $O(CH_2CH_2O)_mCH_2CH_2$, where m is selected from 1 to 4 and wherein X' is $CH_2O(CH_2CH_2O)_nCH_2$, 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 38 glycoconjugate comprising a serotype 38 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (XII), wherein X is $O(CH_2CH_2O)_mCH_2CH_2$, where m is selected from 1 to 4 and wherein X' is $CH_2O(CH_2CH_2O)_m(CH_2CH_2O)_nCH_2$, 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 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 of the present invention, the serotype 38 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 38 glycoconjugate, as disclosed herein above.

In an embodiment, click chemistry may comprise three steps, (a) reacting an isolated serotype 38 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 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.

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".

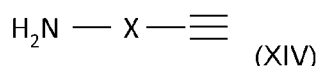
As mentioned above, before the activation (a), sizing of the serotype 38 saccharide to a target molecular weight (MW) range may be performed. Therefore, in an embodiment, the isolated serotype 38 saccharide is sized before activation with a carbonic acid derivative and an alkyne linker. In an embodiment, the isolated serotype 38 saccharide is sized to any of the target molecular weight (MW) range defined above. In an embodiment, the isolated serotype 38 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 (XIV),



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 (XIV), wherein X is CH_2 .

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

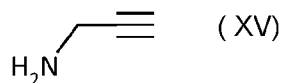
5 In an embodiment, said alkyne linker is a compound of formula (XIV), wherein X is $\text{NHCO}(\text{CH}_2)_n$, and n is selected from 1 to 10. In an embodiment, n is selected from 1 to 5. In an embodiment, n is selected from 1 to 4. In an embodiment, n is selected from 1 to 3. In an embodiment, n 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
10 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 (XIV), 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
15 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 (XIV), 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
20 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
25 is 10.

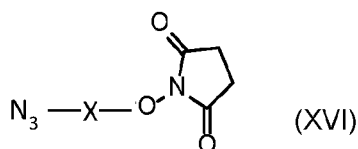
In an embodiment, said alkyne linker is a compound of formula (XIV), wherein X is $\text{O}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$, where m is selected from 1 to 4. In an embodiment, m is selected from 1 to 3. In an embodiment, m is selected from 1 to 2. In a particular embodiment,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

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



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

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

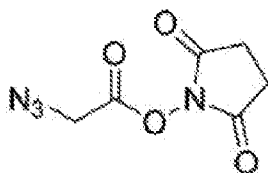


where X is selected from the group consisting of $(\text{CH}_2)_n\text{CH}_2\text{C}=\text{O}$ and $(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2=\text{O}$ where n is selected from 0 to 10 and m is selected from 0 to 4.

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



(XVII)

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.

5 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.

10 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.

15 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.

20 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.

25 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.

30 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.

35 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.

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

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

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

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

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

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

It has been surprisingly found that reacting the isolated saccharide with CDI in an environment with a moisture level of about 0.1% to 1% (v/v) allows to avoid side reactions. Therefore, in one embodiment the isolated saccharide is reacted with CDI in an aprotic solvent comprising 0.1% to 1% (v/v) water. In one embodiment the isolated saccharide is reacted with CDI in an aprotic solvent comprising 0.1% to 0.8% (v/v) water. In one embodiment the isolated saccharide is reacted with CDI in an aprotic solvent comprising 0.1% to 0.5% (v/v) water. In one embodiment the isolated saccharide is reacted with CDI in an aprotic solvent comprising 0.1% to 0.4% (v/v) water. In one embodiment the isolated saccharide is reacted with CDI in an aprotic solvent comprising 0.1% to 0.3% (v/v) water. 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 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 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 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.

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

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

15 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
20 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)
25 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.

30 Therefore, in an embodiment, carbonic acid derivative activation is followed by the addition of water. In an embodiment, water is added to bring the total water content in the mixture to between about 1% to about 10% (v/v). In an embodiment, water is added to bring the total water content in the mixture to between about 1% 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
35 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.

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 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.

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.

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%.

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%.

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

a) is about 2%. In an embodiment the degree of activation of the activated saccharide following step a) is about 3%. In an embodiment the degree of activation of the activated saccharide following step a) is about 4%. In an embodiment the degree of activation of the activated saccharide following step a) is about 5%. In an embodiment the degree of activation of the activated saccharide following step a) is about 6%. In an embodiment the degree of activation of the activated saccharide following step a) is about 7%. In an embodiment the degree of activation of the activated saccharide following step a) is about 8%. In an embodiment the degree of activation of the activated saccharide following step a) is about 9%. In an embodiment the degree of activation of the activated saccharide following step a) is about 10%. In an embodiment the degree of activation of the activated saccharide following step a) is about 11%. In an embodiment the degree of activation of the activated saccharide following step a) is about 12%. In an embodiment the degree of activation of the activated saccharide following step a) is about 13%. In an embodiment the degree of activation of the activated saccharide following step a) is about 14%. In an embodiment the degree of activation of the activated saccharide following step a) is about 15%. In an embodiment the degree of activation of the activated saccharide following step a) is about 16%. In an embodiment the degree of activation of the activated saccharide following step a) is about 17%. In an embodiment the degree of activation of the activated saccharide following step a) is about 18%. In an embodiment the degree of activation of the activated saccharide following step a) is about 19%. In an embodiment the degree of activation of the activated saccharide following step a) is about 20%.

In one embodiment step b) comprises reacting the carrier protein with an amount of agent bearing an N-Hydroxysuccinimide (NHS) moiety and an 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.

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.

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.

5 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.

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 about 0.5 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 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 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.

20 In an embodiment, the carrier protein is CRM₁₉₇, which contains 39 lysine residues. In said embodiment the degree of activation of the activated carrier following step b) may be between 1 to 30. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is between 5 to 20. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is between 9 to 18. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is between 8 to 11. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is between 15 to 20. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 5. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 6. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 7. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 8. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 9. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 10. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 11. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 12. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 13. In another embodiment the degree of activation of the activated carrier (CRM₁₉₇) following step b) is about 14. In another embodiment the degree of

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

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

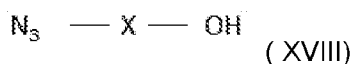
In an embodiment, the conjugation reaction c) is carried out in aqueous buffer. In an embodiment, the conjugation reaction c) is carried out in aqueous buffer in the presence of copper (I) as catalyst. In an embodiment, the conjugation reaction c) is carried out in aqueous buffer in the presence an oxidant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



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.

5 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.

10 In an embodiment the capping of the unreacted alkyne groups is performed with an amount of capping agent that is between 0.5 to 1 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 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.

20 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.

25 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.

30 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.

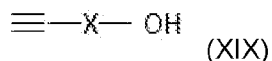
In an embodiment the capping of the unreacted alkyne groups is performed with an amount of capping agent that about 2.5 molar equivalents to the amount of polysaccharide repeat unit of the activated saccharide.

35 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 (XIX),



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.

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

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

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

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

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.

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.

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.

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

In an aspect, the invention provides a serotype 38 glycoconjugate produced according to any of the methods disclosed herein.

2.4 Carrier Proteins

A component of the glycoconjugate is a carrier protein to which the serotype 38 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 38 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),
 5 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
 10 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
 15 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).
 20 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 38 saccharide glycoconjugate of the invention is selected from the group consisting of TT, DT, DT mutants (such
 25 as CRM₁₉₇), and a C5a peptidase from *Streptococcus* (SCP).

In an embodiment, the carrier protein of the serotype 38 saccharide glycoconjugate is DT (Diphtheria Toxoid). In another embodiment, the carrier protein of the serotype 38 saccharide glycoconjugate is TT (Tetanus Toxoid).

In another embodiment, the carrier protein of the serotype 38 saccharide glycoconjugate
 30 is PD (*H. influenzae* protein D; see, e.g., EP0594610 B).

In a preferred embodiment, the carrier protein of the serotype 38 saccharide glycoconjugate is CRM₁₉₇ or a C5a peptidase from *Streptococcus* (SCP).

In another embodiment, the carrier protein of the serotype 38 saccharide glycoconjugate is rhizavidin [aa 45-179J-GGGGSSS-SP1500- AAA-SP0785] (CP1).

35 In another embodiment, the carrier protein of the serotype 38 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 38 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 38 saccharide is conjugated to CRM₁₉₇ protein. In an embodiment, the serotype 38 saccharide is conjugated to CRM₁₉₇ protein or the A chain of CRM₁₉₇ (see CN103495161). In an embodiment, the serotype 38 saccharide is conjugated the A chain of CRM₁₉₇ obtained via expression by genetically recombinant *E. coli* (see CN103495161). In other preferred embodiments, the carrier protein of the serotype 38 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 sepsis (GBS) have developed a way to defeat this immune response. All human isolates of β -hemolytic streptococci, including GAS and GBS, produce a highly conserved cell-wall protein SCP (Streptococcal C5a Peptidase) that specifically inactivates C5a. The *scp* genes from GAS and GBS encode a polypeptide containing between 1,134 and 1,181 amino acids (Brown *et al.*, PNAS, 2005, vol. 102, no. 51 pages 18391–18396). The first 31 residues are the export signal presequence and are removed upon passing through the cytoplasmic membrane. The next 68 residues serve as a pro-sequence and must be removed to produce active SCP. The next 10 residues can be removed without loss of protease activity. At the other end, starting with Lys-1034, are four consecutive 17-residue motifs followed by a cell sorting and cell-wall attachment signal. This combined signal is composed of a 20-residue hydrophilic sequence containing an LPTTND sequence, a 17-residue hydrophobic sequence, and a short basic carboxyl terminus. SCP can be divided in domains (see figure 1B of Brown *et al.*, PNAS, 2005, vol. 102, no. 51 pages 18391–18396). These domains are the Pre/Pro domain (which comprises the export signal presequence (commonly the first 31 residues) and the pro-sequence (commonly the next 68 residues)), the protease domain (which is splitted in two part (protease part 1 commonly residues 89–333/334 and protease domain part 2 and commonly residues 467/468–583/584), the protease-associated domain (PA domain) (commonly residues 333/334–467/468), three

fibronectin type III (Fn) domains (Fn1, commonly residues 583/584–712/713; Fn2, commonly residues 712/713–928/929/930; commonly Fn3, residues 929/930–1029/1030/1031) and a cell wall anchor domain (commonly residues 1029/1030/1031 to the C-terminus).

In an embodiment, the carrier protein of the serotype 38 saccharide glycoconjugate of the invention is an SCP from GBS (SCP_B). An example of SCP_B is provided at SEQ. ID.NO: 3 of WO97/26008. See also SEQ ID NO: 3 of WO00/34487.

In another preferred embodiment, the carrier protein of the serotype 38 saccharide glycoconjugate of the invention is an SCP from GAS (SCP_A).

Examples of SCP_A 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 38 saccharide glycoconjugate of the invention is an enzymatically inactive SCP.

In other preferred embodiments, the carrier protein of the serotype 38 saccharide glycoconjugate of the invention is an enzymatically inactive SCP from GBS (SCP_B).

In another preferred embodiment, the carrier protein of the serotype 38 saccharide glycoconjugate of the invention is an enzymatically inactive SCP from GAS (SCP_A).

In an embodiment, the carrier protein of the serotype 38 saccharide glycoconjugate of the invention is a fragment of an SCP. In an embodiment, the carrier protein of the serotype 38 saccharide glycoconjugate of the invention is a fragment of an SCP_A. Preferably, the carrier protein of the serotype 38 saccharide glycoconjugate of the invention is a fragment of an SCP_B.

In an embodiment, the carrier protein of the serotype 38 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 38 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 38 saccharide glycoconjugate of the invention is an enzymatically inactive fragment of an SCP which comprises the protease domain, the protease-associated domain (PA domain) and two of the three fibronectin type III (Fn) domains but does not comprise the export signal presequence, the pro-sequence and the cell wall anchor domain.

In an embodiment, the carrier protein of the serotype 38 saccharide glycoconjugate of the invention is an enzymatically inactive fragment of an SCP. In an embodiment, said enzymatically inactive fragment of SCP comprises the protease domain, the protease-associated domain (PA domain) and the three fibronectin type III (Fn) domains but does not comprise the export signal presequence, the pro-sequence and the cell wall anchor domain.

In an embodiment, the carrier protein of the serotype 38 saccharide glycoconjugate of the invention is an enzymatically inactive fragment of an SCPA. In an embodiment, said enzymatically inactive fragment of an SCPA comprises the protease domain, the protease-associated domain (PA domain) and the three fibronectin type III (Fn) domains but does not comprise the export signal presequence, the pro-sequence and the cell wall anchor domain.

In a preferred embodiment, the carrier protein of the serotype 38 saccharide glycoconjugate of the invention is an enzymatically inactive fragment of SCPB. Preferably, said enzymatically inactive fragment of SCPB comprises the protease domain, the protease-associated domain (PA domain) and the three fibronectin type III (Fn) domains but does not comprise the export signal presequence, the pro-sequence and the cell wall anchor domain.

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

Therefore, in an embodiment, the carrier protein of the serotype 38 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, 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 38 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 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 38 saccharide glycoconjugate of the invention is an enzymatically inactive SCPB where said inactivation is accomplished by replacing at least one amino acid of the wild type sequence. Preferably, said replacement of at least one amino acid is in the protease domain. In an embodiment, said replacement of at least one amino acid is in part 1 of the protease domain. In an embodiment, said replacement of at least one amino

acid is in part 2 of the protease domain. In an embodiment, said replacement is selected from the group consisting of D130A, H193A, N295A and S512A. In an embodiment, said replacement is D130A. In another embodiment, said replacement is H193A. In another embodiment, said replacement is N295A. In yet another embodiment, said replacement is S512A.

5 In an embodiment, the carrier protein of the serotype 38 saccharide glycoconjugate of the invention is an enzymatically inactive fragment of an SCP where said inactivation is accomplished by replacing at least one amino acid of the wild type sequence. Preferably, said replacement of at least one amino acid is in the protease domain. In an embodiment, said replacement of at least one amino acid is in part 1 of the protease domain. In an embodiment, said replacement of at least one amino acid is in part 2 of the protease domain. In an embodiment, said replacement is selected from the group consisting of D130A, H193A, N295A and S512A. In an embodiment, said replacement is D130A. In another embodiment, said replacement is H193A. In another embodiment, said replacement is N295A. In yet another embodiment, said replacement is S512A.

10 In an embodiment, the carrier protein of the serotype 38 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.

15 In an embodiment, the carrier protein of the serotype 38 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 38 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 38 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 38 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 38 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 38 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 38 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.

5 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
10 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 38 saccharide glycoconjugate of the invention is an enzymatically inactive fragment of SCPA which comprises the protease domain,
15 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
20 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
25 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 38 saccharide glycoconjugate of the
30 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
35 domain. In an embodiment, said replacement of at least two amino acids is in part 1 of the protease domain. In an embodiment, said replacement of at least two amino acids is in part 2 of the protease domain. In an embodiment, said at least two amino acids replacements are selected from the group consisting of D130A, H193A, N295A and S512A. In an embodiment, said at least

two amino acids replacements are D130A and H193A. In an embodiment, said at least two amino acids replacements are D130A and N295A. Preferably, said at least two amino acids replacements are D130A and S512A. In an embodiment, said at least two amino acids replacements are H193A and N295A. In an embodiment, said at least two amino acids replacements are H193A and S512A. In an embodiment, said at least two amino acids replacements are N295A and S512A.

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

Therefore, in an embodiment, the carrier protein of the serotype 38 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, 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 38 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 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 38 saccharide glycoconjugate of the invention is an enzymatically inactive SCPB where said inactivation is accomplished by replacing

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

In an embodiment, the carrier protein of the serotype 38 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 38 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 38 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 38 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 38 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 38 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

5 In an embodiment, the carrier protein of the serotype 38 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

10 In an embodiment, the carrier protein of the serotype 38 saccharide glycoconjugate of the invention is an enzymatically inactive fragment of an SCP where said inactivation is accomplished by replacing at least four amino acids of the wild type sequence. Preferably, said replacement of at least four amino acids is in the protease domain. In an embodiment, said replacement of at least four amino acids is in part 1 of the protease domain. In an embodiment, said replacement of at least four amino acid is in part 2 of the protease domain. In an embodiment, said at least four amino acids replacements are D130A, H193A, N295A and S512A

15 In an embodiment, the carrier protein of the serotype 38 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 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 38 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 type sequence. Preferably, said replacement of at least four amino acids is in the protease domain. In an embodiment, said replacement of at least four amino acids is in part 1 of the protease domain. In an embodiment, said replacement of at least one amino acids is in part 2 of the protease domain. In an embodiment, said at least four amino acids replacements are D130A, H193A, N295A and S512A

In an embodiment, the carrier protein of the serotype 38 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 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 38 saccharide glycoconjugate of the invention is an enzymatically inactive fragment of SCP which consists of SEQ ID NO: 1.

In a particular embodiment, the carrier protein of the serotype 38 saccharide glycoconjugate of the invention is an enzymatically inactive fragment of SCP which consists of SEQ ID NO: 2.

SEQ ID NO: 1 :

MAKTADTPATSKATIRDLNDPSQVKTLQEKGAGTVVAVIAAGFDKNH
 EAWRLTDKAKARYQSKEDLEKAKKEHGITYGEWWNDKVAYYHDYSKDGKT
 AVDQEHGTHVSGILSGNAPSETKEPYRLEGAMPEAQLLLMRVEIVNGLAD
 YARNYAQAIRDAINLGAKVINMSFGNAALAYANLPDETKKAFDYAKSKGV
 SIVTSAGNDSSFSGGKTRLPLADHPDYGVVGTAAADSTLTVASYSPDKQL
 TETVTVKADQQDKEMPVLSTNRFEPNKAYDYAYANRGTKEDDFKDVKGK
 IALIERGIDIFKDKIAKAKKAGAVGVLIYDNQDKGFPIELPNVDQMPPAF
 ISRKDGLLLKDNPKTITFNATPKVLPTASGTKLSRFSSWGLTADGNIKP
 DIAAPGQDILSSVANNKYAKLSGTAMSAPLVAGIMGLLQEQYETQYPMPT
 PSERLDLAKKVLMSATALYDEDEKAYFSPRQQGAGAVDAKKASAATMYV
 TDKDNTSSKVHLNNVSDKFEVTVTVHNKSDKPQELYYQATVQTDKVDGKH
 FALAPKALYETSWQKITIPANSSKQVTPIDASRFSKDLLAQMKNFYFLE
 GFVRFKQDPKKEELMSIPYIGFRGDFGNLSALEKPIYDSKDGSSYYHEAN
 SDAKDQLDGDGLQFYALKNNFTALTTESNPWTIIKAVKEGVENIEDIESS
 EITETIFAGTFAKQDDDDSHYYIHRHANGKPYAAISPNGDGNRDYYVQFQGT
 FLRNAKNLVAEVLDEKGNVVTSEVTEQVVKNNNDLASTLGSTRFEKTR
 WDGKDKDGKVVANGTYTYRVRYTPISSGAKEQHTDFDVIDNTTPEVATS
 ATFSTEDRRLTLASKPKTSQPVYRERIAITYMDEDLPTTEYISPNEGDGTF
 TLPEEAETMEGATVPLKMSDFTYVVEDMAGNITYTPVTKLLEGHSNKPEQ

SEQ ID NO: 1 is 950 amino acids long.

SEQ ID NO: 2 :

AKTADTPATSKATIRDLNDPSQVKTLQEKAGKGAGTVVAVIAAGFDKNH
 EAWRLTDKAKARYQSKEDLEKAKKEHGITYGEWWNDKVAYYHDYSKDGKT
 AVDQEHGTHVSGILSGNAPSETKEPYRLEGAMPEAQLLLMRVEIVNGLAD
 5 YARNYAQAIRDAINLGAKVINMSFGNAALAYANLPDETKKAFDYAKSKGV
 SIVTSAGNDSSFGGKTRLPLADHPDYGVVGTAAADSTLTVASYSPDKQL
 TETVTVKTADQQDKEMPVLSTNRFEPNKAYDYAYANRGTKEDDFKDVKGK
 IALIERGDIKFKDKIAKAKKAGAVGVLIYDNQDKGFPIELPNVDQMPAAF
 ISRKDGLLLKDNPKQTITFNATPKVLPTASGTKLSRFSSWGLTADGNIKP
 10 DIAAPGQDILSSVANNKYAKLSGTAMSAPLVAGIMGLLQEQYETQYPDMT
 PSERLDLAKKVLMSATALYDEDEKAYFSPRQQGAGAVDAKKASAATMYV
 TDKDNTSSKVHLNNVSDKFEVTVTVHNKSDKPQELYYQATVQTDKVDGKH
 FALAPKALYETSWQKITIPANSSKQVTPIDASRFSKDLLAQMKNGYFLE
 GFVRFKQDPKKEELMSIPYIGFRGDFGNLSALEKPIYDSKDGSSYYHEAN
 15 SDAKDQLDGDGLQFYALKNNFTALTTESNPWTIIKAVKEGVENIEDIESS
 EITETIFAGTFAKQDDDSHYIHRHANGKPYAAISPNGDGNRDYYVQFQGT
 FLRNAKNLVAEVLKKEGNVVTSEVTEQVVKNNNDLASTLGSTRFEKTR
 WDGKDKDGKVVANGTYTYRVRYTPISSGAKEQHTDFDVIDNTTPEVATS
 ATFSTEDRRLTLASKPKTSQPVYRERIAITYMDEDLPTTEYISPNEGTGTF
 20 TLPEEAETMEGATVPLKMSDFTYVVEDMAGNITYTPVTKLLEGHSNKPEQ

SEQ ID NO: 2 is 949 amino acids long.

In a particular embodiment, the carrier protein of the serotype 38 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.

25 In a particular embodiment, the carrier protein of the serotype 38 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 38 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.

30 In a particular embodiment, the carrier protein of the serotype 38 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.

35 In a particular embodiment, the carrier protein of the serotype 38 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 38 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 38 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 38 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.

In a particular embodiment, the carrier protein of the serotype 38 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 38 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.

In a particular embodiment, the carrier protein of the serotype 38 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 38 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 38 saccharide of the invention.

In an embodiment the invention relates to an immunogenic composition comprising a *Streptococcus pneumoniae* serotype 38 glycoconjugate of the invention.

In an embodiment the invention relates to an immunogenic composition comprising a *Streptococcus pneumoniae* serotype 38 glycoconjugate of the invention and comprising from 1 to 45 different glycoconjugates.

In an embodiment the invention relates to an immunogenic composition comprising a *Streptococcus pneumoniae* serotype 38 glycoconjugate of the invention and comprising from 1 to 45 glycoconjugates from different serotypes of *S. pneumoniae* (1 to 45 pneumococcal conjugates).

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

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

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 38 glycoconjugate of the invention and comprising from 26 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 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-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.

In an embodiment the invention relates to an immunogenic composition comprising a *Streptococcus pneumoniae* serotype 38 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.

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 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.

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

5 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.

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

In an embodiment the invention relates to an immunogenic composition comprising a *Streptococcus pneumoniae* serotype 38 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.

15 In an embodiment the invention relates to an immunogenic composition comprising a *Streptococcus pneumoniae* serotype 38 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.

20 In an embodiment the invention relates to an immunogenic composition comprising a *S. pneumoniae* serotype 38 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
25 *Streptococcus pneumoniae* serotype 38 glycoconjugate of the invention and further comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F. In an embodiment the immunogenic composition is a 16-valent pneumococcal conjugate compositions.

30 In an embodiment the invention relates to an immunogenic composition comprising a *Streptococcus pneumoniae* serotype 38 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, 23F and 33F. In an embodiment the immunogenic composition is a 21-valent pneumococcal conjugate compositions.

35 In an embodiment the invention relates to an immunogenic composition comprising a *Streptococcus pneumoniae* serotype 38 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, 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 38 glycoconjugate of the invention and further comprising glycoconjugates from *S. pneumoniae* serotypes 1, 2, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F and 33F. In an embodiment the immunogenic composition is a 22-valent pneumococcal conjugate compositions.

In an embodiment the invention relates to an immunogenic composition comprising a *Streptococcus pneumoniae* serotype 38 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 38 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 38 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 38 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 38 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 38 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 38 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 38 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, 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 38 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, 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 38 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, 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 38 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, 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 38 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, 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 38 are conjugated to CRM₁₉₇ and the *S. pneumoniae* 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 38 glycoconjugate of the invention and further comprising
35 at least one glycoconjugate selected from the group consisting of glycoconjugates from *S. pneumoniae* serotypes 2, 3, 7C, 9N, 10B, 15A, 16F, 17F, 20, 21, 22A, 23A, 23B, 24B, 24F, 27, 29, 31, 33B, 34, 35B and 35F.

