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(54) **Title**: SYNTHETIC OLIGOSACCHARIDES FOR STAPHYLOCOCCUS VACCINE

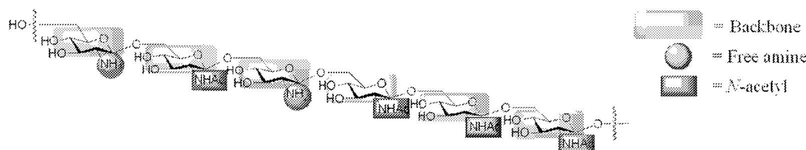

FIG. 1

(57) **Abstract**: The present invention synthetic oligo-β-(1→6)-glucosamine structures and a methodology which essentially allows for the synthesis of any oligo-β-(1→6)-glucosamine species having a definite number of monosaccharide units, including a set pattern of acetylated and non- acetylated residues. The invention further provides antibodies to these synthetic oligo-β-(1→6)-glucosamines as well as compositions thereof and methods for treating and preventing infections caused by bacteria expressing poly-β-(1→6)-glucosamines, such as *Staphylococcus aureus*.

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SYNTHETIC OLIGOSACCHARIDES FOR STAPHYLOCCOCUS VACCINE

CROSS REFERENCE TO RELATED APPLICATIONS

5 **[0001]** This application claims the benefit of U.S. Provisional Patent Application No. 61/478,238, filed April 22, 2011, the entire content of which is hereby incorporated by reference.

FIELD OF THE INVENTION

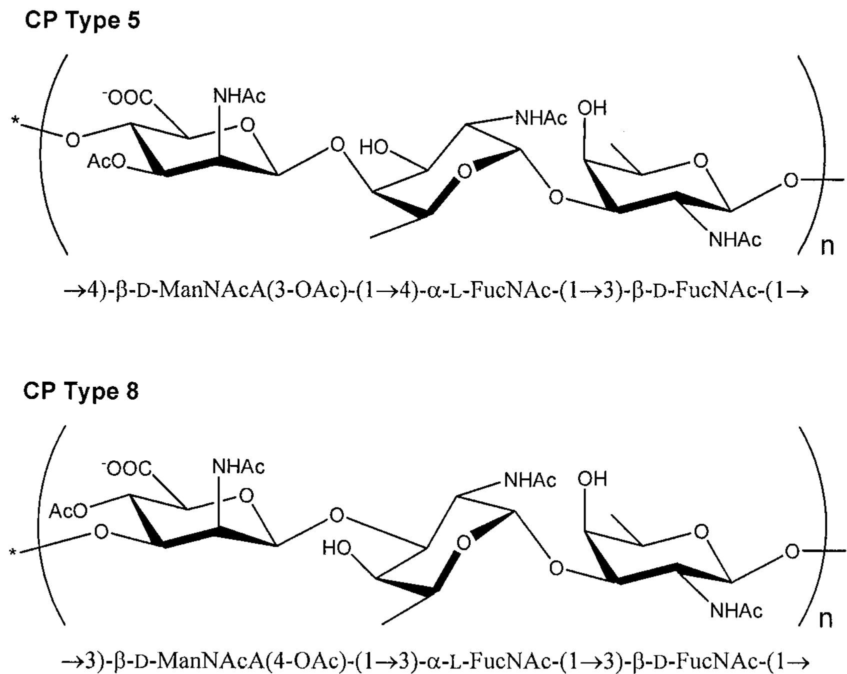
[0002] The present invention relates to synthetic oligo-β-(1→6)-
10 glucosamine structures as found in *Staphylococcus* and other bacterial pathogens.

BACKGROUND

[0003] *Staphylococcus aureus* capsular polysaccharides (CP's) are typically comprised of long linear repeating trisaccharide units, with each
15 repeating unit comprised of variously O-acetylated D-N-acetylmannosaminuronic acid (ManNAcA), L-N-acetyl-fucosamine (L-FucNAc) and D-Nacetyl-fucosamine (D-FucNAc) residues. A ManAcA residue, with a negatively charged carboxylate group at the 6-position, occurs at every third position in the sequence of the CP. The specific region- and stereochemical
20 linkages between units and the specific O-acetylation patterns dictate the particular CP type. The most common CP types related to human staphylococcal infections are CP Types 5 and 8, shown in Scheme 1 below (See e.g., Jean Lee, *International Journal of Antimicrobial Agents* 32S (2008) S71–S78).