In an embodiment the invention relates to an immunogenic composition comprising a *Streptococcus pneumoniae* serotype 38 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, 21, 22A, 23A, 23B, 24B, 24F, 27, 29, 31, 33B, 34, 35B and 35F. In an embodiment the immunogenic composition is a 22-valent pneumococcal conjugate compositions.

5 In an embodiment the invention relates to an immunogenic composition comprising a *Streptococcus pneumoniae* serotype 38 glycoconjugate of the invention and further comprising glycoconjugates from serotypes 2, 3, 7C, 9N, 10B, 15A, 16F, 17F, 20, 21, 22A, 23A, 23B, 24B, 24F, 27, 29, 31, 33B, 34, 35B and 35F. In an embodiment the immunogenic composition is a 23-valent pneumococcal conjugate compositions.

10 In an embodiment the invention relates to an immunogenic composition comprising a *Streptococcus pneumoniae* serotype 38 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, 21, 22A, 23A, 23B, 24B, 24F, 27, 29, 31, 33B, 34, 35B and 35F.

15 In an embodiment the invention relates to an immunogenic composition comprising a *Streptococcus pneumoniae* serotype 38 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, 21, 22A, 23A, 23B, 24B, 24F, 27, 29, 31, 33B, 34, 35B and 35F. In an embodiment the immunogenic composition is a 23-valent pneumococcal conjugate compositions.

20 In an embodiment the invention relates to an immunogenic composition comprising a *Streptococcus pneumoniae* serotype 38 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, 21, 22A, 23A, 23B, 24B, 24F, 27, 29, 31, 33B, 34, 35B and 35F. In an embodiment the immunogenic composition is a 24-valent pneumococcal conjugate compositions.

25 In an embodiment the invention relates to an immunogenic composition comprising a *Streptococcus pneumoniae* serotype 38 glycoconjugate of the invention and further comprising glycoconjugates from serotypes 2, 3, 7C, 9N, 10B, 15A, 16F, 17F, 20, 21, 22A, 23A, 23B, 24B, 24F, 27, 29, 31, 33B, 34, 35B and 35F. In an embodiment the immunogenic composition is a 23-valent pneumococcal conjugate compositions.

30 In an embodiment the invention relates to an immunogenic composition comprising a *Streptococcus pneumoniae* serotype 38 glycoconjugate of the invention and further comprising glycoconjugates from serotypes 2, 3, 7C, 9N, 10B, 15A, 16F, 17F, 19A, 19F, 20, 21, 22A, 23A, 23B, 24B, 24F, 27, 29, 31, 33B, 34, 35B and 35F. 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.

In some embodiments, the immunogenic compositions disclosed herein may further comprise one adjuvant.

In some embodiments, the immunogenic compositions disclosed herein may further comprise two adjuvants.

The term "adjuvant" refers to a compound or mixture that enhances the immune response to an antigen. Antigens may act primarily as a delivery system, primarily as an immune modulator or have strong features of both. Suitable adjuvants include those suitable for use in mammals, including humans.

Examples of known suitable delivery-system type adjuvants that can be used in humans include, but are not limited to, alum (e.g., aluminum phosphate, aluminum sulfate or aluminum hydroxide), calcium phosphate, liposomes, oil-in-water emulsions such as MF59 (4.3% w/v squalene, 0.5% w/v polysorbate 80 (Tween 80), 0.5% w/v sorbitan trioleate (Span 85)), water-in-oil emulsions such as Montanide, and poly(D,L-lactide-co-glycolide) (PLG) microparticles or nanoparticles.

In an embodiment, the immunogenic compositions disclosed herein comprise aluminum salts (alum) as adjuvant (e.g., aluminum phosphate, aluminum sulfate or aluminum hydroxide).

In a preferred embodiment, the immunogenic compositions disclosed herein comprise aluminum phosphate or aluminum hydroxide as adjuvant. In 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-isoglutarninyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine MTP-PE), etc.

In an embodiment of the present invention, the immunogenic compositions as disclosed herein comprise a CpG Oligonucleotide as adjuvant. A CpG oligonucleotide as used herein refers to an immunostimulatory CpG oligodeoxynucleotide (CpG ODN), and accordingly these terms are used interchangeably unless otherwise indicated. Immunostimulatory CpG oligodeoxynucleotides contain one or more immunostimulatory CpG motifs that are unmethylated cytosine-guanine dinucleotides, optionally within certain preferred base contexts. The methylation status of the CpG immunostimulatory motif generally refers to the cytosine residue in the dinucleotide. An immunostimulatory oligonucleotide containing at least one unmethylated CpG dinucleotide is an oligonucleotide which contains a 5' unmethylated cytosine linked by a phosphate bond to a 3' guanine, and which activates the immune system through binding to Toll-like receptor 9 (TLR-9). In another embodiment the immunostimulatory oligonucleotide may contain one or more methylated CpG dinucleotides, which will activate the immune system through TLR9 but not as strongly as if the CpG motif(s) was/were unmethylated. CpG immunostimulatory oligonucleotides may comprise one or more palindromes that in turn may encompass the CpG dinucleotide. CpG oligonucleotides have been described in a number of issued patents, published patent applications, and other publications, including U.S. Patent Nos. 6,194,388; 6,207,646; 6,214,806; 6,218,371; 6,239,116; and 6,339,068.

In an embodiment of the present invention, the immunogenic compositions as disclosed herein comprise any of the CpG Oligonucleotide described at page 3, line 22, to page 12, line 36, of WO 2010/125480.

Different classes of CpG immunostimulatory oligonucleotides have been identified. These are referred to as A, B, C and P class, and are described in greater detail at page 3, line 22, to page 12, line 36, of WO 2010/125480. Methods of the invention embrace the use of these different classes of CpG immunostimulatory oligonucleotides.

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, TRITONTM X-100, octoxynol 40, nonoxynol-9, triethanolamine, triethanolamine polypeptide oleate, polyoxyethylene-660 hydroxystearate (PEG-15, Solutol H 15), polyoxyethylene-35-ricinoleate (CREMOPHOR[®] EL), soy lecithin and a poloxamer.

In one particular embodiment, the surfactant is polysorbate 80. In some said embodiment, the final concentration of polysorbate 80 in the formulation is at least 0.0001% to 10% polysorbate 80 weight to weight (w/w). In some said embodiments, the final concentration of polysorbate 80 in the formulation is at least 0.001% to 1% polysorbate 80 weight to weight (w/w). In some said embodiments, the final concentration of polysorbate 80 in the formulation is at least 0.01% to 1% polysorbate 80 weight to weight (w/w). In other embodiments, the final concentration of polysorbate 80 in the formulation is 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09% or 0.1% polysorbate 80 (w/w). In another embodiment, the final concentration of the polysorbate 80 in the formulation is 0.02% polysorbate 80 (w/w). In another embodiment, the final concentration of the polysorbate 80 in the formulation is 0.01% polysorbate 80 (w/w). In another embodiment, the final concentration of the polysorbate 80 in the formulation is 0.03% polysorbate 80 (w/w). In another embodiment, the final concentration of the polysorbate 80 in the formulation is 0.04% polysorbate 80 (w/w). In another embodiment, the final concentration of the polysorbate 80 in the formulation is 0.05% polysorbate 80 (w/w). In another embodiment, the final concentration of the polysorbate 80 in the formulation is 1% polysorbate 80 (w/w).

In one particular embodiment, the surfactant is polysorbate 20. In some said embodiment, the final concentration of polysorbate 20 in the formulation is at least 0.0001% to 10% polysorbate 20 weight to weight (w/w). In some said embodiments, the final concentration of polysorbate 20 in the formulation is at least 0.001% to 1% polysorbate 20 weight to weight (w/w). In some said embodiments, the final concentration of polysorbate 20 in the formulation is at least 0.01% to 1% polysorbate 20 weight to weight (w/w). In other embodiments, the final concentration of polysorbate 20 in the formulation is 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09% or 0.1% polysorbate 20 (w/w). In another embodiment, the final concentration of the polysorbate 20 in the formulation is 0.02% polysorbate 20 (w/w). In another embodiment, the final concentration of the polysorbate 20 in the formulation is 0.01% polysorbate 20 (w/w). In another embodiment, the final concentration of the polysorbate 20 in the formulation is 0.03% polysorbate 20 (w/w). In another embodiment, the final concentration of the polysorbate 20 in the formulation is 0.04% polysorbate 80 (w/w). In another embodiment, the final concentration of the polysorbate

20 in the formulation is 0.05% polysorbate 20 (w/w). In another embodiment, the final concentration of the polysorbate 20 in the formulation is 1% polysorbate 20 (w/w).

In one particular embodiment, the surfactant is polysorbate 40. In some said embodiment, the final concentration of polysorbate 40 in the formulation is at least 0.0001% to 10% polysorbate 40 weight to weight (w/w). In some said embodiments, the final concentration of polysorbate 40 in the formulation is at least 0.001% to 1% polysorbate 40 weight to weight (w/w). In some said embodiments, the final concentration of polysorbate 40 in the formulation is at least 0.01% to 1% polysorbate 40 weight to weight (w/w). In other embodiments, the final concentration of polysorbate 40 in the formulation is 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09% or 0.1% polysorbate 40 (w/w). In another embodiment, the final concentration of the polysorbate 40 in the formulation is 1% polysorbate 40 (w/w).

In one particular embodiment, the surfactant is polysorbate 60. In some said embodiment, the final concentration of polysorbate 60 in the formulation is at least 0.0001% to 10% polysorbate 60 weight to weight (w/w). In some said embodiments, the final concentration of polysorbate 60 in the formulation is at least 0.001% to 1% polysorbate 60 weight to weight (w/w). In some said embodiments, the final concentration of polysorbate 60 in the formulation is at least 0.01% to 1% polysorbate 60 weight to weight (w/w). In other embodiments, the final concentration of polysorbate 60 in the formulation is 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09% or 0.1% polysorbate 60 (w/w). In another embodiment, the final concentration of the polysorbate 60 in the formulation is 1% polysorbate 60 (w/w).

In one particular embodiment, the surfactant is polysorbate 65. In some said embodiment, the final concentration of polysorbate 65 in the formulation is at least 0.0001% to 10% polysorbate 65 weight to weight (w/w). In some said embodiments, the final concentration of polysorbate 65 in the formulation is at least 0.001% to 1% polysorbate 65 weight to weight (w/w). In some said embodiments, the final concentration of polysorbate 65 in the formulation is at least 0.01% to 1% polysorbate 65 weight to weight (w/w). In other embodiments, the final concentration of polysorbate 65 in the formulation is 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09% or 0.1% polysorbate 65 (w/w). In another embodiment, the final concentration of the polysorbate 65 in the formulation is 1% polysorbate 65 (w/w).

In one particular embodiment, the surfactant is polysorbate 85. In some said embodiment, the final concentration of polysorbate 85 in the formulation is at least 0.0001% to 10% polysorbate 85 weight to weight (w/w). In some said embodiments, the final concentration of polysorbate 85 in the formulation is at least 0.001% to 1% polysorbate 85 weight to weight (w/w). In some said embodiments, the final concentration of polysorbate 85 in the formulation is at least 0.01% to 1% polysorbate 85 weight to weight (w/w). In other embodiments, the final concentration of polysorbate 85 in the formulation is 0.01%, 0.02%, 0.03%, 0.04%, 0.05%, 0.06%, 0.07%, 0.08%, 0.09% or 0.1% polysorbate 85 (w/w). In another embodiment, the final concentration of the polysorbate 85 in the formulation is 1% polysorbate 85 (w/w).

In certain embodiments, the immunogenic composition of the disclosure has a pH of 5.5 to 7.5, more preferably a pH of 5.6 to 7.0, even more preferably a pH of 5.8 to 6.0.

In one embodiment, the present disclosure provides a container filled with any of the immunogenic compositions disclosed herein. In one embodiment, the container is selected from the group consisting of a vial, a syringe, a flask, a fermentor, a bioreactor, a bag, a jar, an ampoule, a cartridge and a disposable pen. In certain embodiments, the container is siliconized.

In an embodiment, the container of the present disclosure is made of glass, metals (e.g., steel, stainless steel, aluminum, etc.) and/or polymers (e.g., thermoplastics, elastomers, thermoplastic-elastomers). In an embodiment, the container of the present disclosure is made of glass.

In one embodiment, the present disclosure provides a syringe filled with any of the immunogenic compositions disclosed herein. In certain embodiments, the syringe is siliconized and/or is made of glass.

A typical dose of the immunogenic composition of the invention for injection has a volume of 0.1 mL to 2 mL. In an embodiment, the immunogenic composition of the invention for injection has a volume of 0.2 mL to 1 mL, even more preferably a volume of about 0.5 mL.

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 as a medicament.

In an embodiment, the immunogenic compositions of the invention are for use as a vaccine.

Therefore, in an embodiment, the immunogenic compositions described herein are for use in generating an immune response in a subject. In one aspect, the subject is a mammal, such as a human, non-human primate, cat, sheep, pig, horse, bovine or dog. 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 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 ameliorating an infection, disease or condition associated with *S. pneumoniae* serotype 38 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, bacteremia, 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 38 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 38 infection in a subject. Thus, in one aspect, the invention provides a method of preventing an infection by *S. pneumoniae* 38 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, bacteremia, 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 38 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.

5 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
10 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
15 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
20 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
25 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.

30 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),
35 spleen dysfunction (such as sickle cell disease), lack of spleen function (asplenia), blood malignancy, leukemia, multiple myeloma, Hodgkin's disease, lymphoma, kidney failure, nephrotic syndrome and asthma.

In an embodiment of the present invention, the immunocompromised subject to be vaccinated suffers from malnutrition.

In a particular embodiment of the present invention, the immunocompromised subject to be vaccinated is taking a drug or treatment that lowers the body's resistance to infection. In an embodiment, said drug is selected from the one disclosed on page 26, line 33, to page 26, line 4, of WO 2010/125480.

In a particular embodiment of the present invention, the immunocompromised subject to be vaccinated is a smoker.

In a particular embodiment of the present invention, the immunocompromised subject to be vaccinated has a white blood cell count (leukocyte count) below 5×10^9 cells per liter, or below 4×10^9 cells per liter, or below 3×10^9 cells per liter, or below 2×10^9 cells per liter, or below 1×10^9 cells per liter, or below 0.5×10^9 cells per liter, or below 0.3×10^9 cells per liter, or below 0.1×10^9 cells per liter.

White blood cell count (leukocyte count): The number of white blood cells (WBC) in the blood. The WBC is usually measured as part of the CBC (complete blood count). White blood cells are the infection-fighting cells in the blood and are distinct from the red (oxygen-carrying) blood cells known as erythrocytes. There are different types of white blood cells, including neutrophils (polymorphonuclear leukocytes; PMN), band cells (slightly immature neutrophils), T-type lymphocytes (T-cells), B-type lymphocytes (B-cells), monocytes, eosinophils, and basophils. All the types of white blood cells are reflected in the white blood cell count. The normal range for the white blood cell count is usually between 4,300 and 10,800 cells per cubic millimeter of blood. This can also be referred to as the leukocyte count and can be expressed in international units as $4.3 - 10.8 \times 10^9$ cells per liter.

In a particular embodiment of the present invention, the immunocompromised subject to be vaccinated suffers from neutropenia. In a particular embodiment of the present invention, the immunocompromised subject to be vaccinated has a neutrophil count below 2×10^9 cells per liter, or below 1×10^9 cells per liter, or below 0.5×10^9 cells per liter, or below 0.1×10^9 cells per liter, or below 0.05×10^9 cells per liter.

A low white blood cell count or "neutropenia" is a condition characterized by abnormally low levels of neutrophils in the circulating blood. Neutrophils are a specific kind of white blood cell that help to prevent and fight infections. The most common reason that cancer patients experience neutropenia is as a side effect of chemotherapy. Chemotherapy-induced neutropenia increases a patient's risk of infection and disrupts cancer treatment.

In a particular embodiment of the present invention, the immunocompromised subject to be vaccinated has a CD4+ cell count below $500/\text{mm}^3$, or CD4+ cell count below $300/\text{mm}^3$, or CD4+ cell count below $200/\text{mm}^3$, CD4+ cell count below $100/\text{mm}^3$, CD4+ cell count below $75/\text{mm}^3$, or CD4+ cell count below $50/\text{mm}^3$.

CD4 cell tests are normally reported as the number of cells in 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
5 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
10 can receive one or several booster immunizations adequately spaced.

In an embodiment, the schedule of vaccination of the immunogenic composition according to the invention is a single dose. In a particular embodiment, said single dose schedule is for healthy persons being at least 2 years of age.

In an embodiment, the schedule of vaccination of the immunogenic composition according
15 to the invention is a multiple dose schedule. In a particular embodiment, said multiple dose schedule consists of a series of 2 doses separated by an interval of about 1 month to about 2 months. In a particular embodiment, said multiple dose schedule consists of a series of 2 doses separated by an interval of about 1 month, or a series of 2 doses separated by an interval of about 2 months.

In another embodiment, said multiple dose schedule consists of a series of 3 doses
20 separated by an interval of about 1 month to about 2 months. In another embodiment, said multiple dose schedule consists of a series of 3 doses separated by an interval of about 1 month, or a series of 3 doses separated by an interval of about 2 months.

In another embodiment, said multiple dose schedule consists of a series of 3 doses
25 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
30 13 months after the first dose.

In an embodiment, the multiple dose schedule consists of at least one dose (e.g., 1, 2 or 3 doses) in the first year of age followed by at least one toddler dose.

In an embodiment, the multiple dose schedule consists of a series of 2 or 3 doses separated by an interval of about 1 month to about 2 months (for example 28-56 days between
35 doses), starting at 2 months of age, and followed by a toddler dose at 12-18 months of age. In an embodiment, said multiple dose schedule consists of a series of 3 doses separated by an interval of about 1 month to about 2 months (for example 28-56 days between doses), starting at 2 months

of age, and followed by a toddler dose at 12-15 months of age. In another embodiment, said multiple dose schedule consists of a series of 2 doses separated by an interval of about 2 months, starting at 2 months of age, and followed by a toddler dose at 12-18 months of age.

In an embodiment, the multiple dose schedule consists of a 4-dose series of vaccine at 2, 4, 6, and 12-15 months of age.

In an embodiment, a prime dose is given at day 0 and one or more boosts are given at intervals that range from about 2 to about 24 weeks, preferably with a dosing interval of 4-8 weeks.

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

9. Analytical methods

In an embodiment the invention relates to a method of detecting the presence of D-2-acetamido-2,6-dideoxy-xylo-hexos-4-ulose (D-Sug) residues in an isolated *S. pneumoniae* serotype 38 polysaccharide, said method comprising the step of: a) isolating an *S. pneumoniae* serotype 38 polysaccharide and b) detecting the presence of D-2-acetamido-2,6-dideoxy-xylo-hexos-4-ulose residues in said polysaccharide.

In an embodiment the presence of D-Sug residues is detected by NMR or Mass Spectrometry (MS). In an embodiment the presence of D-Sug residues is detected by NMR. In an embodiment, the presence of D-Sug residues is detected by 1D NMR. In an embodiment, the presence of D-Sug residues is detected by 1D ^1H or 1D ^{13}C NMR. In an embodiment, the presence of D-Sug residues is detected by 2D NMR. In an embodiment, the presence of D-Sug residues is detected by Heteronuclear Single Quantum Coherence Spectroscopy (HSQC), Heteronuclear multiple-bond correlation spectroscopy (HMBC), Nuclear Overhauser Effect Spectroscopy (NOESY), Correlation spectroscopy (COSY), Total Correlation Spectroscopy (TOCSY) or Heteronuclear Single Quantum Coherence Spectroscopy-Total Correlation Spectroscopy (HSQC-TOCSY).

In an embodiment, the presence of D-Sug residues is detected by 1D ^1H , 2D ^1H - ^{13}C Heteronuclear Single Quantum Coherence Spectroscopy (HSQC), 2D ^1H - ^{13}C Heteronuclear multiple-bond correlation spectroscopy (HMBC), 2D ^1H - ^{13}C Nuclear Overhauser Effect Spectroscopy (NOESY), 2D ^1H - ^{13}C Correlation spectroscopy (COSY), 2D ^1H - ^{13}C Total Correlation Spectroscopy (TOCSY), 2D ^1H - ^{13}C Heteronuclear Single Quantum Coherence Spectroscopy-Total Correlation Spectroscopy (HSQC-TOCSY) or 1D ^{13}C NMR.

In a preferred embodiment, the presence of D-Sug residues is detected by 1D ^1H , 2D ^1H - ^{13}C Heteronuclear Single Quantum Coherence Spectroscopy (HSQC), or 1D ^{13}C NMR.

In an embodiment, the presence of D-Sug residues is detected by 2D ^1H - ^{13}C Heteronuclear Single Quantum Coherence Spectroscopy (HSQC).

In an embodiment the presence of D-Sug residues is detected by Mass Spectrometry (MS). In an embodiment the presence of D-Sug residues is detected by Tandem Mass

Spectrometry (MS/MS). In an embodiment the presence of D-Sug residues is detected by Gas Chromatography-Mass Spectrometry (GC-MS), Liquid Chromatography-Mass Spectrometry (LC-MS), Capillary Electrophoresis-Mass Spectrometry (CE-MS) or Ion Mobility Spectrometry-Mass Spectrometry (IMS/MS or IMMS). In an embodiment the presence of D-Sug residues is detected by Size-Exclusion Chromatography combined with Mass Spectrometry (SEC/MS).

In an embodiment the presence of D-Sug residues is detected by Gas Chromatography-Mass Spectrometry (GC-MS). In an embodiment the presence of D-Sug residues is detected by Liquid Chromatography-Mass Spectrometry (LC-MS). In an embodiment the presence of D-Sug residues is detected by Capillary Electrophoresis-Mass Spectrometry (CE-MS). In an embodiment the presence of D-Sug residues is detected by Ion Mobility Spectrometry-Mass Spectrometry (IMS/MS). In an embodiment the presence of D-Sug residues is detected by Hydrophilic Interaction Liquid Chromatography -Mass Spectrometry (HILIC-LC/MS).

In an embodiment, the invention relates to a method of detecting the presence of N-acetyl-D-fucosamine (D-FucNAc) residues in a reduced serotype 38 polysaccharide, said method comprising the step of: a) reacting an isolated *S. pneumoniae* serotype 38 polysaccharide with a reducing agent and b) detecting the presence of N-acetyl-D-fucosamine (D-FucNAc) residues in said reduced polysaccharide.

In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) residues is detected by NMR. In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) residues is detected by 2D NMR.

In a preferred embodiment, the presence of N-acetyl-D-fucosamine (D-FucNAc) residues is detected by 2D ^1H - ^{13}C HSQC NMR.

In an embodiment, the presence of N-acetyl-D-fucosamine (D-FucNAc) residues is detected by Mass Spectrometry (MS). In an embodiment, the presence of N-acetyl-D-fucosamine (D-FucNAc) residues is detected by Tandem Mass Spectrometry (MS/MS). In an embodiment, the presence of N-acetyl-D-fucosamine (D-FucNAc) residues is detected by Gas Chromatography-Mass Spectrometry (GC-MS), Liquid Chromatography-Mass Spectrometry (LC-MS), Capillary Electrophoresis-Mass Spectrometry (CE-MS) or Ion Mobility Spectrometry-Mass Spectrometry (IMS/MS or IMMS). In an embodiment, the presence of N-acetyl-D-fucosamine (D-FucNAc) residues is detected by Size-Exclusion Chromatography combined with Mass Spectrometry (SEC/MS).

In an embodiment, the presence of N-acetyl-D-fucosamine (D-FucNAc) residues is detected by Gas Chromatography-Mass Spectrometry (GC-MS). In an embodiment, the presence of N-acetyl-D-fucosamine (D-FucNAc) residues is detected by Liquid Chromatography-Mass Spectrometry (LC-MS). In an embodiment, the presence of N-acetyl-D-fucosamine (D-FucNAc) residues is detected by Capillary Electrophoresis-Mass Spectrometry (CE-MS). In an embodiment, the presence of N-acetyl-D-fucosamine (D-FucNAc) residues is detected by Ion Mobility Spectrometry-Mass Spectrometry (IMS/MS). In an embodiment, the presence of N-acetyl-D-

fucosamine (D-FucNAc) residues is detected by Hydrophilic Interaction Liquid Chromatography - Mass Spectrometry (HILIC-LC/MS).

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

In an embodiment, said isolated *S. pneumoniae* serotype 38 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 38 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.

5 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 N-acetyl-D-quinovosamine (D-QuiNAc) residues in a reduced serotype 38 polysaccharide, said method
10 comprising the step of: a) reacting an isolated *S. pneumoniae* serotype 38 polysaccharide with a reducing agent and b) detecting the presence of N-acetyl-D-quinovosamine (D-QuiNAc) residues in said reduced polysaccharide.

In an embodiment the presence of N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by NMR. In an embodiment the presence of N-acetyl-D-quinovosamine (D-QuiNAc) residues is
15 detected by 2D NMR.

In an embodiment the presence of N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Heteronuclear Single Quantum Coherence Spectroscopy (HSQC), Heteronuclear multiple-bond correlation spectroscopy (HMBC), Correlation spectroscopy (COSY), and/or Heteronuclear Single Quantum Coherence Spectroscopy-Total Correlation Spectroscopy (HSQC-TOCSY).

20 In a preferred embodiment, the presence of N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by 2D ^1H - ^{13}C HSQC NMR.

In an embodiment the presence of N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Mass Spectrometry (MS). In an embodiment the presence of N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Tandem Mass Spectrometry (MS/MS). In an embodiment the
25 presence of N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Gas Chromatography-Mass Spectrometry (GC-MS), Liquid Chromatography-Mass Spectrometry (LC-MS), Capillary Electrophoresis-Mass Spectrometry (CE-MS) or Ion Mobility Spectrometry-Mass Spectrometry (IMS/MS or IMMS). In an embodiment the presence of N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Size-Exclusion Chromatography combined with Mass
30 Spectrometry (SEC/MS).

In an embodiment the presence of N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Gas Chromatography-Mass Spectrometry (GC-MS). In an embodiment the presence of N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Liquid Chromatography-Mass Spectrometry (LC-MS). In an embodiment the presence of N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Capillary Electrophoresis-Mass Spectrometry (CE-MS). In an
35 embodiment the presence of N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Ion Mobility Spectrometry-Mass Spectrometry (IMS/MS). In an embodiment the presence of N-acetyl-

D-quinovosamine (D-QuiNAc) residues is detected by Hydrophilic Interaction Liquid Chromatography -Mass Spectrometry (HILIC-LC/MS).

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

In an embodiment, said isolated *S. pneumoniae* serotype 38 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 38 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.

5 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 N-acetyl-D-fucosamine (D-FucNAc) and N-acetyl-D-quinovosamine (D-QuiNAc) residues in a reduced
 10 serotype 38 polysaccharide, said method comprising the step of: a) reacting an isolated *S. pneumoniae* serotype 38 polysaccharide with a reducing agent and b) detecting the presence of N-acetyl-D-fucosamine (D-FucNAc) and N-acetyl-D-quinovosamine (D-QuiNAc) residues in said reduced polysaccharide.

In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) and N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by NMR.
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In a preferred embodiment, the presence of N-acetyl-D-fucosamine (D-FucNAc) and N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by 2D-NMR.

In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) and N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Mass Spectrometry (MS). In an embodiment
 20 the presence of N-acetyl-D-fucosamine (D-FucNAc) and N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Tandem Mass Spectrometry (MS/MS). In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) and N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Gas Chromatography-Mass Spectrometry (GC-MS), Liquid Chromatography-Mass Spectrometry (LC-MS), Capillary Electrophoresis-Mass Spectrometry (CE-MS) or Ion Mobility
 25 Spectrometry-Mass Spectrometry (IMS/MS or IMMS). In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) and N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Size-Exclusion Chromatography combined with Mass Spectrometry (SEC/MS).

In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) and N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Gas Chromatography-Mass Spectrometry
 30 (GC-MS). In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) and N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Liquid Chromatography-Mass Spectrometry (LC-MS). In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) and N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Capillary Electrophoresis-Mass Spectrometry (CE-MS). In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) and N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Ion Mobility
 35 Spectrometry-Mass Spectrometry (IMS/MS). In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) and N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Hydrophilic Interaction Liquid Chromatography -Mass Spectrometry (HILIC-LC/MS).

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

In an embodiment, said isolated *S. pneumoniae* serotype 38 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 38 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 3β -DOXYL- 5α -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- 5α -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 N-acetyl-D-fucosamine (D-FucNAc) and/or N-acetyl-D-quinovosamine (D-QuiNAc) residues in *S. pneumoniae* serotype 38 glycoconjugate, said method comprising the step of: a) preparing a *S. pneumoniae* serotype 38 glycoconjugate and b) detecting the presence of N-acetyl-D-fucosamine (D-FucNAc) and/or N-acetyl-D-quinovosamine (D-QuiNAc) residues in said glycoconjugate.

In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) and/or N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by NMR. In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) and/or N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by 2D NMR.

In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) and/or N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Heteronuclear Single Quantum Coherence Spectroscopy (HSQC), Heteronuclear multiple-bond correlation spectroscopy (HMBC), Correlation spectroscopy (COSY), and/or Heteronuclear Single Quantum Coherence Spectroscopy-Total Correlation Spectroscopy (HSQC-TOCSY).

In a preferred embodiment, the presence of N-acetyl-D-fucosamine (D-FucNAc) and/or N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by 2D ^1H - ^{13}C HSQC NMR.

In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) and/or N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Mass Spectrometry (MS). In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) and/or N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Tandem Mass Spectrometry (MS/MS). In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) and/or N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Gas Chromatography-Mass Spectrometry (GC-MS), Liquid Chromatography-Mass Spectrometry (LC-MS), Capillary Electrophoresis-Mass Spectrometry (CE-MS) or Ion Mobility Spectrometry-Mass Spectrometry (IMS/MS or IMMS). In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) and/or N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Size-Exclusion Chromatography combined with Mass Spectrometry (SEC/MS).

In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) and/or N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Gas Chromatography-Mass Spectrometry (GC-MS). In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) and/or N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Liquid Chromatography-Mass Spectrometry (LC-MS). In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) and/or N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Capillary Electrophoresis-

Mass Spectrometry (CE-MS). In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) and/or N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Ion Mobility Spectrometry-Mass Spectrometry (IMS/MS). In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) and/or N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Hydrophilic Interaction Liquid Chromatography -Mass Spectrometry (HILIC-LC/MS).

In an embodiment the invention relates to a method of detecting the presence of N-acetyl-D-fucosamine (D-FucNAc) and N-acetyl-D-quinovosamine (D-QuiNAc) residues in *S. pneumoniae* serotype 38 glycoconjugate, said method comprising the step of: a) preparing a *S. pneumoniae* serotype 38 glycoconjugate and b) detecting the presence of N-acetyl-D-fucosamine (D-FucNAc) and N-acetyl-D-quinovosamine (D-QuiNAc) residues in said glycoconjugate.

In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) and N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by NMR. In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) and N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by 2D NMR.

In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) and N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Heteronuclear Single Quantum Coherence Spectroscopy (HSQC), Heteronuclear multiple-bond correlation spectroscopy (HMBC), Correlation spectroscopy (COSY), and/or Heteronuclear Single Quantum Coherence Spectroscopy-Total Correlation Spectroscopy (HSQC-TOCSY).

In a preferred embodiment, the presence of N-acetyl-D-fucosamine (D-FucNAc) and N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by 2D ^1H - ^{13}C HSQC NMR.

In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) and N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Mass Spectrometry (MS). In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) and N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Tandem Mass Spectrometry (MS/MS). In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) and N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Gas Chromatography-Mass Spectrometry (GC-MS), Liquid Chromatography-Mass Spectrometry (LC-MS), Capillary Electrophoresis-Mass Spectrometry (CE-MS) or Ion Mobility Spectrometry-Mass Spectrometry (IMS/MS or IMMS). In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) and N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Size-Exclusion Chromatography combined with Mass Spectrometry (SEC/MS).

In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) and N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Gas Chromatography-Mass Spectrometry (GC-MS). In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) and N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Liquid Chromatography-Mass Spectrometry (LC-MS). In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) and N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Capillary Electrophoresis-Mass Spectrometry (CE-MS). In an embodiment the presence of N-acetyl-D-fucosamine (D-

FucNAc) and N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Ion Mobility Spectrometry-Mass Spectrometry (IMS/MS). In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) and N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Hydrophilic Interaction Liquid Chromatography -Mass Spectrometry (HILIC-LC/MS).

5 In an embodiment the invention relates to a method of detecting the presence of N-acetyl-D-fucosamine (D-FucNAc) residues in *S. pneumoniae* serotype 38 glycoconjugate, said method comprising the step of: a) preparing a *S. pneumoniae* serotype 38 glycoconjugate and b) detecting the presence of N-acetyl-D-fucosamine (D-FucNAc) residues in said glycoconjugate.

In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) residues is detected by
10 NMR. In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) residues is detected by 2D NMR.

In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) residues is detected by Heteronuclear Single Quantum Coherence Spectroscopy (HSQC), Heteronuclear multiple-bond correlation spectroscopy (HMBC), Correlation spectroscopy (COSY), and/or Heteronuclear
15 Single Quantum Coherence Spectroscopy-Total Correlation Spectroscopy (HSQC-TOCSY).

In a preferred embodiment, the presence of N-acetyl-D-fucosamine (D-FucNAc) residues is detected by 2D ^1H - ^{13}C HSQC NMR.

In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) residues is detected by Mass Spectrometry (MS). the presence of N-acetyl-D-fucosamine (D-FucNAc) residues is
20 detected by Tandem Mass Spectrometry (MS/MS). In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) residues is detected by Gas Chromatography-Mass Spectrometry (GC-MS), Liquid Chromatography-Mass Spectrometry (LC-MS), Capillary Electrophoresis-Mass Spectrometry (CE-MS) or Ion Mobility Spectrometry-Mass Spectrometry (IMS/MS or IMMS). In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) residues is detected by Size-
25 Exclusion Chromatography combined with Mass Spectrometry (SEC/MS).

In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) residues is detected by Gas Chromatography-Mass Spectrometry (GC-MS). In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) residues is detected by Liquid Chromatography-Mass Spectrometry (LC-MS). In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) residues is
30 detected by Capillary Electrophoresis-Mass Spectrometry (CE-MS). In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) residues is detected by Ion Mobility Spectrometry-Mass Spectrometry (IMS/MS). In an embodiment the presence of N-acetyl-D-fucosamine (D-FucNAc) residues is detected by Hydrophilic Interaction Liquid Chromatography - Mass Spectrometry (HILIC-LC/MS).

35 In an embodiment the invention relates to a method of detecting the presence of N-acetyl-D-quinovosamine (D-QuiNAc) residues in *S. pneumoniae* serotype 38 glycoconjugate, said method comprising the step of: a) preparing a *S. pneumoniae* serotype 38 glycoconjugate and b) detecting the presence of N-acetyl-D-quinovosamine (D-QuiNAc) residues in said glycoconjugate.

In an embodiment the presence of N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by NMR. In an embodiment the presence of N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by 2D NMR.

In an embodiment the presence of N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Heteronuclear Single Quantum Coherence Spectroscopy (HSQC), Heteronuclear multiple-bond correlation spectroscopy (HMBC), Correlation spectroscopy (COSY), and/or Heteronuclear Single Quantum Coherence Spectroscopy-Total Correlation Spectroscopy (HSQC-TOCSY).

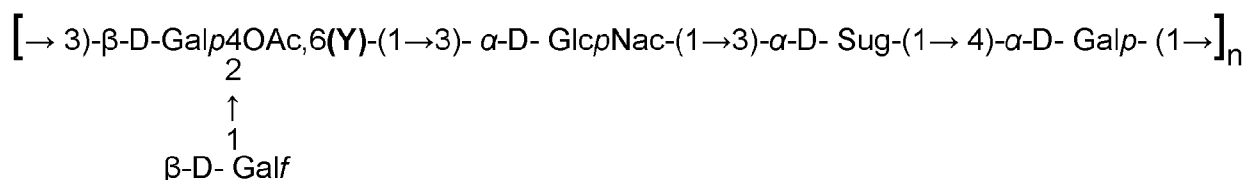
In a preferred embodiment, the presence of N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by 2D ^1H - ^{13}C HSQC NMR.

In an embodiment the presence of N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Mass Spectrometry (MS). In an embodiment the presence of N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Tandem Mass Spectrometry (MS/MS). In an embodiment the presence of N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Gas Chromatography-Mass Spectrometry (GC-MS), Liquid Chromatography-Mass Spectrometry (LC-MS), Capillary Electrophoresis-Mass Spectrometry (CE-MS) or Ion Mobility Spectrometry-Mass Spectrometry (IMS/MS or IMMS). In an embodiment the presence of N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Size-Exclusion Chromatography combined with Mass Spectrometry (SEC/MS).

In an embodiment the presence of N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Gas Chromatography-Mass Spectrometry (GC-MS). In an embodiment the presence of N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Liquid Chromatography-Mass Spectrometry (LC-MS). In an embodiment the presence of N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Capillary Electrophoresis-Mass Spectrometry (CE-MS). In an embodiment the presence of N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Ion Mobility Spectrometry-Mass Spectrometry (IMS/MS). In an embodiment the presence of N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Hydrophilic Interaction Liquid Chromatography -Mass Spectrometry (HILIC-LC/MS).

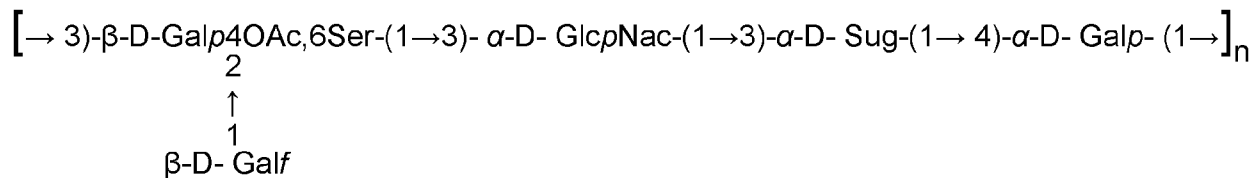
10 Particular embodiments of the invention are set forth in the following numbered paragraphs 1 to 330:

1. An isolated *S. pneumoniae* serotype 38 saccharide with the following repeating unit:



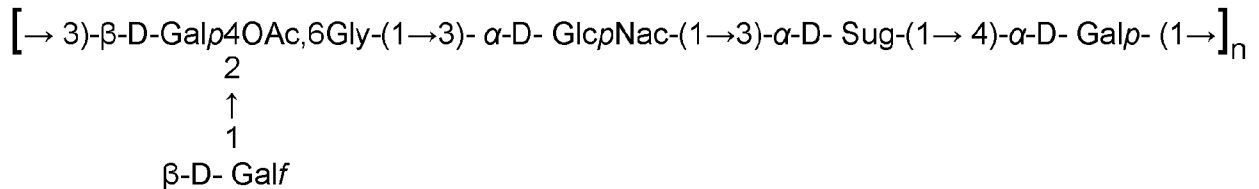
where n represents the number of repeating units, wherein Y represents either a Serine or a Glycine residue and wherein all the repeating units comprise the same Y residue.

2. An isolated *S. pneumoniae* serotype 38 saccharide with the following repeating unit:



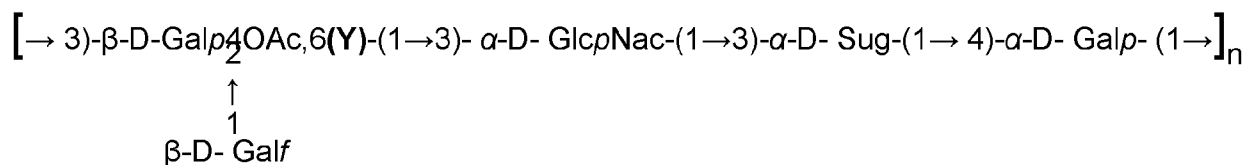
where n represents the number of repeating units.

3. An isolated *S. pneumoniae* serotype 38 saccharide with the following repeating unit:



where n represents the number of repeating units.

4. An isolated *S. pneumoniae* serotype 38 saccharide with the following repeating unit:



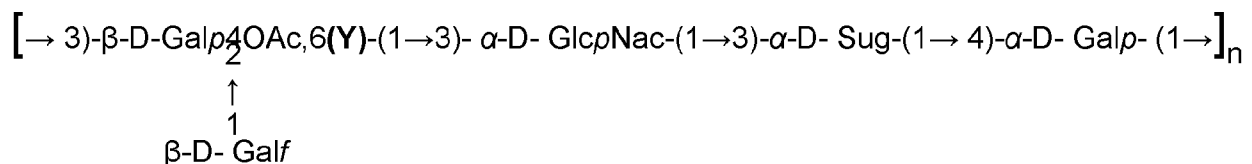
where n represents the number of repeating units, wherein Y represents either a Serine or a Glycine residue, wherein all the repeating units comprise the same Y residue and wherein the O-acetyl group at position 4 of $\beta\text{-D-Galp4OAc,6(Y)}$ is present in about 0% to about 100% of the repeating units.

5. The isolated *S. pneumoniae* serotype 38 saccharide of paragraph 4 wherein said O-acetyl group is present in about 50% to about 100% of the repeating units.

6. The isolated *S. pneumoniae* serotype 38 saccharide of paragraph 4 wherein said O-acetyl group is present in about 80% to about 100% of the repeating units

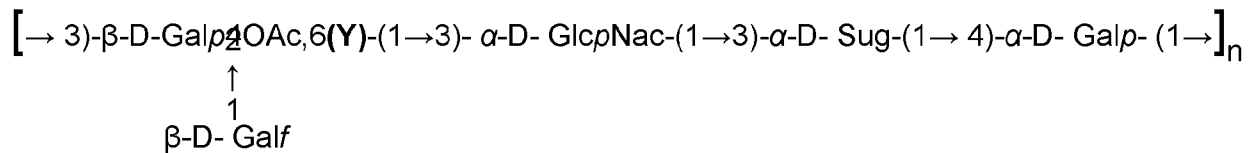
7. The isolated *S. pneumoniae* serotype 38 saccharide of paragraph 4 wherein said O-acetyl group is present in about 90% to about 100% of the repeating units.

8. An isolated *S. pneumoniae* serotype 38 saccharide with the following repeating unit:



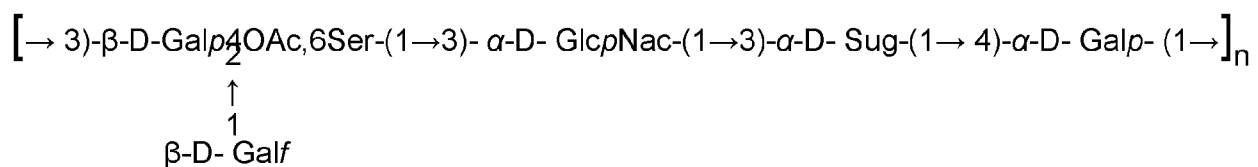
where n represents the number of repeating units, wherein Y represents either a Serine or a Glycine residue, wherein all the repeating units comprise the same Y residue and wherein the O-acetyl group at position 4 of $\beta\text{-D-Galp4OAc,6(Y)}$ is present in about 100% of the repeating units.

9. An isolated *S. pneumoniae* serotype 38 saccharide with the following repeating unit:



where n represents the number of repeating units, wherein Y represents either a Serine or a Glycine residue, wherein all the repeating units comprise the same Y residue and wherein the O-acetyl group at position 4 of $\beta\text{-D-Galp4OAc,6(Y)}$ is present in about 95% of the repeating units.

10. An isolated *S. pneumoniae* serotype 38 saccharide with the following repeating unit:



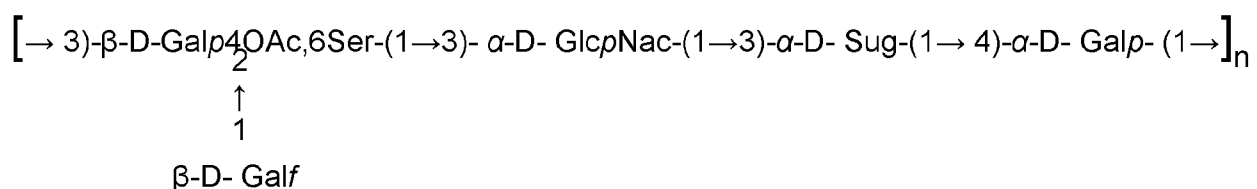
where n represents the number of repeating units and wherein the O-acetyl group at position 4 of $\beta\text{-D-Galp4OAc,6Ser}$ is present in about 0% to about 100% of the repeating units.

11. The isolated *S. pneumoniae* serotype 38 saccharide of paragraph 10 wherein said O-acetyl group is present in about 50% to about 100% of the repeating units.

12. The isolated *S. pneumoniae* serotype 38 saccharide of paragraph 10 wherein said O-acetyl group is present in about 80% to about 100% of the repeating units

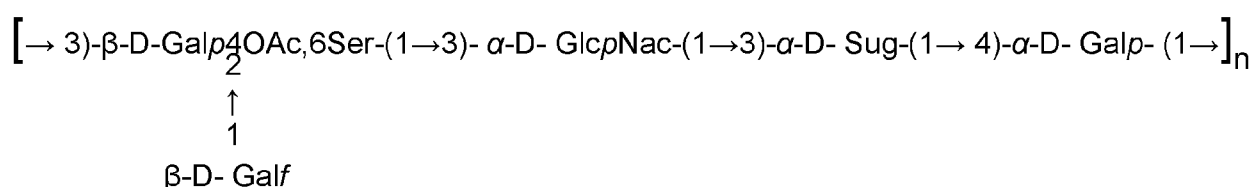
13. The isolated *S. pneumoniae* serotype 38 saccharide of paragraph 10 wherein said O-acetyl group is present in about 90% to about 100% of the repeating units.

14. An isolated *S. pneumoniae* serotype 38 saccharide with the following repeating unit:



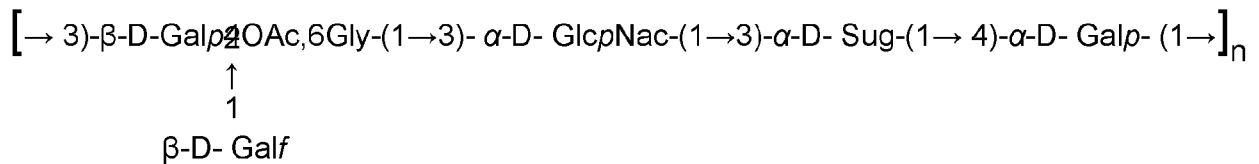
where n represents the number of repeating units and wherein the O-acetyl group at position 4 of $\beta\text{-D-Galp4OAc,6Ser}$ is present in about 100% of the repeating units.

15. An isolated *S. pneumoniae* serotype 38 saccharide with the following repeating unit :



where n represents the number of repeating units and wherein the O-acetyl group at position 4 of $\beta\text{-D-Galp4OAc,6Ser}$ is present in about 95% of the repeating units.

16. An isolated *S. pneumoniae* serotype 38 saccharide with the following repeating unit:



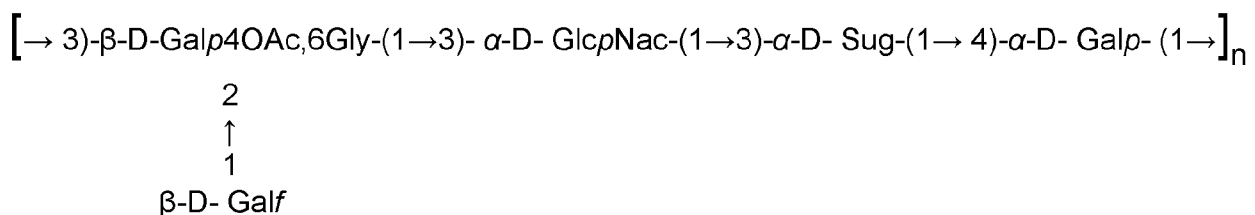
where n represents the number of repeating units and wherein the O-acetyl group at position 4 of $\beta\text{-D-Galp4OAc,6Gly}$ is present in about 0% to about 100% of the repeating units.

17. The isolated *S. pneumoniae* serotype 38 saccharide of paragraph 16 wherein said O-acetyl group is present in about 50% to about 100% of the repeating units.

18. The isolated *S. pneumoniae* serotype 38 saccharide of paragraph 16 wherein said O-acetyl group is present in about 80% to about 100% of the repeating units

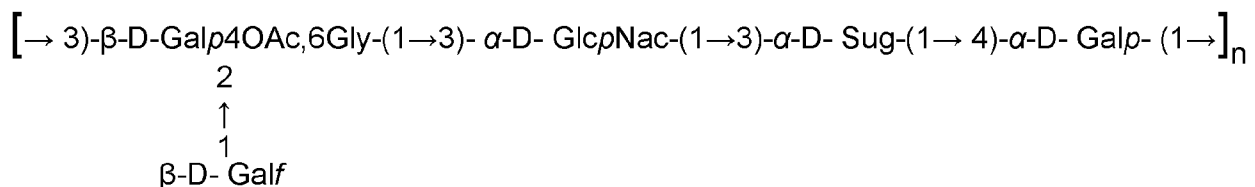
19. The isolated *S. pneumoniae* serotype 38 saccharide of paragraph 16 wherein said O-acetyl group is present in about 90% to about 100% of the repeating units.