25

Scheme 1

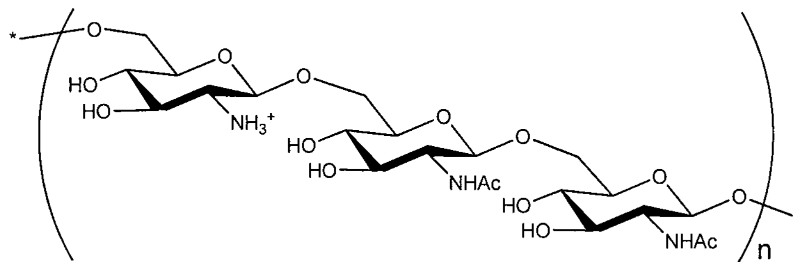


[0004] Infection involving a CP-producing strain of *S. aureus*, or vaccination with a *S. aureus* CP glycoconjugate, will result in the production of anti-CP antibodies by the host. The most productive anti-CP antibodies recognize the negatively charged 6-carboxylate group of the ManNAcA as a critical part of the epitope. As a result, these antibodies are expected to have complementary functional groups in their Fv regions that are capable of recognizing, and therefore binding to, the ManNAcA 6-COO- group in the context of the repeating sequence. Furthermore, it is expected that the linear epitope may span up to two repeating units (hexasaccharide) in length.

[0005] dPNAG sequences, including the example shown in Scheme 2, contain free amino groups that will be protonated, hence positively charged, at physiological pH. Infections involving a biofilm-producing (ica locus positive) strain of *S. aureus*, or vaccination with a *S. aureus* PNAG or dPNAG glycoconjugate, will result in the production of anti-PNAG and/or anti-dPNAG antibodies by the host. The most protective antibodies are raised against the dPNAG sequences and recognize the positively charged 2-ammonium group

of the $GlcNH_2$ residues as a critical part of the epitope. Depending on the degree of de-N-acetylation present in the antigen, the productive epitope may contain multiple positive charges, one at each de-N-acetylated residue. As a result, these anti-dPNAG antibodies are expected to have complementary functional groups in their Fv regions that are capable of recognizing, and therefore binding to, the $GlcNH_3^+$ ammonium groups in the context of the dPNAG sequence. Furthermore, it is expected that the linear epitope may span up to two repeating units (hexasaccharide) in length.

Scheme 2



[0006] Staphylococcal infection can lead to a wide range of severe clinical disease manifestations and represents a major public healthcare concern. The PNAG molecule has been used in vaccine studies (See Pier et al., U.S. Publication 2005/0118198). Data generated using purified PNAG-based material demonstrates the viability of this carbohydrate-based vaccine approach (Maira-Litran et al., *Infect. Immun.*, 73:6752, 2005). However, in spite of the aforementioned PNAG-focused studies revealing that functional protection and opsonization activity is dependent on the presence of an amine-rich modified PNAG structure (<50% acetylated positions), the major component of biofilms is secreted PNAG that contains mostly (>95%) N-acetylated positions. Accordingly, carbohydrate-based vaccine development is in need of a better understanding of the requirements for maintaining an appropriate acetylation-amine balance in lead PNAG-based vaccine target selection.

[0007] Methods for preparing synthetic PNAGs have been described (Gening et al., *Infect. Immun.*, 78:764, 2010, epub 11/30/2009; WO

2010/011284 A2; 1. Gening et al., *Carbohydrate Research* 342:567-575, 2007; Gening et al., *Russian J Bioorganic Chem.* 32(4):389-399, 2006; Yang et al., *Carbohydrate Research*, 338:495-502, 2003; Yang et al., *Carbohydrate Research* 338:1313-1318, 2003; Fridman et al., *Organic Letters* 4(2):281-
5 283, 2002). The described synthetic PNAGs were limited to homogenous PNAG compositions that are fully N-acetylated or fully non-N-acetylated; neither of these references, nor those cited therein, described a methodology for synthesizing homogenous mixed PNAGs having a predetermined number and arrangement of N-acetylated and N-deacetylated residues. Moreover,
10 although the previous purification-chemical process utilizing purified PNAG material that was subsequently chemically treated to yield an appropriate range of *N*-acetylated versus free amine positions on the PNAG molecule (Maira-Litran et al., *supra*), the purification-chemical process is only able to provide a heterogeneous mixture poorly defined at the molecular level.
15 Accordingly, the prior art processes failed to provide oligosaccharides with spatially defined acetyl-amine positions, such as an amine every third position on the glucosamine polymer, for example. As such, the identification of a precise acetyl-amine sequence required to generate a desired immune response can neither be achieved by this method nor predicted *a priori*.