20. An isolated *S. pneumoniae* serotype 38 saccharide with the following repeating unit:



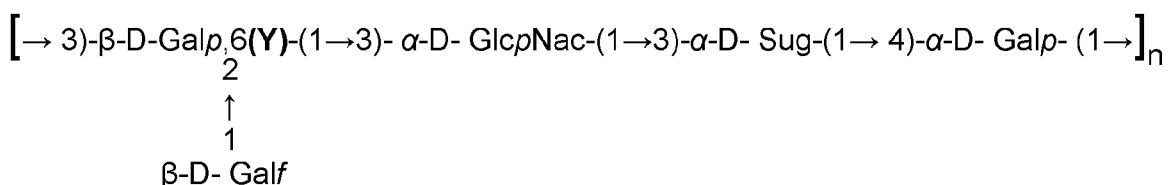
where n represents the number of repeating units and wherein the O-acetyl group at position 4 of $\beta\text{-D-Galp4OAc,6Gly}$ is present in about 100% of the repeating units.

21. An isolated *S. pneumoniae* serotype 38 saccharide with the following repeating unit:



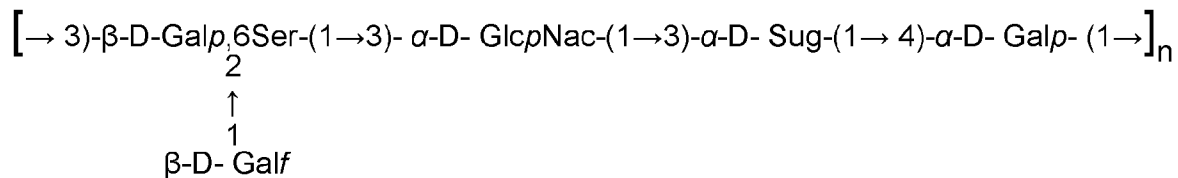
where n represents the number of repeating units and wherein the O-acetyl group at position 4 of $\beta\text{-D-Galp4OAc,6Gly}$ is present in 95% of the repeating units.

22. An isolated *S. pneumoniae* serotype 38 saccharide with the following repeating unit:



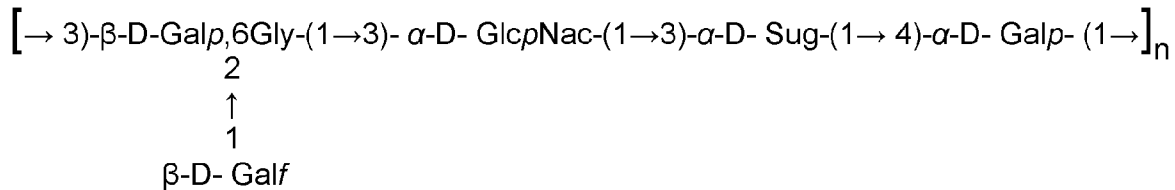
where n represents the number of repeating units, wherein Y represents either a Serine or a Glycine residue and wherein all the repeating units comprise the same Y residue.

23. An isolated *S. pneumoniae* serotype 38 saccharide with the following repeating unit:



where n represents the number of repeating units.

24. An isolated *S. pneumoniae* serotype 38 saccharide with the following repeating unit:



where n represents the number of repeating units.

24. The isolated *S. pneumoniae* serotype 38 saccharide of any one of paragraphs 1-23 having between 10 and 5,000 repeating units.

25. The isolated *S. pneumoniae* serotype 38 saccharide of any one of paragraphs 1-23 having between 50 and 4,500 repeating units.

26. The isolated *S. pneumoniae* serotype 38 saccharide of any one of paragraphs 1-23 having between 100 and 4,500 repeating units.

27. The isolated *S. pneumoniae* serotype 38 saccharide of any one of paragraphs 1-23 having between 150 and 2,000 repeating units.

28. The isolated *S. pneumoniae* serotype 38 saccharide of any one of paragraphs 1-23 having a weight average molecular weight between 5 kDa and 5000 kDa.

29. The isolated *S. pneumoniae* serotype 38 saccharide of any one of paragraphs 1-23 having a weight average molecular weight between 5 kDa and 2000 kDa.

30. The isolated *S. pneumoniae* serotype 38 saccharide of any one of paragraphs 1-23 having a weight average molecular weight between 50 kDa and 5000 kDa.

31. The isolated *S. pneumoniae* serotype 38 saccharide of any one of paragraphs 1-23 having a weight average molecular weight between 50 kDa and 1000 kDa.

32. The isolated *S. pneumoniae* serotype 38 saccharide of any one of paragraphs 1-23 having a weight average molecular weight between 100 kDa and 5000 kDa.

33. The isolated *S. pneumoniae* serotype 38 saccharide of any one of paragraphs 1-23 having a weight average molecular weight between 100 kDa and 1000 kDa.

34. The isolated *S. pneumoniae* serotype 38 saccharide of any one of paragraphs 1-23 having a weight average molecular weight between 100 kDa and 500 kDa.

35. The isolated *S. pneumoniae* serotype 38 saccharide of any one of paragraphs 1-23 having a weight average molecular weight between 300 kDa and 5000 kDa.

5 36. The isolated *S. pneumoniae* serotype 38 saccharide of any one of paragraphs 1-23 having a weight average molecular weight between 300 kDa and 1000 kDa.

37. The isolated *S. pneumoniae* serotype 38 saccharide of any one of paragraphs 1-23 having a weight average molecular weight between 500 kDa and 3000 kDa.

10 38. The isolated *S. pneumoniae* serotype 38 saccharide of any one of paragraphs 1-23 having a weight average molecular weight between 500 kDa and 2000 kDa.

39. The isolated *S. pneumoniae* serotype 38 saccharide of any one of paragraphs 1-23 having a weight average molecular weight between 500 kDa and 1000 kDa.

40. The isolated *S. pneumoniae* serotype 38 saccharide of any one of paragraphs 1-23 sized to a weight average molecular weight between 10 kDa and 1000 kDa.

15 41. The isolated *S. pneumoniae* serotype 38 saccharide of any one of paragraphs 1-23 sized to a weight average molecular weight between 50 kDa and 500 kDa.

42. The isolated *S. pneumoniae* serotype 38 saccharide of any one of paragraphs 1-23 sized to a weight average molecular weight between 100 kDa and 400 kDa.

20 43. A glycoconjugate comprising an isolated *S. pneumoniae* serotype 38 saccharide of any one of paragraphs 1-42 conjugated to a carrier protein.

44. A glycoconjugate consisting of an isolated *S. pneumoniae* serotype 38 saccharide of any one of paragraphs 1-42 conjugated to a carrier protein.

25 45. A serotype 38 glycoconjugate comprising a serotype 38 capsular saccharide comprising between about 1 to about 70 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

46. The serotype 38 glycoconjugate of paragraph 45 comprising between about 1 to about 68 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

30 46. The serotype 38 glycoconjugate of paragraph 45 comprising between about 1 to about 65 N-acetyl-D-fucosamine (D-FucNAc) residues in every 100 saccharide repeat units of the saccharide.

47. A serotype 38 glycoconjugate comprising a serotype 38 capsular saccharide comprising between about 0.5 to about 35 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

48. The serotype 38 glycoconjugate of paragraph 47 comprising between about 0.5 to about 32 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

49. The serotype 38 glycoconjugate of paragraph 47 comprising between about 0.5 to about 30 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

50. A serotype 38 glycoconjugate comprising a serotype 38 capsular saccharide comprising between about 1 to about 70 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 0.5 to about 30 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

51. A serotype 38 glycoconjugate comprising a serotype 38 capsular saccharide comprising between about 1 to about 68 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 0.5 to about 32 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

52. A serotype 38 glycoconjugate comprising a serotype 38 capsular saccharide comprising between about 1 to about 65 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 0.5 to about 32.5 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

53. A serotype 38 glycoconjugate comprising a serotype 38 capsular saccharide comprising between about 1 to about 60 N-acetyl-D-fucosamine (D-FucNAc) residues and between about 0.5 to about 30 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

54. A serotype 38 glycoconjugate comprising a serotype 38 capsular saccharide comprising about 68 N-acetyl-D-fucosamine (D-FucNAc) residues and about 32 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

55. The serotype 38 glycoconjugate of any one of paragraphs 45-54 comprising a serotype 38 capsular saccharide comprising N-acetyl-D-fucosamine (D-FucNAc) residues and N-acetyl-D-quinovosamine (D-QuiNAc) residues where the number of D-FucNAc residues is about the double of the number of D-QuiNAc residues.

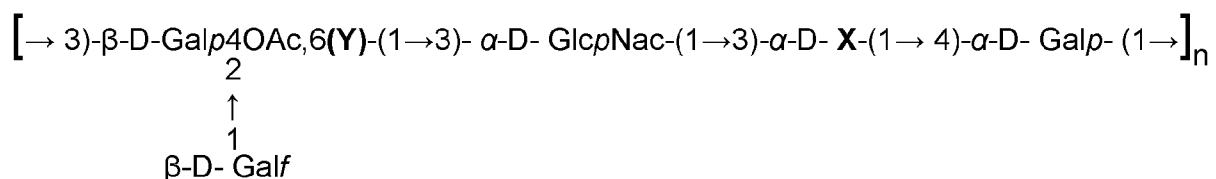
56. A serotype 38 glycoconjugate comprising a serotype 38 capsular saccharide comprising N-acetyl-D-fucosamine (D-FucNAc), N-acetyl-D-quinovosamine (D-QuiNAc) and D-2-acetamido-2,6-dideoxy-xylo-hexos-4-ulose (D-Sug) residues.

57. The serotype 38 glycoconjugate of paragraph 56 comprising a serotype 38 capsular saccharide comprising at the same position of the repeat unit either a N-acetyl-D-fucosamine (D-

FucNAc) residue, a N-acetyl-D-quinovosamine (D-QuiNAc) residue or a D-2-acetamido-2,6-dideoxy-xylo-hexos-4-ulose (D-Sug) residue.

58. The serotype 38 glycoconjugate of paragraph 56 or 57 wherein the number of D-FucNAc residues is about the double of the number of D-QuiNAc residues.

59. A serotype 38 glycoconjugate comprising a serotype 38 capsular saccharide with the following repeating unit:

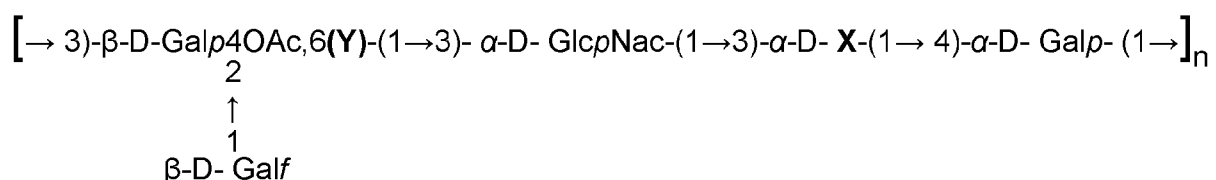


where n represents the number of repeating units, wherein Y represents either a Serine or a Glycine residue, wherein all the repeating units comprise the same Y residue and where X represents either a N-acetyl-D-fucosamine (D-FucNAc) residue or a N-acetyl-D-quinovosamine (D-QuiNAc) residue.

60. The serotype 38 glycoconjugate of paragraph 59 wherein said serotype 38 capsular saccharide comprises about 70 N-acetyl-D-fucosamine (D-FucNAc) residues and about 30 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

61. The serotype 38 glycoconjugate of paragraph 59 wherein said serotype 38 capsular saccharide comprises about 68 N-acetyl-D-fucosamine (D-FucNAc) residues and about 32 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

62. A serotype 38 glycoconjugate comprising a serotype 38 capsular saccharide with the following repeating unit:

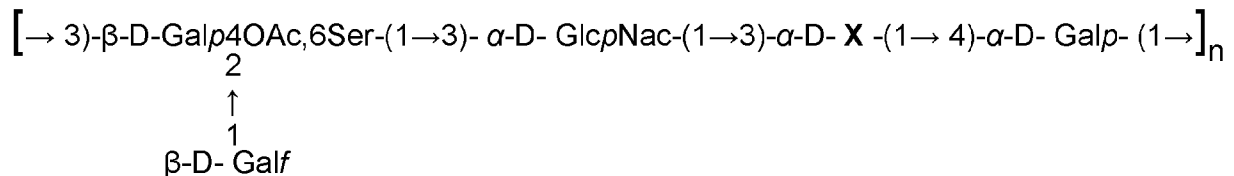


where n represents the number of repeating units, wherein Y represents either a Serine or a Glycine residue wherein all the repeating units comprise the same Y residue and where X represents either a N-acetyl-D-fucosamine (D-FucNAc) residue or a N-acetyl-D-quinovosamine (D-QuiNAc) residue.

63. The serotype 38 glycoconjugate of paragraph 62 wherein said serotype 38 capsular saccharide comprises about 70 N-acetyl-D-fucosamine (D-FucNAc) residues and about 30 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

64. The serotype 38 glycoconjugate of paragraph 62 wherein said serotype 38 capsular saccharide comprises about 68 N-acetyl-D-fucosamine (D-FucNAc) residues and about 32 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

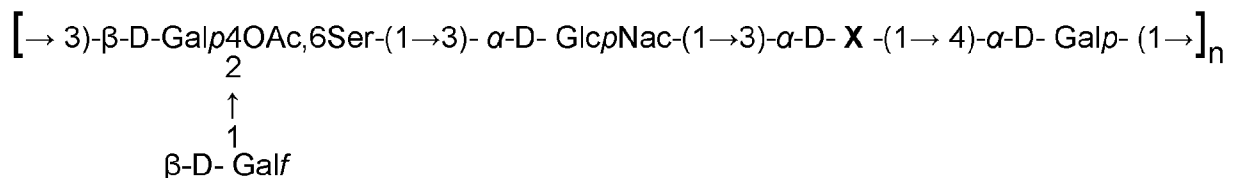
65. A serotype 38 glycoconjugate comprising a serotype 38 capsular saccharide with the following repeating unit:



where n represents the number of repeating units and where X represents either a N-acetyl-D-fucosamine (D-FucNAc) residue, a N-acetyl-D-quinovosamine (D-QuiNAc) residue or a D-2-acetamido-2,6-dideoxy-xylo-hexos-4-ulose (D-Sug) residue.

66. The serotype 38 glycoconjugate of paragraph 65 wherein said serotype 38 capsular saccharide comprises between about 1 to about 70 N-acetyl-D-fucosamine (D-FucNAc) residues, between about 0.5 to about 30 N-acetyl-D-quinovosamine (D-QuiNAc) residues and between about 0 to about 98.5 D-2-acetamido-2,6-dideoxy-xylo-hexos-4-ulose (D-Sug) residues in every 100 saccharide repeat units of the saccharide.

67. A serotype 38 glycoconjugate comprising a serotype 38 capsular saccharide with the following repeating unit:

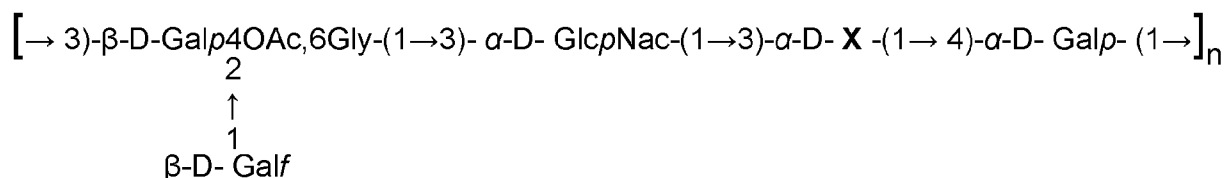


where n represents the number of repeating units and where X represents either a N-acetyl-D-fucosamine (D-FucNAc) residue or a N-acetyl-D-quinovosamine (D-QuiNAc) residue.

68. The serotype 38 glycoconjugate of paragraph 67 wherein said serotype 38 capsular saccharide comprises about 70 N-acetyl-D-fucosamine (D-FucNAc) residues and about 30 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

69. The serotype 38 glycoconjugate of paragraph 67 wherein said serotype 38 capsular saccharide comprises about 68 N-acetyl-D-fucosamine (D-FucNAc) residues and about 32 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

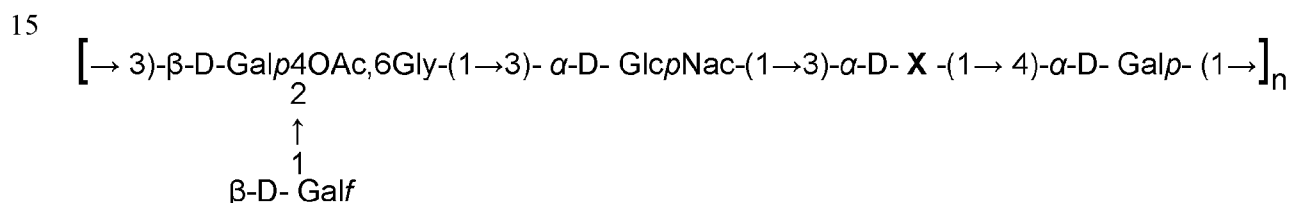
70. A serotype 38 glycoconjugate comprising a serotype 38 capsular saccharide with the following repeating unit:



5 where n represents the number of repeating units and where X represents either a N-acetyl-D-fucosamine (D-FucNAc) residue, a N-acetyl-D-quinovosamine (D-QuiNAc) residue or a D-2-acetamido-2,6-dideoxy-xylo-hexos-4-ulose (D-Sug) residue.

71. The serotype 38 glycoconjugate of paragraph 70 wherein said serotype 38 capsular saccharide comprises between about 1 to about 70 N-acetyl-D-fucosamine (D-FucNAc) residues,
10 between about 0.5 to about 30 N-acetyl-D-quinovosamine (D-QuiNAc) residues and between about 0 to about 98.5 D-2-acetamido-2,6-dideoxy-xylo-hexos-4-ulose (D-Sug) residues in every 100 saccharide repeat units of the saccharide.

72. A serotype 38 glycoconjugate comprising a serotype 38 capsular saccharide with the following repeating unit:



15 where n represents the number of repeating units and where X represents either a N-acetyl-D-fucosamine (D-FucNAc) residue or a N-acetyl-D-quinovosamine (D-QuiNAc) residue.

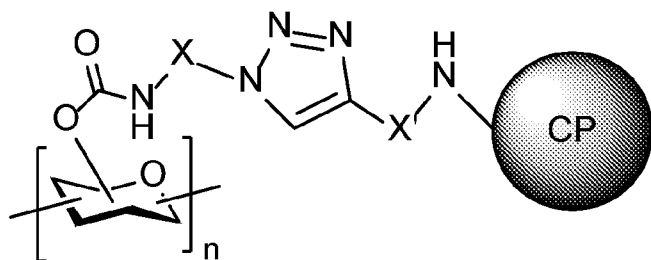
73. The serotype 38 glycoconjugate of paragraph 72 wherein said serotype 38 capsular saccharide comprises about 70 N-acetyl-D-fucosamine (D-FucNAc) residues and about 30 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

25 74. The serotype 38 glycoconjugate of paragraph 72 wherein said serotype 38 capsular saccharide comprises about 68 N-acetyl-D-fucosamine (D-FucNAc) residues and about 32 N-acetyl-D-quinovosamine (D-QuiNAc) residues in every 100 saccharide repeat units of the saccharide.

75. The serotype 38 glycoconjugate of paragraph 43 or 44 wherein said serotype 38 glycoconjugate comprises a serotype 38 saccharide covalently conjugated to a carrier protein
30 through a (2-((2-oxoethyl)thio)ethyl)carbamate (eTEC) spacer.

75. The serotype 38 glycoconjugate of paragraph 43 or 44 wherein said serotype 38 glycoconjugate is prepared by click chemistry.

76. A serotype 38 glycoconjugate comprising a serotype 38 saccharide covalently
35 conjugated to a carrier protein (CP) through a spacer and having the general formula (IV):



(IV),

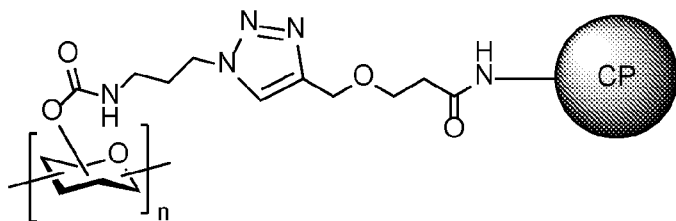
wherein X is selected from the group consisting of $\text{CH}_2(\text{CH}_2)_{n'}$, $(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$, $\text{NHCO}(\text{CH}_2)_{n'}$, $\text{NHCO}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$, $\text{OCH}_2(\text{CH}_2)_{n'}$ and $\text{O}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$;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

- 5 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 38 saccharide and wherein n represents the number of repeating units.

- 10 77. A serotype 38 glycoconjugate comprising a serotype 38 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.

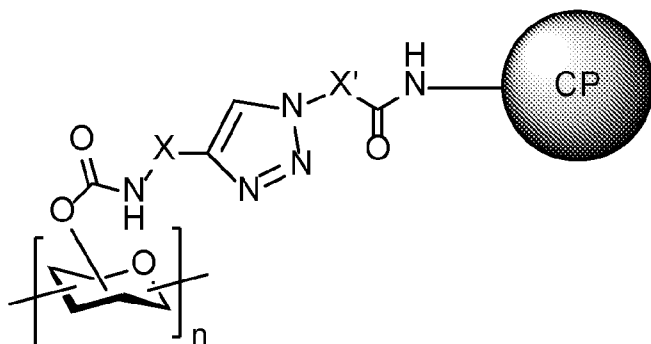
78. A serotype 38 glycoconjugate comprising a serotype 38 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (V),



(V)

wherein the structure in square bracket represents a repeat unit of the serotype 38 saccharide and wherein n represents the number of repeating units.

79. A serotype 38 glycoconjugate comprising a serotype 38 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (XII):



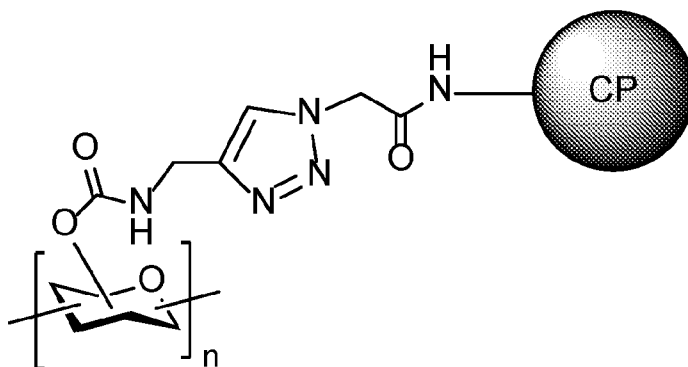
(XII),

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 38 saccharide and wherein n represents the number of repeating units.

80. A serotype 38 glycoconjugate comprising a serotype 38 saccharide covalently
10 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 0 and wherein X' is $\text{CH}_2(\text{CH}_2)_{n''}$ where n'' is 0.

81. A serotype 38 glycoconjugate comprising a serotype 38 saccharide covalently
conjugated to a carrier protein (CP) through a spacer and having the general formula (XIII),



(XIII)

- 15 wherein the structure in square bracket represents a repeat unit of the serotype 38 saccharide and wherein n represents the number of repeating units.

80. The glycoconjugate of any one of paragraphs 45 to 81 wherein said a serotype 38 saccharide is an isolated *S. pneumoniae* serotype 38 saccharide of any one of paragraphs 1-42.

81. The glycoconjugate of any one of paragraphs 43 to 81 wherein the weight average
20 molecular weight (Mw) of said *S. pneumoniae* serotype 38 saccharide before conjugation is between 50 kDa and 1,000 kDa.

82. The glycoconjugate of any one of paragraphs 43 to 81 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 38 saccharide before conjugation is between 100 kDa and 600 kDa.

83. The glycoconjugate of any one of paragraphs 43 to 81 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 38 saccharide before conjugation is between 100 kDa and 400 kDa.

84. The glycoconjugate of any one of paragraphs 43 to 81 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 38 saccharide before conjugation is between 150 kDa and 300 kDa.

85. The glycoconjugate of any one of paragraphs 43 to 84 wherein said serotype 38 glycoconjugate has a weight average molecular weight (Mw) of between 250 kDa and 20,000 kDa.

86. The glycoconjugate of any one of paragraphs 43 to 84 wherein said serotype 38 glycoconjugate has a weight average molecular weight (Mw) of between 500 kDa and 15,000 kDa.

87. The glycoconjugate of any one of paragraphs 43 to 84 wherein said serotype 38 glycoconjugate has a weight average molecular weight (Mw) of between 500 kDa and 10,000 kDa.

87a. The glycoconjugate of any one of paragraphs 43 to 84 wherein said serotype 38 glycoconjugate has a weight average molecular weight (Mw) of between 1,000 kDa and 10,000 kDa.

87b. The glycoconjugate of any one of paragraphs 43 to 81 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 38 saccharide before conjugation is between 150 kDa and 300 kDa and wherein said serotype 38 glycoconjugate has a weight average molecular weight (Mw) of between 1,000 kDa and 10,000 kDa.

88. The glycoconjugate of any one of paragraphs 43 to 84 wherein said serotype 38 glycoconjugate has a weight average molecular weight (Mw) of between 750 kDa and 7,500 kDa.

89. The glycoconjugate of any one of paragraphs 43 to 84 wherein said serotype 38 glycoconjugate has a weight average molecular weight (Mw) of between 1,000 kDa and 5,000 kDa.

90. The glycoconjugate of any one of paragraphs 43 to 89 wherein the degree of conjugation of said serotype 38 glycoconjugate is between 2 and 15.

91. The glycoconjugate of any one of paragraphs 43 to 89 wherein the degree of conjugation of said serotype 38 glycoconjugate is between 2 and 10.

92. The glycoconjugate of any one of paragraphs 43 to 89 wherein the degree of conjugation of said serotype 38 glycoconjugate is between 3 and 5.

93. The glycoconjugate of any one of paragraphs 43 to 89 wherein the degree of conjugation of said serotype 38 glycoconjugate is between 2 and 6.

5 94. The glycoconjugate of any one of paragraphs 43 to 89 wherein the degree of conjugation of said serotype 38 glycoconjugate is between 4 and 10.

94a. The glycoconjugate of any one of paragraphs 43 to 81 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 38 saccharide before conjugation is between 150 kDa and 300 kDa, wherein said serotype 38 glycoconjugate has a weight average
10 molecular weight (Mw) of between 1,000 kDa and 10,000 kDa and wherein the degree of conjugation of said serotype 38 glycoconjugate is between 2 and 10.

94b. The glycoconjugate of any one of paragraphs 43 to 81 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 38 saccharide before conjugation is between 150 kDa and 300 kDa, wherein said serotype 38 glycoconjugate has a weight average
15 molecular weight (Mw) of between 1,000 kDa and 10,000 kDa and wherein the degree of conjugation of said serotype 38 glycoconjugate is between 4 and 10.

95. The glycoconjugate of any one of paragraphs 43 to 94 wherein the ratio of serotype 38 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 3.0.

20 97. The glycoconjugate of any one of paragraphs 43 to 94 wherein the ratio of serotype 38 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 2.0.

98. The glycoconjugate of any one of paragraphs 43 to 94 wherein the ratio of serotype 38 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.5.

99. The glycoconjugate of any one of paragraphs 43 to 94 wherein the ratio of serotype 38 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.8 and 1.2.

25 100. The glycoconjugate of any one of paragraphs 43 to 94 wherein the ratio of serotype 38 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.0.

101. The glycoconjugate of any one of paragraphs 43 to 94 wherein the ratio of serotype 38 saccharide to carrier protein in the glycoconjugate (w/w) is between 1.0 and 1.5.

30 102. The glycoconjugate of any one of paragraphs 43 to 94 wherein the ratio of serotype 38 saccharide to carrier protein in the glycoconjugate (w/w) is between 1.0 and 2.0.

103. The glycoconjugate of any one of paragraphs 43 to 94 wherein the ratio of serotype 38 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.8 and 1.2.

104. The glycoconjugate of any one of paragraphs 43 to 94 wherein the ratio of serotype 38 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.7 and 1.1.

104a. The glycoconjugate of any one of paragraphs 43 to 81 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 38 saccharide before conjugation is between 150 kDa and 300 kDa, wherein said serotype 38 glycoconjugate has a weight average molecular weight (Mw) of between 1,000 kDa and 10,000 kDa, wherein the ratio of serotype 38 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.5 and wherein the degree of conjugation of said serotype 38 glycoconjugate is between 4 and 10.

104b. The glycoconjugate of any one of paragraphs 43 to 81 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 38 saccharide before conjugation is between 150 kDa and 300 kDa, wherein said serotype 38 glycoconjugate has a weight average molecular weight (Mw) of between 1,000 kDa and 10,000 kDa, wherein the ratio of serotype 38 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.7 and 1.1 and wherein the degree of conjugation of said serotype 38 glycoconjugate is between 4 and 10.

105. The glycoconjugate of any one of paragraphs 43 to 104 wherein said serotype 38 glycoconjugate comprises less than about 50% of free serotype 38 saccharide compared to the total amount of serotype 38 saccharide.

106. The glycoconjugate of any one of paragraphs 43 to 104 wherein said serotype 38 glycoconjugate comprises less than about 25% of free serotype 38 saccharide compared to the total amount of serotype 38 saccharide.

107. The glycoconjugate of any one of paragraphs 43 to 104 wherein said serotype 38 glycoconjugate comprises less than about 20% of free serotype 38 saccharide compared to the total amount of serotype 38 saccharide.

108. The glycoconjugate of any one of paragraphs 43 to 104 wherein said serotype 38 glycoconjugate comprises less than about 15% of free serotype 38 saccharide compared to the total amount of serotype 38 saccharide.

108a. The glycoconjugate of any one of paragraphs 43 to 81 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 38 saccharide before conjugation is between 150 kDa and 300 kDa, wherein said serotype 38 glycoconjugate has a weight average molecular weight (Mw) of between 1,000 kDa and 10,000 kDa, wherein the ratio of serotype 38 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.5, wherein the degree of conjugation of said serotype 38 glycoconjugate is between 4 and 10 and wherein said serotype 38 glycoconjugate comprises less than about 20% of free serotype 38 saccharide compared to the total amount of serotype 38 saccharide.

108b. The glycoconjugate of any one of paragraphs 43 to 81 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 38 saccharide before conjugation is between 150 kDa and 300 kDa, wherein said serotype 38 glycoconjugate has a weight average molecular weight (Mw) of between 1,000 kDa and 10,000 kDa, wherein the ratio of serotype 38

saccharide to carrier protein in the glycoconjugate (w/w) is between 0.7 and 1.1, wherein the degree of conjugation of said serotype 38 glycoconjugate is between 4 and 10 and wherein said serotype 38 glycoconjugate comprises less than about 20% of free serotype 38 saccharide compared to the total amount of serotype 38 saccharide.

5 109. The glycoconjugate of any one of paragraphs 43 to 108 wherein at least 30% of the serotype 38 glycoconjugate has a K_d below or equal to 0.3 in a CL-4B column.

 110. The glycoconjugate of any one of paragraphs 43 to 108 wherein at least 40% of the glycoconjugate has a K_d below or equal to 0.3 in a CL-4B column.

10 111. The glycoconjugate of any one of paragraphs 43 to 108 wherein at least 60% of the serotype 38 glycoconjugate has a K_d below or equal to 0.3 in a CL-4B column.

 112. The glycoconjugate of any one of paragraphs 43 to 108 wherein between 50% and 80% of the serotype 38 glycoconjugate has a K_d below or equal to 0.3 in a CL-4B column.

 113. The glycoconjugate of any one of paragraphs 43 to 108 wherein between 65% and 80% of the serotype 38 glycoconjugate has a K_d below or equal to 0.3 in a CL-4B column.

15 113a. The glycoconjugate of any one of paragraphs 43 to 81 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 38 saccharide before conjugation is between 150 kDa and 300 kDa, wherein said serotype 38 glycoconjugate has a weight average molecular weight (Mw) of between 1,000 kDa and 10,000 kDa, wherein the ratio of serotype 38 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.5, wherein the
20 degree of conjugation of said serotype 38 glycoconjugate is between 4 and 10, wherein said serotype 38 glycoconjugate comprises less than about 20% of free serotype 38 saccharide compared to the total amount of serotype 38 saccharide and wherein between 65% and 80% of the serotype 38 glycoconjugate has a K_d below or equal to 0.3 in a CL-4B column.

 113b. The glycoconjugate of any one of paragraphs 43 to 81 wherein the weight average
25 molecular weight (Mw) of said *S. pneumoniae* serotype 38 saccharide before conjugation is between 150 kDa and 300 kDa, wherein said serotype 38 glycoconjugate has a weight average molecular weight (Mw) of between 1,000 kDa and 10,000 kDa, wherein the ratio of serotype 38 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.7 and 1.1, wherein the degree of conjugation of said serotype 38 glycoconjugate is between 4 and 10, wherein said
30 serotype 38 glycoconjugate comprises less than about 20% of free serotype 38 saccharide compared to the total amount of serotype 38 saccharide and wherein between 65% and 80% of the serotype 38 glycoconjugate has a K_d below or equal to 0.3 in a CL-4B column.

 114. The glycoconjugate of any one of paragraphs 43 to 113 wherein the carrier protein of the serotype 38 saccharide glycoconjugate is selected in the group consisting of: TT, DT, DT
35 mutants and a C5a peptidase from *Streptococcus* (SCP).

115. The glycoconjugate of any one of paragraphs 43 to 113 wherein the carrier protein of the serotype 38 saccharide glycoconjugate is rhizavidin [aa 45-179J-GGGGSSS-SP1500-AAA-SP0785] (CP1).

115a. The glycoconjugate of any one of paragraphs 43 to 113 wherein the carrier protein of the serotype 38 saccharide glycoconjugate is Rhavi-linker-PdT(G294P)-linker-SP0435 [aa 62-185] fusion protein (SPP2).

116. The glycoconjugate of any one of paragraphs 43 to 113 wherein the carrier protein of the serotype 38 saccharide glycoconjugate is DT.

117. The glycoconjugate of any one of paragraphs 43 to 113 wherein the carrier protein of the serotype 38 saccharide glycoconjugate is TT.

118. The glycoconjugate of any one of paragraphs 43 to 113 wherein the carrier protein of the serotype 38 saccharide glycoconjugate is PD.

119. The glycoconjugate of any one of paragraphs 43 to 113 wherein the carrier protein of the serotype 38 saccharide glycoconjugate is CRM₁₉₇ or a C5a peptidase from *Streptococcus* (SCP).

120. The glycoconjugate of any one of paragraphs 43 to 113 wherein the carrier protein of the serotype 38 saccharide glycoconjugate is CRM₁₉₇.

121. The glycoconjugate of any one of paragraphs 43 to 113 wherein the carrier protein of the serotype 38 saccharide glycoconjugate is SCP.

122. The glycoconjugate of any one of paragraphs 43 to 113 wherein the carrier protein of the serotype 38 saccharide glycoconjugate is an enzymatically inactive SCP.

123. The glycoconjugate of any one of paragraphs 43 to 113 wherein the carrier protein of the serotype 38 saccharide glycoconjugate is an enzymatically inactive SCP from GBS (SCPB).

124. The glycoconjugate of any one of paragraphs 43 to 113 wherein the carrier protein of the serotype 38 saccharide glycoconjugate is an enzymatically inactive SCP from GAS (SCPA).

125. The glycoconjugate of any one of paragraphs 43 to 113 wherein the carrier protein of the serotype 38 saccharide glycoconjugate is a fragment of an SCPB.

126. The glycoconjugate of any one of paragraphs 43 to 113 wherein the carrier protein of the serotype 38 saccharide glycoconjugate is an enzymatically inactive fragment of SCP which consists of SEQ ID NO: 1.

127. The glycoconjugate of any one of paragraphs 43 to 113 wherein the carrier protein of the serotype 38 saccharide glycoconjugate is an enzymatically inactive fragment of SCP which consists of SEQ ID NO: 2.

128. An immunogenic composition comprising a *S. pneumoniae* serotype 38 saccharide according to any one of paragraphs 1-42.

129. An immunogenic composition comprising a *S. pneumoniae* serotype 38 saccharide glycoconjugate according to any one of paragraphs 43-127.

5 130. The immunogenic composition of paragraph 129 comprising from 1 to 45 different glycoconjugates.

131. The immunogenic composition of paragraph 129 comprising from 1 to 45 glycoconjugates from different serotypes of *S. pneumoniae*.

10 132. The immunogenic composition of paragraph 129 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*.

133. The immunogenic composition of paragraph 129 comprising glycoconjugates from 16 or 20 different serotypes of *S. pneumoniae*.

15 134. The immunogenic composition of paragraph 129 which is a 21, 22, 23, 24 or 25-valent pneumococcal conjugate composition.

135. The immunogenic composition of paragraph 129 which is a 21-valent pneumococcal conjugate composition.

136. The immunogenic composition of paragraph 129 which is a 22-valent pneumococcal conjugate composition.

20 137. The immunogenic composition of paragraph 129 which is a 23-valent pneumococcal conjugate composition.

138. The immunogenic composition of paragraph 129 which is a 24-valent pneumococcal conjugate composition.

25 139. The immunogenic composition of paragraph 129 which is a 25-valent pneumococcal conjugate composition.

140. The immunogenic composition of paragraph 129 comprising glycoconjugates from 26 to 45 glycoconjugates from different serotypes of *S. pneumoniae*.

30 141. The immunogenic composition of paragraph 129 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*.

142. The immunogenic composition of paragraph 129 comprising glycoconjugates from 35 or 45 different serotypes of *S. pneumoniae*.

142a. The immunogenic composition of paragraph 129 comprising glycoconjugates from 35 different serotypes of *S. pneumoniae*.

143. The immunogenic composition of paragraph 129 which is a 35, 36, 37, 38, 39, 40, 41, 42, 43, 44 or 45-valent pneumococcal conjugate composition.

5 143a. The immunogenic composition of paragraph 129 which is a 35-valent pneumococcal conjugate composition.

144. The immunogenic composition of paragraph 129 which is a 40, 41, 42, 43, 44 or 45-valent pneumococcal conjugate composition.

10 145. The immunogenic composition of paragraph 129 which is a 40-valent pneumococcal conjugate composition.

146. The immunogenic composition of paragraph 129 which is a 41-valent pneumococcal conjugate composition.

147. The immunogenic composition of paragraph 129 which is a 42-valent pneumococcal conjugate composition.

15 148. The immunogenic composition of paragraph 129 which is a 43-valent pneumococcal conjugate composition.

149. The immunogenic composition of paragraph 129 which is a 44-valent pneumococcal conjugate composition.

20 150. The immunogenic composition of paragraph 129 which is a 45-valent pneumococcal conjugate composition.

151. The immunogenic composition of any one of paragraphs 129-150 further comprising glycoconjugates from *S. pneumoniae* serotypes 4, 6B, 9V, 14, 18C, 19F and 23F.

152. The immunogenic composition of paragraph 151 further comprising glycoconjugates from *S. pneumoniae* serotypes 1, 5 and 7F.

25 153. The immunogenic composition of paragraph 152 further comprising a glycoconjugate from *S. pneumoniae* serotype 3.

154. The immunogenic composition of paragraph 153 further comprising glycoconjugates from *S. pneumoniae* serotypes 6A and 19A.

30 155. The immunogenic composition of paragraph 154 further comprising glycoconjugates from *S. pneumoniae* serotype 22F and 33F.

156. The immunogenic composition of paragraph 155 further comprising glycoconjugates from *S. pneumoniae* serotypes 8, 10A, 11A, 12F and 15B.

157. The immunogenic composition of paragraph 156 further comprising a glycoconjugate from *S. pneumoniae* serotype 2.

158. The immunogenic composition of paragraph 157 further comprising a glycoconjugate from *S. pneumoniae* serotype 9N.

5 159. The immunogenic composition of paragraph 158 further comprising a glycoconjugate from *S. pneumoniae* serotype 17F.

160. The immunogenic composition of paragraph 159 further comprising a glycoconjugate from *S. pneumoniae* serotype 20.

10 161. The immunogenic composition of paragraph 160 further comprising a glycoconjugate from *S. pneumoniae* serotype 2.

162. The immunogenic composition of paragraph 161 further comprising a glycoconjugate from *S. pneumoniae* serotype 15C.

15 163. The immunogenic composition of any one of paragraphs 129-150 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.

164. The immunogenic composition of any one of paragraphs 129-150 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.

20 165. The immunogenic composition of any one of paragraphs 129-150 further comprising glycoconjugates from *S. pneumoniae* serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F and wherein said immunogenic composition is a 14-valent pneumococcal conjugate composition.

25 166. The immunogenic composition of any one of paragraphs 129-150 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 conjugate composition.

30 167. The immunogenic composition of any one of paragraphs 129-150 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.

168. The immunogenic composition of any one of paragraphs 129-150 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.

169. The immunogenic composition of paragraph 168 which is a 21-valent pneumococcal conjugate composition.

170. The immunogenic composition of any one of paragraphs 129-150 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.

171. The immunogenic composition of paragraph 170 which is a 22-valent pneumococcal conjugate composition.

172. The immunogenic composition of any one of paragraphs 129-150 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.

173. The immunogenic composition of paragraph 172 which is a 22-valent pneumococcal conjugate composition.

174. The immunogenic composition of any one of paragraphs 129-150 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.

175. The immunogenic composition of paragraph 174 which is a 22-valent pneumococcal conjugate composition.

176. The immunogenic composition of any one of paragraphs 129-150 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.

177. The immunogenic composition of paragraph 176 which is a 23-valent pneumococcal conjugate composition.

178. The immunogenic composition of any one of paragraphs 129-150 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.

179. The immunogenic composition of paragraph 178 which is a 24-valent pneumococcal conjugate composition.

180. The immunogenic composition of any one of paragraphs 129-150 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.

181. The immunogenic composition of paragraph 180 which is a 25-valent pneumococcal conjugate composition.

182. The immunogenic composition of any one of paragraphs 129-150 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.

183. The immunogenic composition of paragraph 182 which is a 22-valent pneumococcal conjugate composition.

184. The immunogenic composition of any one of paragraphs 129-150 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.

185. The immunogenic composition of paragraph 184 which is a 22-valent pneumococcal conjugate composition.

186. The immunogenic composition of any one of paragraphs 129-150 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.

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

188. The immunogenic composition of any one of paragraphs 129-150 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, 24F and 33F.

189. The immunogenic composition of paragraph 188 which is a 22-valent pneumococcal conjugate composition.

190. The immunogenic composition of any one of paragraphs 129-150 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, 33F and 35B.

191. The immunogenic composition of paragraph 190 which is a 22-valent pneumococcal conjugate composition.

192. The immunogenic composition of any one of paragraphs 129-150 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, 23F and 33F.

193. The immunogenic composition of paragraph 192 which is a 23-valent pneumococcal conjugate composition.

194. The immunogenic composition of any one of paragraphs 129-150 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.

195. The immunogenic composition of paragraph 194 wherein the *S. pneumoniae* saccharides are conjugated to CRM₁₉₇.

196. The immunogenic composition of paragraph 194 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.

197. The immunogenic composition of paragraph 196 which is a 26-valent pneumococcal conjugate composition.

198. The immunogenic composition of any one of paragraphs 129-150 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, 21, 22A, 23A, 23B, 24B, 24F, 27, 29, 31, 33B, 34, 35B and 35F.

199. The immunogenic composition of any one of paragraphs 129-150 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, 21, 22A, 23A, 23B, 24B, 24F, 27, 29, 31, 33B, 34, 35B and 35F.

200. The immunogenic composition of paragraph 199 which is a 22-valent pneumococcal conjugate composition.

201. The immunogenic composition of any one of paragraphs 129-150 further comprising glycoconjugates from serotypes 2, 3, 7C, 9N, 10B, 15A, 16F, 17F, 20, 21, 22A, 23A, 23B, 24B, 24F, 27, 29, 31, 33B, 34, 35B and 35.

202. The immunogenic composition of paragraph 201 which is a 23-valent pneumococcal conjugate composition.

203. The immunogenic composition of any one of paragraphs 129-150 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, 21, 22A, 23A, 23B, 24B, 24F, 27, 29, 31, 33B, 34, 35B and 35F.

204. The immunogenic composition of any one of paragraphs 129-150 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, 21, 22A, 23A, 23B, 24B, 24F, 27, 29, 31, 33B, 34, 35B and 35F.

205. The immunogenic composition of paragraph 204 which is a 23-valent pneumococcal conjugate composition.

206. The immunogenic composition of any one of paragraphs 129-150 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, 21, 22A, 23A, 23B, 24B, 24F, 27, 29, 31, 33B, 34, 35B and 35F.

207. The immunogenic composition of paragraph 206 which is a 24-valent pneumococcal conjugate composition.

5 208. The immunogenic composition of any one of paragraphs 129-150 further comprising glycoconjugates from serotypes 2, 3, 7C, 9N, 10B, 15A, 16F, 17F, 20, 21, 22A, 23A, 23B, 24B, 24F, 27, 29, 31, 33B, 34, 35B and 35F.

209. The immunogenic composition of paragraph 208 which is a 23-valent pneumococcal conjugate composition.

10 210. The immunogenic composition of any one of paragraphs 129-150 further comprising glycoconjugates from serotypes 2, 3, 7C, 9N, 10B, 15A, 16F, 17F, 19A, 19F, 20, 21, 22A, 23A, 23B, 24B, 24F, 27, 29, 31, 33B, 34, 35B and 35F.

211. The immunogenic composition of paragraph 210 which is a 23-valent pneumococcal conjugate composition.

15 212. The immunogenic composition of any one of paragraphs 128-211 further comprising at least one, two or three adjuvants.

213. The immunogenic composition of any one of paragraphs 128-211 further comprising one adjuvant.

20 214. The immunogenic composition of any one of paragraphs 128-211 further comprising two adjuvants.

215. The immunogenic composition of any one of paragraphs 128-211 further comprising aluminum salts (alum) as adjuvant.

216. The immunogenic composition of any one of paragraphs 128-211 further comprising aluminum phosphate as adjuvant.

25 217. The immunogenic composition of any one of paragraphs 128-211 further comprising a saponin based adjuvant.

218. The immunogenic composition of any one of paragraphs 128-211 further comprising a QS21 based adjuvant.

30 219. The immunogenic composition of any one of paragraphs 128-211 further comprising a CpG Oligonucleotide as adjuvant.

220. The immunogenic composition of any one of paragraphs 128-219 for use as a vaccine.

221. The immunogenic composition of any one of paragraphs 128-219 for use in a method of preventing, treating or ameliorating an infection, disease or condition associated with *S. pneumoniae* serotype 38 in a subject.

222. The immunogenic composition of any one of paragraphs 128-219 for use in a method of inducing an immune response to *S. pneumoniae* serotype 38 in a subject said method comprising administering to the subject an immunologically effective amount of said immunogenic composition.

223. The immunogenic composition of any one of paragraphs 128-219 for use in a method of preventing an infection by *S. pneumoniae* 38 in a subject said method comprising administering to the subject an immunologically effective amount of said immunogenic composition.

224. A method of detecting the presence of D-2-acetamido-2,6-dideoxy-xylo-hexos-4-ulose (D-Sug) residues in an isolated *S. pneumoniae* serotype 38 polysaccharide, said method comprising the step of: a) isolating an *S. pneumoniae* serotype 38 polysaccharide and b) detecting the presence of D-2-acetamido-2,6-dideoxy-xylo-hexos-4-ulose residues in said polysaccharide.

225. the method of paragraph 224 wherein the presence of D-Sug residues is detected by NMR or Mass Spectrometry (MS).

226. A method of detecting the presence of N-acetyl-D-fucosamine (D-FucNAc) residues in a reduced serotype 38 polysaccharide, said method comprising the step of: a) reacting an isolated *S. pneumoniae* serotype 38 polysaccharide with a reducing agent and b) detecting the presence of N-acetyl-D-fucosamine (D-FucNAc) residues in said reduced polysaccharide.

226. the method of paragraph 225 wherein the presence of N-acetyl-D-fucosamine (D-FucNAc) residues is detected by NMR.

227. The method of any one of 225-226 wherein said reducing agent is sodium borohydride (NaBH₄).

228. The method of any one of 225-227 wherein said isolated *S. pneumoniae* serotype 38 polysaccharide has been previously treated with an oxidizing agent.

229. A method of detecting the presence of N-acetyl-D-quinovosamine (D-QuiNAc) residues in a reduced serotype 38 polysaccharide, said method comprising the step of: a) reacting an isolated *S. pneumoniae* serotype 38 polysaccharide with a reducing agent and b) detecting the presence of N-acetyl-D-quinovosamine (D-QuiNAc) residues in said reduced polysaccharide

230. The method of paragraph 229 wherein the presence of N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by NMR.

231. The method of paragraph 229 wherein the presence of N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Mass Spectrometry (MS).

232. The method of any one of 229-231 wherein said reducing agent is sodium borohydride (NaBH₄).

5 233. The method of any one of 229-232 wherein said isolated *S. pneumoniae* serotype 38 polysaccharide has been previously treated with an oxidizing agent.

234. A method of detecting the presence of N-acetyl-D-fucosamine (D-FucNAc) and N-acetyl-D-quinovosamine (D-QuiNAc) residues in a reduced serotype 38 polysaccharide, said method comprising the step of: a) reacting an isolated *S. pneumoniae* serotype 38 polysaccharide
10 with a reducing agent and b) detecting the presence of N-acetyl-D-fucosamine (D-FucNAc) and N-acetyl-D-quinovosamine (D-QuiNAc) residues in said reduced polysaccharide.

235. The method of paragraph 234 wherein the presence of N-acetyl-D-fucosamine (D-FucNAc) and N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by NMR.

236. The method of paragraph 234 wherein the presence of N-acetyl-D-fucosamine (D-FucNAc) and N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Mass Spectrometry
15 (MS).

237. The method of any one of 234-236 wherein said reducing agent is sodium borohydride (NaBH₄).

238. The method of any one of 234-237 wherein said isolated *S. pneumoniae* serotype 38
20 polysaccharide has been previously treated with an oxidizing agent.

239. A method of detecting the presence of N-acetyl-D-fucosamine (D-FucNAc) and/or N-acetyl-D-quinovosamine (D-QuiNAc) residues in *S. pneumoniae* serotype 38 glycoconjugate, said method comprising the step of: a) preparing a *S. pneumoniae* serotype 38 glycoconjugate and b) detecting the presence of N-acetyl-D-fucosamine (D-FucNAc) and/or N-acetyl-D-quinovosamine
25 (D-QuiNAc) residues in said glycoconjugate.

240. The method of paragraph 239 wherein the presence of N-acetyl-D-fucosamine (D-FucNAc) and/or N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by NMR.

241. The method of paragraph 239 wherein the presence of N-acetyl-D-fucosamine (D-FucNAc) and/or N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Mass
30 Spectrometry (MS).

242. A method of detecting the presence of N-acetyl-D-fucosamine (D-FucNAc) and N-acetyl-D-quinovosamine (D-QuiNAc) residues in *S. pneumoniae* serotype 38 glycoconjugate, said method comprising the step of: a) preparing a *S. pneumoniae* serotype 38 glycoconjugate and b) detecting the presence of N-acetyl-D-fucosamine (D-FucNAc) and N-acetyl-D-quinovosamine (D-
35 QuiNAc) residues in said glycoconjugate.

243. The method of paragraph 242 wherein the presence of N-acetyl-D-fucosamine (D-FucNAc) and N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by NMR.

244. The method of paragraph 242 wherein the presence of presence of N-acetyl-D-fucosamine (D-FucNAc) and N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by
5 Mass Spectrometry (MS).

245. A method of detecting the presence of N-acetyl-D-fucosamine (D-FucNAc) residues in *S. pneumoniae* serotype 38 glycoconjugate, said method comprising the step of: a) preparing a *S. pneumoniae* serotype 38 glycoconjugate and b) detecting the presence of N-acetyl-D-fucosamine (D-FucNAc) residues in said glycoconjugate.

10 246. The method of paragraph 245 wherein the presence of N-acetyl-D-fucosamine (D-FucNAc) residues is detected by NMR.

247. The method of paragraph 245 wherein the presence of N-acetyl-D-fucosamine (D-FucNAc) residues is detected by Mass Spectrometry (MS).

15 248. A method of detecting the presence of N-acetyl-D-quinovosamine (D-QuiNAc) residues in *S. pneumoniae* serotype 38 glycoconjugate, said method comprising the step of: a) preparing a *S. pneumoniae* serotype 38 glycoconjugate and b) detecting the presence of N-acetyl-D-quinovosamine (D-QuiNAc) residues in said glycoconjugate.

249. The method of paragraph 248 wherein the presence of N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by NMR.

20 250. The method of paragraph 248 wherein the presence of N-acetyl-D-quinovosamine (D-QuiNAc) residues is detected by Mass Spectrometry (MS).

251. The glycoconjugate of any one of paragraphs 43-127 for use as an antigen.

252. The glycoconjugate of any one of paragraphs 43-127 for use as a medicament.

253. The glycoconjugate of any one of paragraphs 43-127 for use as a vaccine.

25 254. The glycoconjugate of any one of paragraphs 43-127 for use in generating an immune response in a subject.

255. The glycoconjugate for use of paragraph 254 wherein said subject is a human.

256. The immunogenic composition of any one of paragraphs 128-219 for use as a medicament.

30 257. The immunogenic composition of any one of paragraphs 128-219 for use as a vaccine.

258. The immunogenic composition for use of paragraph 257 wherein said subject is a human.

259. The immunogenic composition of any one of paragraphs 128-219 for use is a method of preventing, treating or ameliorating a bacterial infection, disease or condition in a subject.

260. The immunogenic composition of any one of paragraphs 128-219 for use is a method of preventing, treating or ameliorating a *S. pneumoniae* serotype 38 infection, disease or condition in a subject.

261. The immunogenic composition of any one of paragraphs 128-219 for use is a method of inducing an immune response to *S. pneumoniae* serotype 38 in a subject.

262. The immunogenic composition of any one of paragraphs 128-219 for use is a method of preventing an infection by *S. pneumoniae* serotype 38 in a subject.

263. The immunogenic composition of any one of paragraphs 128-219 for use is a method of protecting a human susceptible to a *S. pneumoniae* serotype 38 infection.

264. A method of making a *Streptococcus pneumoniae* serotype 38 glycoconjugate, using click chemistry said method comprising the steps of (a) reacting an isolated serotype 38 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.

265. The method of paragraph 264 wherein before the activation (a), sizing of the saccharide to a target molecular weight (MW) range has been performed.

266. The method of paragraph 264 wherein the isolated serotype 38 saccharide is sized before activation with a carbonic acid derivative and an azido linker.

267. The method of paragraph 266 wherein the isolated serotype 38 capsular polysaccharide is sized to a weight average molecular weight between 50 kDa and 500 kDa.

268. The method of paragraph 266 wherein the isolated serotype 38 capsular polysaccharide is sized to a weight average molecular weight between 50 kDa and 400 kDa.

268. The method of paragraph 266 wherein the isolated serotype 38 capsular polysaccharide is sized to a weight average molecular weight between 100 kDa and 500 kDa.

270. The method of paragraph 266 wherein the isolated serotype 38 capsular polysaccharide is sized to a weight average molecular weight between about 100 kDa and about 400 kDa.

271. The method of paragraph 266 wherein the isolated serotype 38 capsular polysaccharide is sized to a weight average molecular weight between about 150 kDa and about 300 kDa.

272. The method of paragraph 264 wherein the isolated serotype 38 capsular polysaccharide is not sized before activation with a carbonic acid derivative and an azido linker.

273. The method of any one of paragraphs 264 to 272 wherein said carbonic acid derivative is selected from the group consisting of 1,1'-carbonyldiimidazole (CDI), 1,1'-carbonyldi-(1,2,4-triazole) (CDT), N,N'-Disuccinimidyl carbonate (DSC) and N-hydroxysuccinimidyl chloroformate.

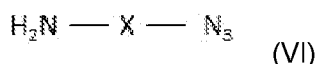
274. The method of any one of paragraphs 264 to 272 wherein said carbonic acid derivative is 1,1'-carbonyldiimidazole (CDI).

275. The method of any one of paragraphs 264 to 272 wherein said carbonic acid derivative is 1,1'-Carbonyl-di-(1,2,4-triazole) (CDT).

276. The method of any one of paragraphs 264 to 272 wherein said carbonic acid derivative is N,N'-Disuccinimidyl carbonate (DSC).

277. The method of any one of paragraphs 264 to 272 wherein said carbonic acid derivative is N-hydroxysuccinimidyl chloroformate.

278. The method of any one of paragraphs 264 to 277 wherein said azido linker is a compound of formula (VI),



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.

279. The method of any one of paragraphs 264 to 277 wherein said azido linker is a compound of formula (VI), wherein X is $\text{CH}_2(\text{CH}_2)_n$, and n is selected from 1 to 10.

280. The method of any one of paragraphs 264 to 277 wherein said azido linker is a compound of formula (VI), 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.

281. The method of any one of paragraphs 264 to 277 wherein said azido linker is a compound of formula (VI), wherein X is $\text{NHCO}(\text{CH}_2)_n$, and n is selected from 1 to 10.

282. The method of any one of paragraphs 264 to 277 wherein said azido linker is a compound of formula (VI), 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.

283. The method of any one of paragraphs 264 to 277 wherein said azido linker is a compound of formula (VI), wherein X is $\text{OCH}_2(\text{CH}_2)_n$, and n is selected from 1 to 10.

284. The method of any one of paragraphs 264 to 277 wherein said azido linker is a compound of formula (VI), 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.

5 285. The method of any one of paragraphs 264 to 277 wherein said azido linker is a compound of formula (VII),

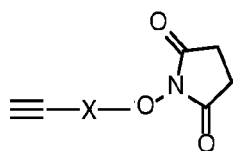


286. The method of any one of paragraphs 264 to 277 wherein said azido linker is 3-azido-propylamine.

10 287. The method of any one of paragraphs 264 to 286 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.

288. The method of any one of paragraphs 264 to 286 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.

15 289. The method of any one of paragraphs 264 to 286 wherein said agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group is a compound of formula (VIII),



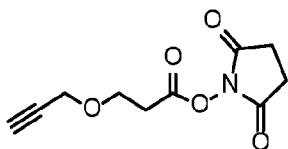
(VIII)

20 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.

25 290. The method of any one of paragraphs 264 to 286 wherein said agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group is a compound of formula (VIII), 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.

291. The method of any one of paragraphs 264 to 286 wherein said agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group is a compound of formula (VIII),
30 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.

292; The method of any one of paragraphs 264 to 286 wherein said agent bearing an N-Hydroxysuccinimide (NHS) moiety and an alkyne group is a compound of formula (IX):



(IX).

293. The method of any one of paragraphs 264 to 292 wherein, 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.

294. The method of any one of paragraphs 264 to 293 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.

295. The method of any one of paragraphs 264 to 294 wherein at step a) the isolated saccharide is reacted with a carbonic acid derivative in an aprotic solvent.

296. The method of any one of paragraphs 264 to 295 wherein the isolated saccharide is reacted with CDI in an aprotic solvent comprising 0.1% to 1% (v/v) water.

297. The method of any one of paragraphs 264 to 296 wherein step a) further comprises reacting the carbonic acid derivative-activated saccharide with an amount of azido linker that is between 0.01-10 molar equivalent to the amount of polysaccharide Repeat Unit of the activated saccharide (molar equivalent of RU).

298. The method of any one of paragraphs 264 to 297 wherein the degree of activation of the activated saccharide following step a) is between 1.0 to 100%.

299. The method of any one of paragraphs 264 to 297 wherein the degree of activation of the activated saccharide following step a) is between 5 to 70%.

300. The method of any one of paragraphs 264 to 297 wherein the degree of activation of the activated saccharide following step a) is between 15 to 50%.

301. The method of any one of paragraphs 264 to 297 wherein the degree of activation of the activated saccharide following step a) is between 10 to 40%.

302. The method of any one of paragraphs 264 to 297 wherein the degree of activation of the activated saccharide following step a) is between 5 to 15%.

303. The method of any one of paragraphs 264 to 297 wherein the degree of activation of the activated saccharide following step a) is between 15 to 35%.

304. The method of any one of paragraphs 264 to 297 wherein the degree of activation of the activated saccharide following step a) is between 15 to 25%.

305. The method of any one of paragraphs 264 to 297 wherein the degree of activation of the activated saccharide following step a) is about 25%.

306. The method of any one of paragraphs 264 to 305 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.

307. The method of any one of paragraphs 264 to 306 wherein the degree of activation of the activated carrier following step b) is between 1 and 50.

308. The method of any one of paragraphs 264 to 306 wherein the degree of activation of the activated carrier following step b) may be between 5 to 50.

309. The method of any one of paragraphs 264 to 306 wherein the degree of activation of the activated carrier following step b) is between 10 to 40.

310. The method of any one of paragraphs 264 to 306 wherein the degree of activation of the activated carrier following step b) is between 15 to 25.

311. The method of any one of paragraphs 264 to 310 wherein the conjugation reaction c) is carried out in aqueous buffer.

312. The method of any one of paragraphs 264 to 310 wherein the conjugation reaction c) is carried out in aqueous buffer in the presence of copper (I) as catalyst.

313. The method of any one of paragraphs 264 to 310 wherein the conjugation reaction c) is carried out in aqueous buffer in the presence an oxidant and of copper (I) as catalyst.

314. The method of any one of paragraphs 264 to 313 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.

315. The method of any one of paragraphs 264 to 314 wherein following step c), unreacted azido groups in the conjugates, are capped using a suitable azido group capping agent.

316. The method of any one of paragraphs 264 to 315 wherein following step c) unreacted alkyne groups are capped using a suitable alkyne group capping agent.

317. The method of any one of paragraphs 283 to 336 wherein said Streptococcus pneumoniae serotype 38 glycoconjugate is according to any one of paragraphs 1-57.

318. A method of making a Streptococcus pneumoniae serotype 38 glycoconjugate, using reductive amination said method comprising the steps of (1) oxidation (activation) of the serotype 38 purified saccharide, (2) reduction of the activated saccharide and the carrier protein to form a glycoconjugate.

319. The method of paragraph 318 wherein the isolated serotype 38 polysaccharide is sized before oxidation.

320. A method of making a *Streptococcus pneumoniae* serotype 38 glycoconjugate, comprising the step of: (a) reacting said serotype 38 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.

321. The method of paragraph 320 wherein the oxidizing agent is periodate.

322. The method of paragraph 321 wherein the degree of oxidation of the activated serotype 38 saccharide is between 2 and 30.

323. The method of paragraph 321 wherein the degree of oxidation (DO) of the activated serotype 38 saccharide is between 10 and 25.

324. The method of paragraph 321 wherein the degree of oxidation (DO) of the activated serotype 38 saccharide is between 10 and 25 and wherein the initial input ratio (weight by weight) of activated serotype 38 saccharide to carrier protein at step b) is between 1.5:1 and 0.5:1.

325. The method of paragraph 321 wherein the degree of oxidation (DO) of the activated serotype 38 saccharide is between 10 and 25 and wherein the initial input ratio (weight by weight) of activated serotype 38 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.

326. The method of paragraph 321 wherein the degree of oxidation (DO) of the activated serotype 38 saccharide is between 10 and 25 and wherein the initial input ratio (weight by weight) of activated serotype 38 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).

327. The method of paragraph 321 wherein the degree of oxidation (DO) of the activated serotype 38 saccharide is between 10 and 25 and wherein the initial input ratio (weight by weight) of activated serotype 38 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.

328. The method of paragraph 321 wherein the degree of oxidation (DO) of the activated serotype 38 saccharide is between 10 and 25 and wherein the initial input ratio (weight by weight) of activated serotype 38 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.

329. The method of any one of paragraphs 320 to 328 wherein said *Streptococcus pneumoniae* serotype 38 glycoconjugate is according to any one of paragraphs 44-74.

330. The method of paragraph 328 wherein said *Streptococcus pneumoniae* serotype 38 glycoconjugate is according to any one of paragraphs 44-74.

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.

Examples

Example 1: Serotype 38 capsular saccharides, samples preparation

***Streptococcus pneumoniae* serotype 38 polysaccharide :**

25mg of lyophilized native *Streptococcus pneumoniae* serotype 38 was weighed and dissolved in 1000μL 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 38 polysaccharide:

25mg of native serotype 38 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.

Reduced *Streptococcus pneumoniae* serotype 38 polysaccharide:

25mg of native serotype 38 polysaccharide was completely reduced after incubating with 1 molar equivalent of NaBH₄ at room temperature for 18 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.

Example 2: Structural elucidation of *Streptococcus pneumoniae* serotype 38 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 protons and carbons in the molecule, to map the backbone of the representative sugars in the serotype 38 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 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: total number of point in the ¹H and ¹³C dimensions, respectively, with 2 scans, recycle delay 1s), ¹H-¹³C HSQC (Heteronuclear Single Quantum Coherence, 2048 x 256: total number of point in the ¹H and ¹³C dimensions, respectively, with 4 scans, recycle delay 1s), ¹H-¹³C HSQC-TOCSY (Heteronuclear Single Quantum Coherence - Total Correlation Spectroscopy, 2048 x 256: total number of point in the ¹H and ¹³C dimensions, respectively, with 48 scans, recycle delay 1s mixing time of 120 ms), ¹H-¹³C HMBC (Heteronuclear Multiple Bond Correlation, 2048 x 256: total number of point in the ¹H and ¹³C dimensions, respectively, long range J ~ 5-10 Hz, with 48 scans, recycle delay 1s) and ¹H-¹³C HSQC-COSY (Heteronuclear Single Quantum Coherence – Correlation Spectroscopy, 2048 x 256: total number of point in the ¹H and ¹³C dimensions, respectively, with 48 scans, recycle delay 1s), ¹H-¹³C CLIP-HSQC (¹H decoupled Heteronuclear Single Quantum Coherence, 2048 x 256: total number of point in the ¹H and ¹³C dimensions, respectively, with 8 scans, recycle delay 1s). 1D ³¹P 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).

It has been found two structures depending on the starting strain (two forms: named serine and glycine form). The chemical structure of *Streptococcus pneumoniae* serotype 38 serine and glycine forms is shown in Figure 1A and 1B respectively. The serotype 38 polysaccharide is a penta-saccharide: α -D-glucosamine (A), α -D-2-acetamido-2,6-dideoxy-xylo-hexos-4-ulose (α -D-Sug) (B), O-acetylated β -D-galactose (C), α -D-galactose (D), and β -D-galactofuranose (E). Residue C (O-acetylated β -D-galactose) is further linked at the 6 position to serine or glycine amino acid, thus representing the serotype 38 serine and glycine forms of polysaccharide.

The β -D-Galp4OAc,6Ser (residue C) of serotype 38 polysaccharide is O-acetylated at carbon 4 position and the level of acetylation is about 94%.

2D NMR analysis of the serotype 38 polysaccharide

The complete structural elucidation of sized serotype 38 (serine and glycine forms) 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 $^2J_{\text{C,H}}$ or $^3J_{\text{C,H}}$ 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 neighboring carbon through the glycosidic bond, which are helpful in sequential assignment of the sugar units and their connectivity.

All the sugar backbone resonances of the hydrate and keto state were assigned by comparing the resonances from the short range (HSQC-COSY) and long range (HSQC-TOCSY).

The ^1H - ^{13}C HSQC correlation spectrum of the serotype 38 polysaccharide with complete assignment of the resonances of all sugars of the serotype 38 repeating unit is tabulated in Table 1 for serine-form and glycine-form.

Table 1. Chemical shift assignment of all sugars of the serotype 38 repeating unit (for serine and glycine forms):

		Serine form		Glycine form	
		1H (ppm)	13C (ppm)	1H (ppm)	13C (ppm)
D-a-GlcNAc (A)	A1	5.24	99.9	5.27	99.7
(Hydrate form)	A2	4.11	53.9	4.12	53.9
	A3	4.11	77.6	4.05	77.3
	A4	3.71	68.6	3.71	68.5
	A5	3.71	72.9	3.71	72.9
	A6	3.88	60.9	3.89	60.9
	ANAc	2.08	22.8	2.07	22.7
	CO		174.4		174.4
D-a-Sug (B)	B1	4.80	98.7	4.80	98.7
(Hydrate form)	B2	4.24	51.8	4.24	51.8
	B3	3.91	78.7	3.93	78.3
	B4		94.8		94.9
	B5	4.33	69.6	4.33	69.6
	B6	1.19	11.2	1.19	11.2
	BNAc	2.06	22.8	2.07	22.7
	CO		174.3		174.0
D-b-Galp4OAc,6Ser (C)	C1	4.85	101.5	4.83	101.3
D-b-Galp4OAc,6Gly (C)	C2	3.89	73.6	3.89	73.6
(Hydrate form)	C3	4.25	74.2	4.23	74.2
	C4	5.87	66.8	5.86	66.8
	C5	4.40	73.8	4.41	73.8
	C4OAc	2.12	20.5	2.12	20.6
	CO		172.8		172.9
	Ser-Ca	4.27	57.5		
	Ser-Cb	3.83	62.7		
	Gly-H1			3.86	43.7
	Gly-H2			3.79	43.7
D-a-Galp (D)	D1	5.24	94.6	5.25	94.6
(Hydrate form)	D2	3.93	68.3	3.91	68.3
	D3	3.94	69.3	3.94	69.4
	D4	4.01	78.2	4.00	78.1
	D5	4.12	72.3	4.11	72.3
	D6	3.70	60.8	3.71	60.8
D-b-Gal (E)	G1	5.16	108.3	5.16	108.3
(Hydrate form)	G2	4.03	83.0	4.02	83.0
	G3	4.00	78.1	4.00	78.1
	G4	4.06	81.6	4.06	81.7

	G5	3.87	70.6	3.87	70.7
	G6	3.74	64.1	3.74	64.1
D-a-GlcNAc (A)	A1_{keto}	5.06	97.3	5.07	97.2
(Keto form)	A2_{keto}	4.15	53.6	4.15	53.5
	A3_{keto}	3.98	78.2	3.99	78.3
	A4_{keto}	3.68	68.7	3.67	68.6
	A5_{keto}	3.67	73.2	3.67	73.2
D-a-Sug (B)	B1_{keto}	5.05	98.6	5.05	98.6
(Keto form)	B2_{keto}	4.48	55.6	4.50	55.6
	B3_{keto}	4.69	77.1	4.69	76.9
	B4_{keto}		204.8		204.8
	B5_{keto}	5.06	70.7	5.08	70.7
	B6_{keto}	1.22	12.7	1.22	12.8

Example 3: Structural comparison of O-acetylated and de-O-acetylated serotype 38 (serine form) polysaccharide by NMR experiments

The comparison of 1D ¹H and ¹³C NMR spectrum of acetylated (bottom panel) and deacetylated (top panel) serotype 38 polysaccharides is shown at Figure 3 and Figure 4. All the resonances are annotated in the 1D spectrum, and some key resonances are annotated in the ¹³C spectrum. The methyl, carbonyl carbon resonances of the acetate group and the C4 of resonance β-D-Galp4OAc,6Ser sugar are all absent in the deacetylated serotype 38 polysaccharide.

Example 4: Structural characterization of reduced serotype 38 polysaccharide (serine form) by NMR experiments

The activation of polysaccharides for conjugation can be conducted using sodium periodate (NaIO₄) which generates primary aldehyde at selective sugars in the polymer chain. This activated polysaccharide is then conjugated with a carrier protein. One of the critical unit operations after the conjugation is to reduce the unconjugated activated polysaccharide using sodium borohydrate (NaBH₄). However, it has been found that during this process the keto sugar (Sug) present in serotype 38 will be reduced. There is a need to understand the potential structural change in the serotype 38 after reducing with NaBH₄ and its potential impact on the glycoconjugates.

Figure 5 shows the 1D ¹H spectrum of reduced serotype 38 polysaccharide (obtained as disclosed at example 1). Upon reduction with sodium borohydrate (NaBH₄) the keto sugar (Sug) is reduced to form: FucpNAc and QuipNAc sugars in different ratios. The area under the methyl signals from FucpNAc and QuipNAc residue has been used in determining the populations of

polysaccharide chains having either FucpNAc or QuipNAc (using the 2D ^1H - ^{13}C HSQC spectra). The methyl signal from Sug residue has completely disappeared in the 2D HSQC spectra of reduced 38 polysaccharide, suggesting complete reduction of the polysaccharide. The reduced serotype 38 polysaccharide shows a ratio of 68% and 32% of FucpNAc and QuipNAc sugars, respectively in the repeating units.

Comparison of the 1D ^{13}C spectra of non-reduced and reduced serotype 38 polysaccharide clearly illustrated the difference in the chemical resonances observed upon reduction with NaBH_4 .

Structures of reduced 38 serotype is illustrated at figure 6.

Example 5: Conjugation of *S. pneumoniae* serotype 38 Capsular Polysaccharide using click chemistry

1. Mechanical sizing of Serotype 38 Capsular Polysaccharide

The native polysaccharide was subjected to mechanical 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 38 Capsular Polysaccharide with azido linker

Serotype 38 polysaccharide (173 kDa) 400 mg was mixed with 1.2 g imidazole then frozen and lyophilized. After lyophilization, the lyophilized polysaccharide was reconstituted with 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 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 or CRM₁₉₇ to alkyne-SCP or alkyne-CRM₁₉₇ with Alkyne NHS ester

Activation of SCP

To the SCP solution (2 g, 116 mL), 284 mL WFI and 100 mL 0.5 M Sodium Phosphate Buffer (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 $\pm$ 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 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 μ m (Millipak 60) filtration. The retentate was analyzed using Lowry assay for protein quantification and SEC-MALLS for Mw.

Activation of CRM₁₉₇

Activation of CRM₁₉₇ was similar to activation of SCP except that the amount of linker was adapted to 0.5 MEq to lysins on CRM₁₉₇.

4. Click Conjugation

To the mixture of Serotype 38 azido polysaccharide (as obtained at point 2 above) (62.5 mL) and 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 μ mol, 6.2 mL) and Tris(3-hydroxypropyltriazolylmethyl)amine (THPTA) (155 μ mol, 6.2 mL), aminoguanidine (1240 μ mol, 12.4 mL), and sodium ascorbate (1240 μ mol, 12.4 mL). The reaction mixture was stirred for 1.5 hours at room temperature.

After 1.5 hours, propargyl alcohol 36 μ L (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.

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 analyzed.

Table 2 shows attributes of some of the conjugates obtained (column 4 and 5).

Example 6: Conjugation of *S. pneumoniae* serotype 38 Capsular Polysaccharide using Reductive amination chemistry

1. Oxidation of Serotype 38 Capsular Polysaccharide using sodium periodate (Oxidized Polysaccharide)

Mechanically sized serotype 38 polysaccharide 870 mL (173kDa, 3.0 g) was mixed with 555 mL WFI and 75 mL 1M potassium phosphate buffer (KPB) (pH 6) to make 2 mg/mL concentration and 50 mM ionic strength solution. After the pH was adjusted to 6.0, sodium periodate (0.1 MEq) was added to the reaction mixture. The reaction mixture was stirred at 5 °C for 20 hours, followed by purification by UF/DF using 10K MWCO PES membrane against WFI (20X, v/v) and analyzed.

2. Conjugation using Reductive Amination Chemistry (RAC) in aqueous system (RAC-Aq) Serotype 38 oxidized polysaccharide (see step 1) (150 mg, 25x sucrose) was mixed with CRM₁₉₇ 300 mg (SPR input ratio: 0.5) and then lyophilized (Co-lyophilized).

Lyophilized mixture was reconstituted in 30 mL of 0.1 M KPB (pH 7.5). pH of the solution was adjusted to 6. After the reaction solution was heated to 30 °C, sodium cyanoborohydride (100 mg/mL in 0.15M KPB, pH 7.5, aged 3 hours) 91 µL (1 MEq) was added. The reaction was monitored by HPLC and after 72 hours another molar equivalent of sodium cyanoborohydride (100 mg/mL in 0.15M KPB, pH 7.5, aged 3 hours) 91 µL (1 MEq) was added. The capping was done for 24 hours.

The reaction mixture was then diluted with pre-cooled 5 mM succinate in saline (pH 6.0) 300 mL and purified by UF/DF using 100K MWCO RC membrane against 5 mM succinate in saline (pH 6.0) (50X diavolume).

3. Conjugation using Reductive Amination Chemistry (RAC) in DMSO (RAC-DMSO) with only NaCNBH₃ as a reducing and capping agent (about 30% reduced, column 2 of Table 2)

Serotype 38 oxidized polysaccharide (see step 1) (200 mg) was mixed with Sucrose 5.0 g and then lyophilized. The lyophilized activated oxidized polysaccharide was then reconstituted with DMSO 100 mL at room temperature. And lyophilized CRM₁₉₇ (200 mg) was also reconstituted with DMSO 100 mL.

Oxidized polysaccharide and CRM₁₉₇ were mixed, and the reaction mixture was stirred for 1 hour at room temperature (SPR input: 1, 1 mg/mL reaction concentration). Sodium cyanoborohydride (100 mg/mL in 0.15M SPB, pH 7.5) 120 µL (1 MEq) was added to the reaction and stirred at room temperature for 20 hrs. After 20 hours, sodium cyanoborohydride (100 mg/mL in 0.15M SPB, pH 7.5) 120 µL (1 MEq) was added to quench and then stirred (200 rpm) at room temperature for 48 hours. The reaction mixture was then diluted to pre-cooled 5 mM succinate in saline (pH 6.0) 800 mL and purified by UF/DF using 300K MWCO RC membrane against 5 mM succinate in saline (pH 6.0) (50X diavolume). After UF/DF, the retentate was filtered through 0.22 µm filter (Millipak 40) and analyzed.

4. Conjugation using Reductive Amination Chemistry (RAC) in DMSO (RAC-DMSO) with NaCNBH₃ as a reducing agent and NaBH₄ as a capping agent (fully reduced, column 1 of Table 2)

Serotype 38 oxidized polysaccharide (see step 1) (250 mg) was mixed with Sucrose 6.25 g and then lyophilized. The lyophilized polysaccharide was then reconstituted with DMSO 125 mL at room temperature. The lyophilized CRM₁₉₇ (600 mg) was also reconstituted with DMSO 240 mL. 125 mg oxidized polysaccharide and 157 mg K-CRM was mixed, and the reaction mixture was stirred for 1 hour at room temperature (SPR input:0.8). Sodium cyanoborohydride (100 mg/mL in 0.15M SPB, pH 7.5, aged 3 hours) 76 µL (1 MEq) was added to the reaction and stirred at room temperature for 20 hrs. After 20 hours, sodium borohydride (100 mg/mL in WFI) 46 µL (1 MEq) was added to quench the reaction and then stirred (200 rpm) at room temperature for 3 hours.

The reaction mixture was then diluted to pre-cooled 5 mM succinate in saline (pH 6.0) 600 mL and purified by UF/DF using 100K MWCO RC membrane against 5 mM succinate in saline (pH 6.0) (50X diavolume). After UF/DF, the retentate was filtered through 0.22 um filter (Millipak 40) and analyzed.

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Table 2 below comprises characterizing data for serotype 38 polysaccharide conjugates obtained by the methods of this invention.

Table 2

Serotype 38 Conjugates					
Conjugate#	1	2	3	4	5
Native Poly MW (KDa)	831.5	684	684	684	684
Sized Poly MW (KDa)	166	173	173	173	173
Chemistry	RAC-DMSO	RAC-DMSO	RAC-Aq	Click	Click
% of keto sugar (Sug) in reduced to form	Fully reduced	~50% reduced	~40% reduced	Not reduced	Not reduced
Degree of Activation of Act poly	DO 17	DO 16	DO 16	23%	23%
Act Poly Mw (kDa)	157	169	169	220	220
Conjugate data					
Carrier protein	CRM ₁₉₇	CRM ₁₉₇	CRM ₁₉₇	CRM ₁₉₇	SCP
Conjugation yield (%)	47	19	55	46	62
SPR (Output Ratio)	0.84	0.56	1.42	0.79	0.76
Free Saccharide (%)	8	22	18	20	16
Conjugate Mw (kDa)	1456	4964	3504	2121	2288

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

Example 7: Evaluation of immunogenicity in mice vaccinated with *S. pneumoniae* serotype 38 glycoconjugates using different chemistries and carrier proteins (Figure 7)

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The opsonophagocytic geomean titers (OPA GMT) for serotype 38 conjugates in mice, generated using different chemistries (RAC-Aq, RAC-DMSO or Click) with different carrier proteins (CRM₁₉₇ or SCP) were determined under standard conditions.

The five conjugates have been tested (see attributes of the tested conjugates at Table 2).

Groups of twenty-five 6-8 weeks old female Swiss Webster mice were immunized with 0.01 µg/animal or 0.1 µg/animal of test conjugates via the subcutaneous route on week 0. The mice were boosted with the same dose of conjugate on week 3 and then bled at week 5. Each vaccination was formulated with 100 µg/dose of AlPO₄ as an adjuvant. All preclinical immunogenicity studies were powered to detect a 4 to 5-fold difference in OPA titers using 25 mice per group. Whole blood was collected from mice two weeks after the second vaccination (Week 5, PD 2) and sera used for analyses. Serotype-specific OPAs were performed on week 5 sera samples.

Opsonophagocytic activity (OPA) assays are used to measure functional antibodies in murine sera specific for *S. pneumonia* serotype 38. Test serum is set up in assay reactions that measure the ability of capsular polysaccharide specific immunoglobulin to opsonize bacteria, trigger complement deposition, thereby facilitating phagocytosis and killing of bacteria by phagocytes. The OPA titer is defined as the reciprocal dilution that results in a 50% reduction in bacterial count over control wells without test serum. The OPA titer is interpolated from the two dilutions that encompass this 50% killing cut-off.

OPA procedures were based on methods described in Hu *et al.* (2005) Clin Diagn Lab Immunol 12 (2):287–295 with the following modifications. Test serum was serially diluted 2.5-fold and added to microtiter assay plates. Live serotype 38 target bacterial strains were added to the wells and the plates were shaken at 25°C for 30 minutes. Differentiated HL-60 cells (phagocytes) and baby rabbit serum (3- to 4-week old, PEL-FREEZ®, 12% final concentration) were added to the wells, and the plates were shaken at 37°C for 45 minutes. Following incubation, 10 µL aliquot were transferred to the wells of MULTISCREEN® HTS filter plates (MILLIPORE®) containing 50 µL of water. Liquid was filtered through the plates under vacuum, and 50 µL of HYSOY® medium was added to each well and filtered through. The filter plates were then incubated at 37°C, 5% CO₂ overnight and were then fixed with 70% EtOH solution. The plates were then stained with Coomassie Blue and destained once. Colonies were imaged and enumerated on a Cellular Technology Limited (CTL) (Shaker Heights, OH) IMMUNOSPOT® Analyzer. Raw colony counts were used to plot kill curves and calculate OPA titers.

OPA titers (geometric mean titer (GMT) with 95% confidence interval (CI)) at five weeks at different doses are shown in Table 3. The results are also presented in Figure 7.

Table 3 - OPA titers following vaccination with Pn338 conjugates using RAC-Aq, RAC-DMSO or Click chemistry and CRM ₁₉₇ or SCP carrier protein . The conjugates were used to vaccinate animals in the presence of adjuvant. Female Swiss-Webster mice, 6-8 weeks old; Doses: 0.01 or 0.1 µg/animal + AlPO ₄ ; Vaccinate: 0 and 3 wk.; terminal bleed wk. 5 Readout: OPA			
		<u>0.01</u>	<u>0.1</u>
RAC/DMSO-CRM Fully reduced (conjugate #1 of Table 2)	GMT	15	175
	% of non-responders	68	13
RAC/DMSO-CRM ~50% reduced (conjugate #2 of Table 2)	GMT	12	498
	% of non-responders	75	0
RAC/Aq-CRM ~40% reduced (conjugate #3 of Table 2)	GMT	14	4095
	% of non-responders	67	4
Click-CRM (conjugate #4 of Table 2)	GMT	6	77
	% of non-responders	96	36
Click-SCP (conjugate #5 of Table 2)	GMT	53	900
	% of non-responders	33	0

GMT: Geomean Titers

Example 8: Additional *S. pneumoniae* serotype 38 conjugate using Reductive Amination Chemistry in Aqueous system (RAC-Aq)

Additional serotype 38 conjugate has been generated using RAC-Aq (see example 6).

- 5 Table 4 below comprises characterizing data for serotype 38 polysaccharide conjugates obtained by the methods of this invention.

Table 4

Serotype 38 Conjugates	
Conjugate#	6
Native Poly MW (KDa)	376
Sized Poly MW (KDa)	140
Chemistry	RAC-Aq
Degree of Activation of Act poly	DO 15
Act Poly Mw (kDa)	149
Conjugate data	
Carrier protein	CRM ₁₉₇
Conjugation yield (%)	67

SPR (Output Ratio)	1.07
Free Saccharide (%)	29
Conjugate Mw (kDa)	3722

Mw: molecular weight; SPR: Saccharide to protein ratio; Act Poly: Activated Polysaccharide

Example 9: Additional *S. pneumoniae* serotype 38 conjugates using Click chemistry (Click)

Additional serotype 38 conjugate has been generated using Click (see example 5).

Table 5 below comprises characterizing data for serotype 38 polysaccharide conjugates obtained by the methods of this invention.

Table 5

Serotype 38 Conjugates				
Conjugate#	7	8	9	10
Sized Poly MW (KDa)	140	211	146	211
Activated polysaccharide attributes				
Degree of Activation of Act poly	12%	12%	20%	20%
Act Poly Mw (kDa)	149	194	151	214
Activated carrier attributes				
Degree of Activation (DoA)	20	20	20	20
Activated carrier MW, kDa	104.1	104.1	104.1	104.1
Conjugate data				
Carrier protein	SCP	SCP	SCP	SCP
Conjugation yield (%)	56	91	73	92
SPR (Output Ratio)	0.63	0.99	0.91	0.97
Free Saccharide (%)	15	17	16	11
Conjugate Mw (kDa)	1061	5611	3382	9598

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

Example 10: Evaluation of immunogenicity in mice vaccinated with *S. pneumoniae* serotype 38 glycoconjugates using different chemistries and carrier proteins (Figure 8)

The opsonophagocytic geomean titers (OPA GMT) for serotype 38 conjugates in mice, generated using different chemistries (RAC-Aq or Click) and difference carrier proteins (CRM₁₉₇ or SCP) were determined under standard conditions.

Seven conjugates have been tested (see attributes of the tested conjugates at Tables 2,4 and 5). Groups of twenty-five 6-8 weeks old female Swiss Webster mice were immunized with 0.01 µg/animal or 0.1 µg/animal of test conjugates via the subcutaneous route on week 0. The mice were boosted with the same dose of conjugate on week 3 and then bled at week 5. Each vaccination was formulated with 100 µg/dose of AlPO₄ as an adjuvant. All preclinical immunogenicity studies were powered to detect a 4 to 5-fold difference in OPA titers using 25 mice per group. Whole blood was collected from mice two weeks after the second vaccination (Week 5, PD 2) and sera used for analyses. Serotype-specific OPAs were performed on week 5 sera samples.

- 10 Opsonophagocytic activity (OPA) assays are used to measure functional antibodies in murine sera specific for *S. pneumonia* serotype 38 (see example 7).
OPA titers (geometric mean titer (GMT) with 95% confidence interval (CI)) at five weeks at different doses are shown in Table 6. The results are also presented in Figure 8.

Table 6 - OPA titers following vaccination with Pn38 conjugates using RAC-Aq or Click chemistry and CRM₁₉₇ or SCP carrier protein. The conjugates were used to vaccinate animals in the presence of adjuvant. Female Swiss-Webster mice, 6-8 weeks old; Doses: 0.01 or 0.1 µg/animal + AlPO₄; Vaccinate: 0 and 3 wk.; terminal bleed wk. 5 Readout: OPA			
		<u>0.01</u>	<u>0.1</u>
RAC/Aq-CRM (conjugate #3 of Table 2)	GMT	NA	3334
	%of non-responders	NA	4
RAC/Aq-CRM (conjugate #6 of Table 4)	GMT	NA	1023
	%of non-responders	NA	4
Click-SCP (conjugate #5 of Table 2)	GMT	40	663
	%of non-responders	48	8
Click-SCP (conjugate #7 of Table 5)	GMT	9	74
	%of non-responders	79	28
Click-SCP (conjugate #9 of Table 5)	GMT	113	264
	%of non-responders	24	16
Click-SCP (conjugate #10 of Table 5)	GMT	29	869
	%of non-responders	44	0
Click-SCP (conjugate #8 of Table 5)	GMT	18	1002
	%of non-responders	56	4

- 15 NA: Not Available
GMT: Geomean Titers

Example 11: Additional *S. pneumoniae* serotype 38 conjugates using Click chemistry (Click)

Additional serotype 38 conjugate has been generated using Click (see example 5), however N,N'-Disuccinimidyl carbonate (DSC) (1.5 MEq) was used instead of CDI. Table 7 show characterizing data.

Table 7

Serotype 38 Conjugates	
Conjugate#	11
Sized Poly MW (KDa)	202
Activated polysaccharide attributes	
Degree of Activation of Act poly	30%
Act Poly Mw (kDa)	246
Conjugate data	
Carrier protein	SCP
Conjugation yield (%)	83
SPR (Output Ratio)	0.8
Free Saccharide (%)	>5
Conjugate Mw (kDa)	5291

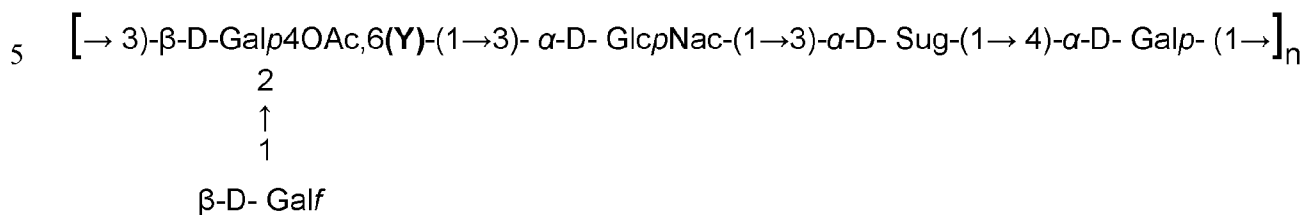
Mw: molecular weight; SPR: Saccharide to protein ratio; NA: Not Available; Act Poly: Activated Polysaccharide

All publications and patent applications mentioned in the specification are indicative of the level of those skilled in the art to which this invention pertains. All publications and patent applications are hereby incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, certain changes and modifications may be practiced within the scope of the appended claims.

Claims

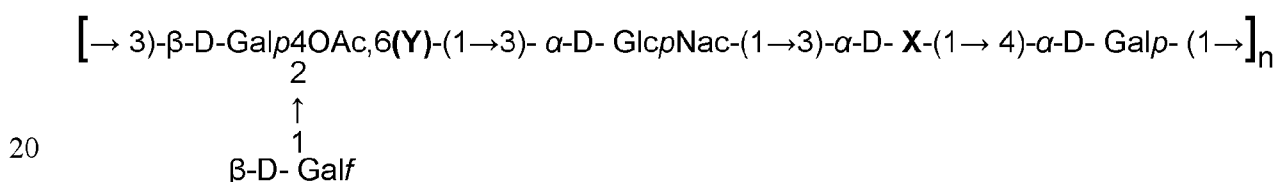
1. A glycoconjugate comprising an isolated *S. pneumoniae* serotype 38 saccharide with the following repeating unit:



where n represents the number of repeating units, wherein Y represents either a Serine or a Glycine residue, wherein all the repeating units comprise the same Y residue and wherein the O-acetyl group at position 4 of $\beta\text{-D-Galp4OAc,6(Y)}$ is present in about 0% to about 100% of the repeating units, conjugated to a carrier protein.

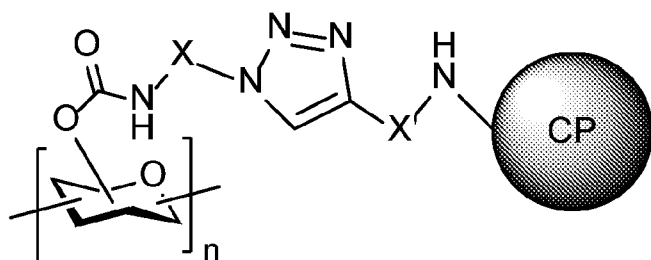
2. The serotype 38 glycoconjugate of claim 1 wherein said serotype 38 glycoconjugate is prepared by click chemistry.

3. A serotype 38 glycoconjugate comprising a serotype 38 capsular saccharide with the following repeating unit:



where n represents the number of repeating units, wherein Y represents either a Serine or a Glycine residue, wherein all the repeating units comprise the same Y residue and where X represents either a N-acetyl-D-fucosamine (D-FucNAc) residue or a N-acetyl-D-quinovosamine (D-QuiNAc) residue.

4. A serotype 38 glycoconjugate comprising a serotype 38 saccharide covalently conjugated to a carrier protein (CP) through a spacer and having the general formula (IV):



(IV),

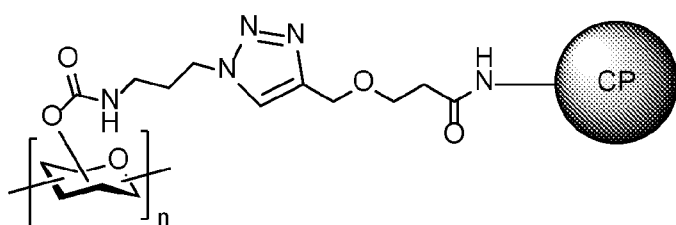
wherein X is selected from the group consisting of $\text{CH}_2(\text{CH}_2)_{n'}$, $(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$, $\text{NHCO}(\text{CH}_2)_{n'}$, $\text{NHCO}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$, $\text{OCH}_2(\text{CH}_2)_{n'}$ and $\text{O}(\text{CH}_2\text{CH}_2\text{O})_m\text{CH}_2\text{CH}_2$; 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 38 saccharide and
 5 wherein n represents the number of repeating units.

5. A serotype 38 glycoconjugate comprising a serotype 38 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 6. A serotype 38 glycoconjugate comprising a serotype 38 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 38 saccharide and
 wherein n represents the number of repeating units.

15 7. The glycoconjugate of any one of claims 1 to 6 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 38 saccharide before conjugation is between 150 kDa and 300 kDa.

8. The glycoconjugate of any one of claims 1 to 7 wherein said serotype 38 glycoconjugate has a weight average molecular weight (Mw) of between 1,000 kDa and 10,000 kDa.

20 9. The glycoconjugate of any one of claims 1 to 8 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 38 saccharide before conjugation is between 150 kDa and 300 kDa and wherein said serotype 38 glycoconjugate has a weight average molecular weight (Mw) of between 1,000 kDa and 10,000 kDa.

10. The glycoconjugate of any one of claims 1 to 9 wherein the degree of conjugation of said serotype 38 glycoconjugate is between 2 and 15.

25 11. The glycoconjugate of any one of claims 1 to 10 wherein the degree of conjugation of said serotype 38 glycoconjugate is between 4 and 10.

12. The glycoconjugate of any one of claims 1 to 6 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 38 saccharide before conjugation is between 150 kDa and 300 kDa, wherein said serotype 38 glycoconjugate has a weight average molecular

weight (Mw) of between 1,000 kDa and 10,000 kDa and wherein the degree of conjugation of said serotype 38 glycoconjugate is between 4 and 10.

13. The glycoconjugate of any one of claims 1 to 12 wherein the ratio of serotype 38 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.5.

5 14. The glycoconjugate of any one of claims 1 to 12 wherein the ratio of serotype 38 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.7 and 1.1.

15. The glycoconjugate of any one of claims 1 to 6 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 38 saccharide before conjugation is between 150 kDa and 300 kDa, wherein said serotype 38 glycoconjugate has a weight average molecular weight (Mw) of between 1,000 kDa and 10,000 kDa, wherein the ratio of serotype 38 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.5 and wherein the degree of conjugation of said serotype 38 glycoconjugate is between 4 and 10.

16. The glycoconjugate of any one of claims 1 to 6 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 38 saccharide before conjugation is between 150 kDa and 300 kDa, wherein said serotype 38 glycoconjugate has a weight average molecular weight (Mw) of between 1,000 kDa and 10,000 kDa, wherein the ratio of serotype 38 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.7 and 1.1 and wherein the degree of conjugation of said serotype 38 glycoconjugate is between 4 and 10.

17. The glycoconjugate of any one of claims 1 to 16 wherein said serotype 38 glycoconjugate comprises less than about 20% of free serotype 38 saccharide compared to the total amount of serotype 38 saccharide.

18. The glycoconjugate of any one of claims 1 to 6 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 38 saccharide before conjugation is between 150 kDa and 300 kDa, wherein said serotype 38 glycoconjugate has a weight average molecular weight (Mw) of between 1,000 kDa and 10,000 kDa, wherein the ratio of serotype 38 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.5, wherein the degree of conjugation of said serotype 38 glycoconjugate is between 4 and 10 and wherein said serotype 38 glycoconjugate comprises less than about 20% of free serotype 38 saccharide compared to the total amount of serotype 38 saccharide.

19. The glycoconjugate of any one of claims 1 to 6 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 38 saccharide before conjugation is between 150 kDa and 300 kDa, wherein said serotype 38 glycoconjugate has a weight average molecular weight (Mw) of between 1,000 kDa and 10,000 kDa, wherein the ratio of serotype 38 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.7 and 1.1, wherein the degree of conjugation of said serotype 38 glycoconjugate is between 4 and 10 and wherein said serotype

38 glycoconjugate comprises less than about 20% of free serotype 38 saccharide compared to the total amount of serotype 38 saccharide.

20. The glycoconjugate of any one of claims 1 to 19 wherein at least 60% of the serotype 38 glycoconjugate has a K_d below or equal to 0.3 in a CL-4B column.

5 21. The glycoconjugate of any one of claims 1 to 19 wherein between 65% and 80% of the serotype 38 glycoconjugate has a K_d below or equal to 0.3 in a CL-4B column.

22. The glycoconjugate of any one of claims 1 to 6 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 38 saccharide before conjugation is between 150 kDa and 300 kDa, wherein said serotype 38 glycoconjugate has a weight average molecular weight (Mw) of between 1,000 kDa and 10,000 kDa, wherein the ratio of serotype 38 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.5 and 1.5, wherein the degree of conjugation of said serotype 38 glycoconjugate is between 4 and 10, wherein said serotype 38 glycoconjugate comprises less than about 20% of free serotype 38 saccharide compared to the total amount of serotype 38 saccharide and wherein between 65% and 80% of the serotype 38 glycoconjugate has a K_d below or equal to 0.3 in a CL-4B column.

23. The glycoconjugate of any one of claims 1 to 6 wherein the weight average molecular weight (Mw) of said *S. pneumoniae* serotype 38 saccharide before conjugation is between 150 kDa and 300 kDa, wherein said serotype 38 glycoconjugate has a weight average molecular weight (Mw) of between 1,000 kDa and 10,000 kDa, wherein the ratio of serotype 38 saccharide to carrier protein in the glycoconjugate (w/w) is between 0.7 and 1.1, wherein the degree of conjugation of said serotype 38 glycoconjugate is between 4 and 10, wherein said serotype 38 glycoconjugate comprises less than about 20% of free serotype 38 saccharide compared to the total amount of serotype 38 saccharide and wherein between 65% and 80% of the serotype 38 glycoconjugate has a K_d below or equal to 0.3 in a CL-4B column.

24. The glycoconjugate of any one of claims 1 to 23 wherein the carrier protein of the serotype 38 saccharide glycoconjugate is selected in the group consisting of: TT, DT, DT mutants and a C5a peptidase from *Streptococcus* (SCP).

25. The glycoconjugate of any one of claims 1 to 23 wherein the carrier protein of the serotype 38 saccharide glycoconjugate is rhizavidin [aa 45-179J-GGGGSSS-SP1500- AAA-SP0785] (CP1).

26. The glycoconjugate of any one of claims 1 to 23 wherein the carrier protein of the serotype 38 saccharide glycoconjugate is Rhavi-linker-PdT(G294P)-linker-SP0435 [aa 62-185] fusion protein (SPP2).

27. The glycoconjugate of any one of claims 1 to 23 wherein the carrier protein of the serotype 38 saccharide glycoconjugate is SCP.

28. An immunogenic composition comprising a *S. pneumoniae* serotype 38 saccharide glycoconjugate according to any one of claims 1-27.

29. The immunogenic composition of claim 28 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,
5 22F, 23F and 33F.

30. The immunogenic composition of claim 28 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.

31. The immunogenic composition of claim 30 wherein the *S. pneumoniae* saccharides
10 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.

32. The immunogenic composition of any one of claims 28 to 31 further comprising one adjuvant.

15 33. The immunogenic composition of any one of claims 28 to 32 for use as a vaccine.

34. The immunogenic composition of any one of claims 28 to 32 for use in a method of preventing, treating or ameliorating an infection, disease or condition associated with *S. pneumoniae* serotype 38 in a subject.

20

Figure 1B

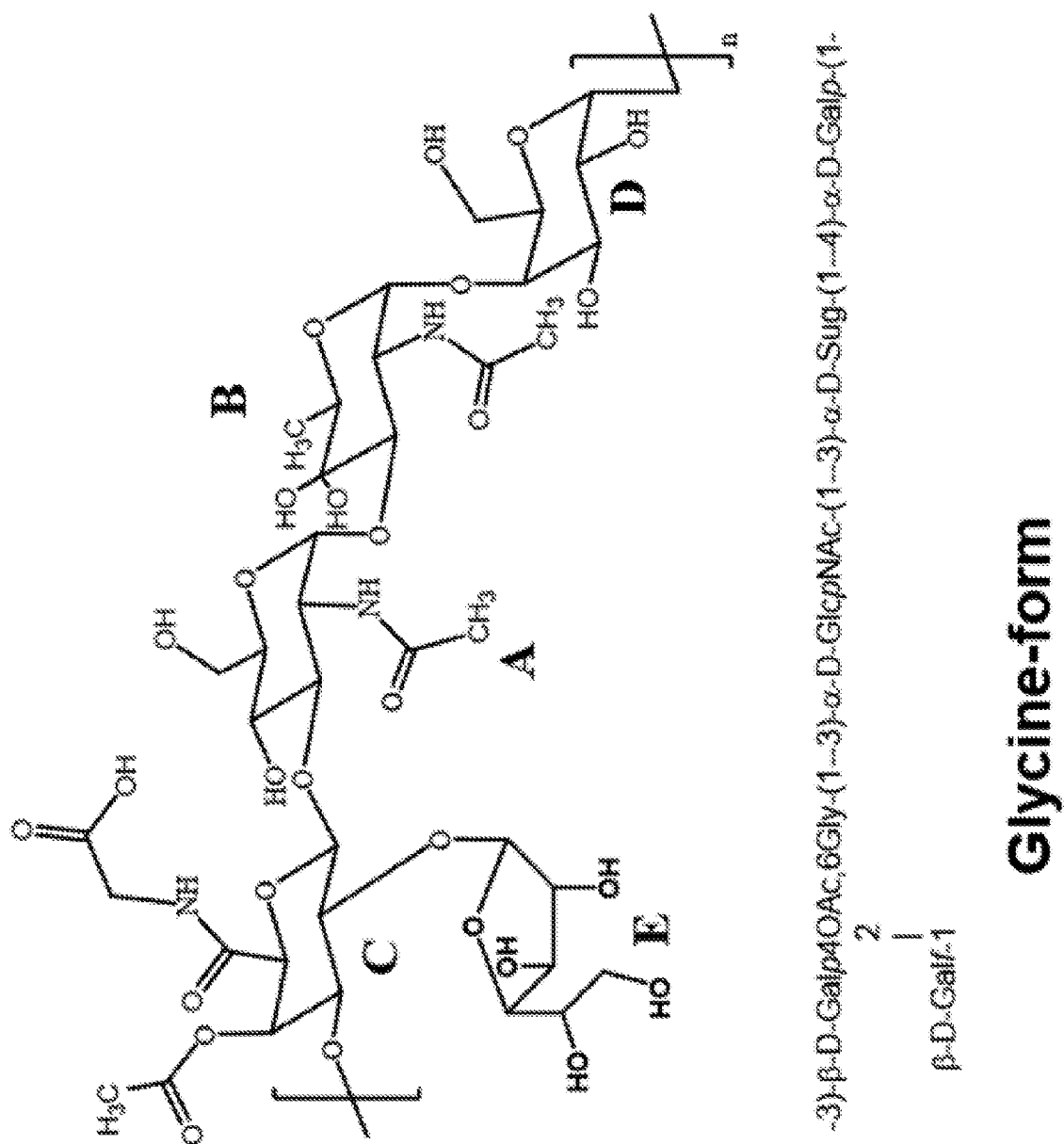


Figure 2

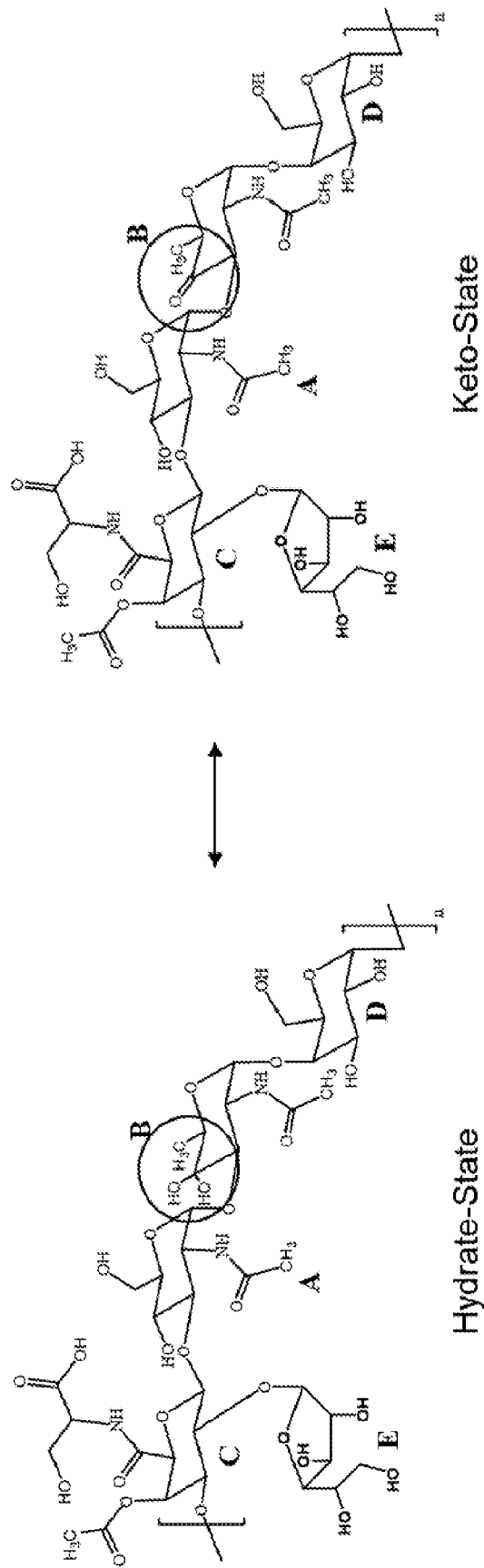


Figure 3

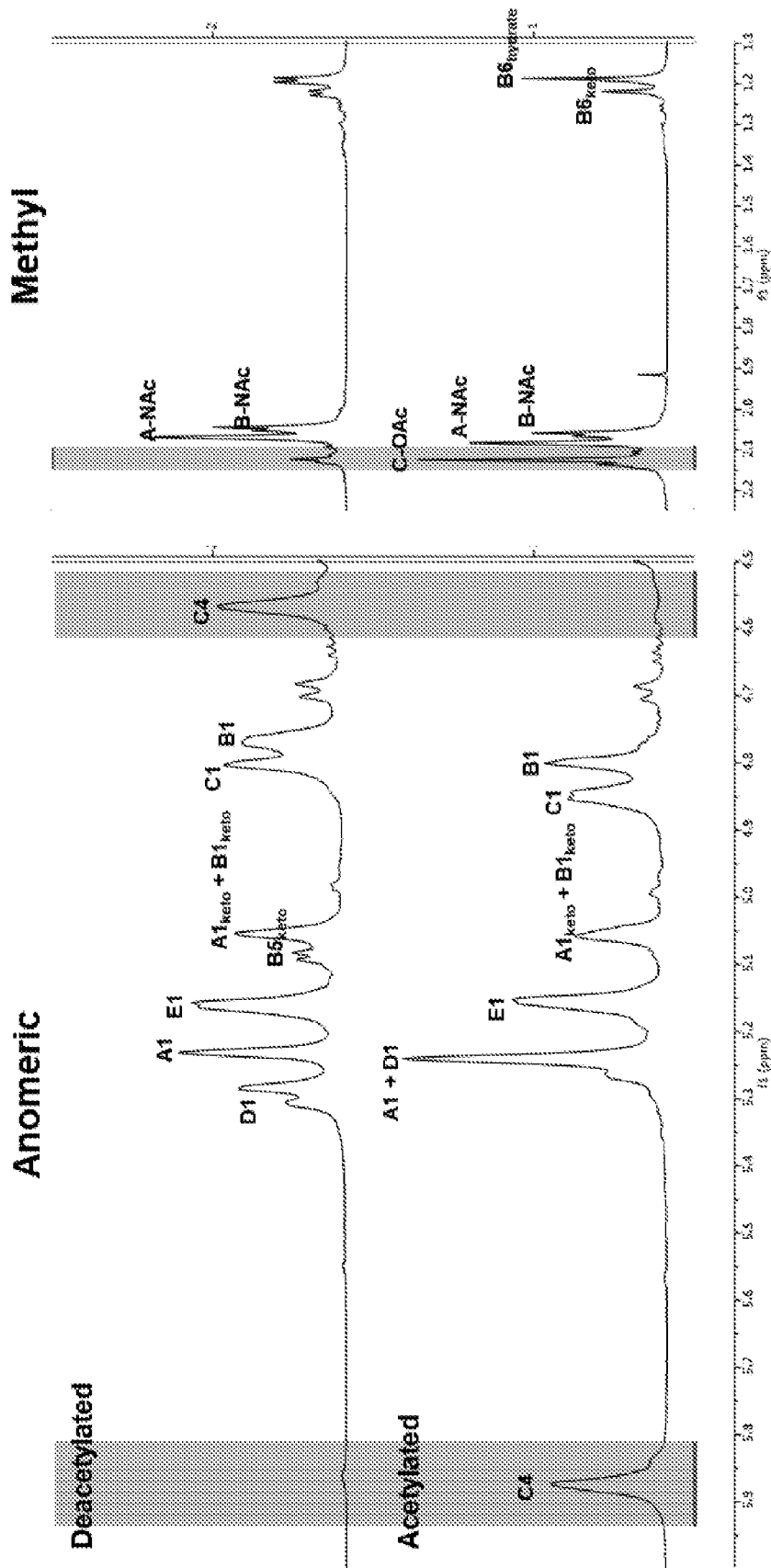


Figure 4

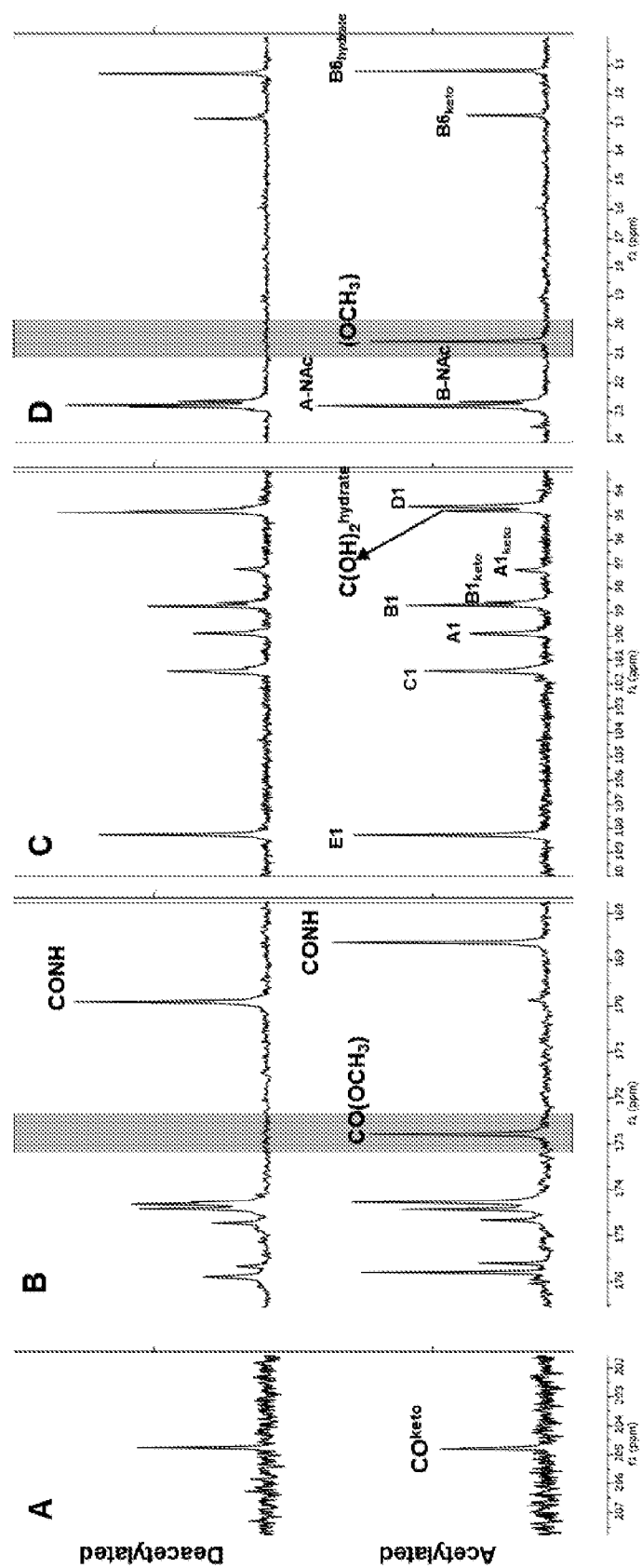


Figure 5

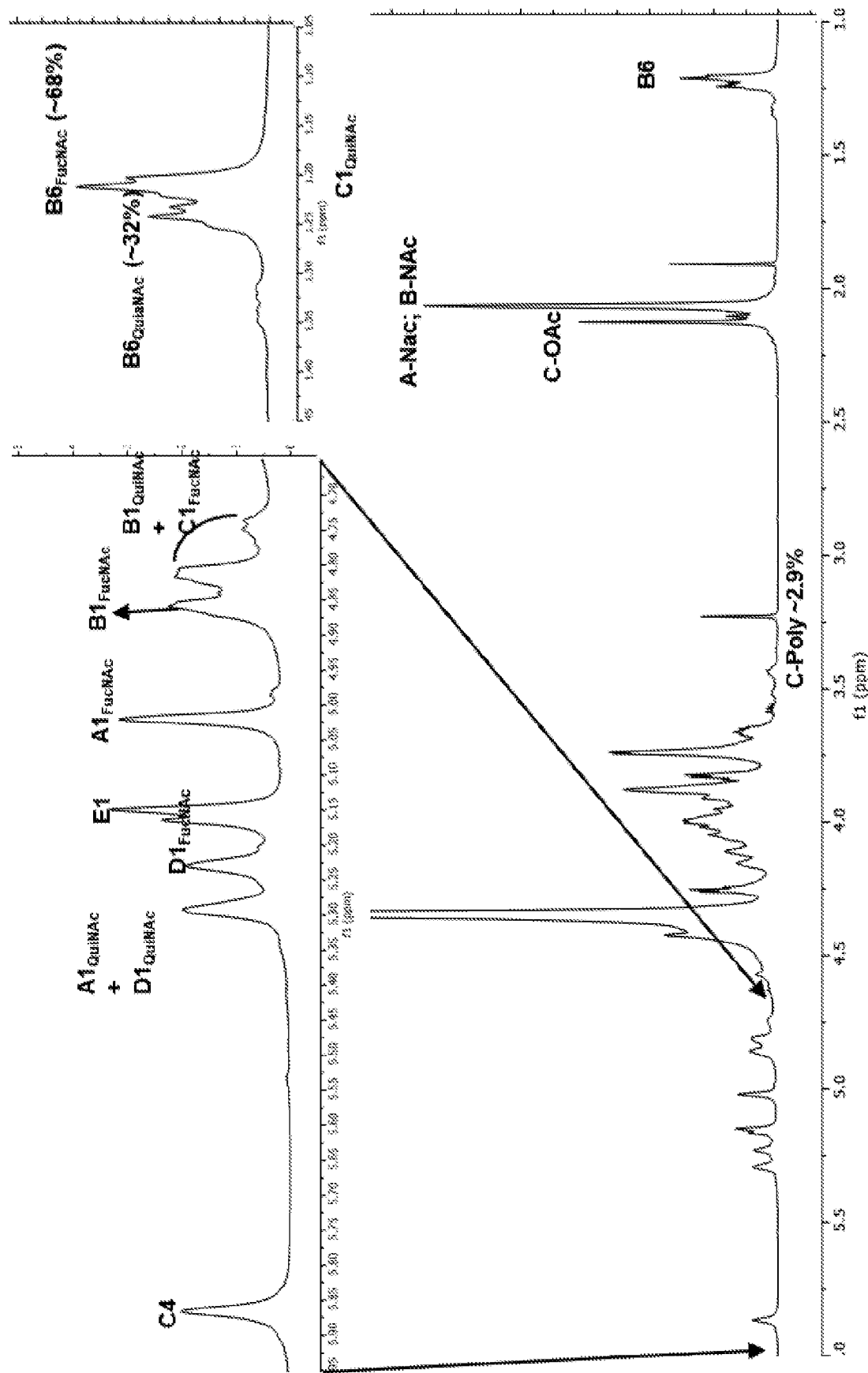


Figure 6

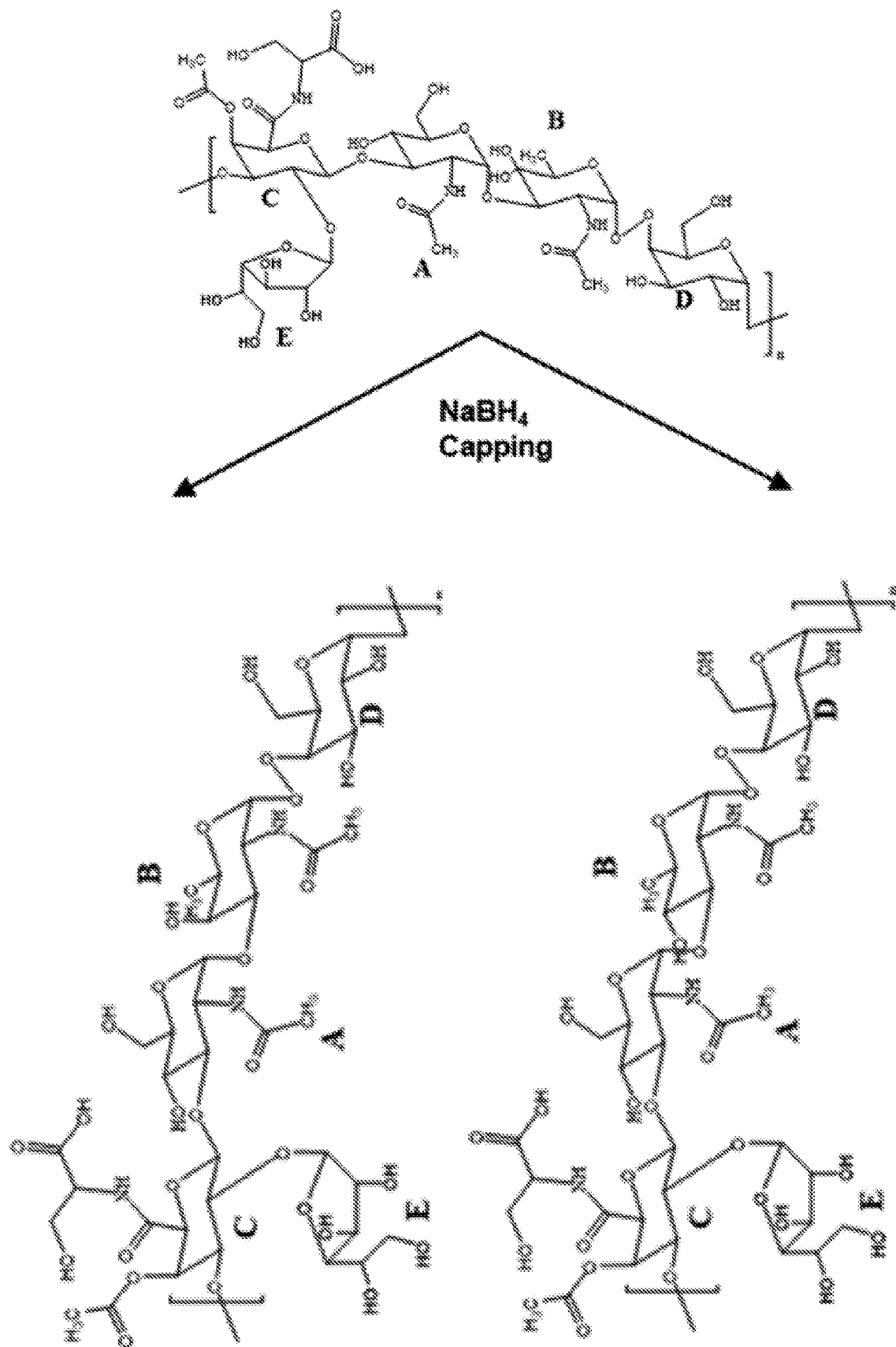


Figure 7