20 **[0008]** The present inventors have previously described methods for synthesizing oligosaccharides with spatially defined acetyl-amine positions in U.S. Serial No. 61/327,621, incorporated by reference herein, and exemplified below.

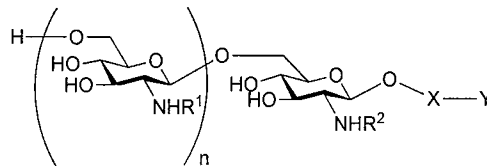
[0009] In view of the above, the present invention provides several
25 benefits for vaccine development, especially against *S. aureus*, including specific homogenous antigen compositions with acetyl-amine sequences predicted to generate a desired immune response.

SUMMARY

[0010] The most productive antibodies to each antigen class, if
30 simultaneously generated in response to a staphylococcal infection, could bind to each other in a head-to-head, or idiotype-anti-idiotype fashion. This

phenomenon was demonstrated by Pier and coworkers (*J. Clin. Invest,* 10.1172/JCI42748, 2010) who showed competitive binding between anti-CP antibodies and anti-dPNAG antibodies. The result in this case was that when both types of antibodies were present the infection course by Staphylococcus
5 was unaffected. This was shown both *in vitro* (opsonophagocytosis assays, binding studies) and *in vivo* (animal infection models). Thus, when used alone or in combination, a vaccine against staphylococcal CP's or a vaccine against a dPNAG containing a CP-complementary sequence may result in suboptimal immunoprotection from a *S. aureus* infection. The present
10 invention avoids competitive binding by developing synthetic oligosaccharides (oligo-β-(1→6)-glucosamine structures) that will lead to antibodies that cannot bind in neutralizing head-to-head fashion.

[0011] The present invention is a subset of oligosaccharides have the formula **1a**



15

1a

where R^1 and R^2 are each independently H or $COCH_3$, where at least one at least one R^1 or R^2 in the oligosaccharide is H and at least another is $COCH_3$; n is an integer from 5-11, X is a bond or a linker, and Y is H or a carrier; and
20 where each occurrence of R^1 can be the same or different. The subset is defined more particularly below in the Table A below.

[0012] The present invention also provides compositions and methods for synthesizing the subset of oligo-β-(1→6)-glucosamine structures.

[0013] The present invention further provides immunogenic and
25 immunoprotective compositions containing the subset of synthetic oligo-β-(1→6)-glucosamines **1a** and antibodies derived therefrom for diagnosing, treating, and preventing infections caused by bacteria such as *Staphylococcus aureus* and others.

[0014] The present invention further provides compositions consisting essentially of one of the oligosaccharides from the subset, compositions consisting essentially of two of the oligosaccharides from the subset, compositions consisting essentially of three of the oligosaccharides from the subset, etc. (including compositions consisting essentially of all or less than all of the oligosaccharides from the subset). The compositions may be homogeneous and/or synthetic.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] FIG. 1 depicts an exemplary core structure of an oligo-β-(1→6)-glucosamine comprised of a glucosamine backbone unique to bacterial pathogens. The backbone structure can be modified with N-acetyl groups with variable spacing.

[0016] FIG. 2A depicts naturally-derived PNAG polysaccharides and FIG. 2B depicts a synthetic PNAG oligosaccharide, including potential conjugation sites in FIGs. 2A and 2B.

[0017] FIG. 3 depicts a reaction scheme for synthesizing four monosaccharide building blocks which can be used to synthesize any dPNAG/PNAG oligosaccharide.

[0018] FIGs. 4A-4D depict a series of reaction schemes for assembling disaccharide building blocks from the four monosaccharide building blocks depicted in FIG. 3.

[0019] FIGs. 5A and 5B depict a reaction scheme for synthesizing a 6-mer thiol oligosaccharide **37**.

[0020] FIGs. 6A and 6B depict a reaction scheme for synthesizing a 12-mer thiol oligosaccharide **40.**

[0021] FIG. 7 depicts a reaction scheme for synthesizing an 18-mer thiol oligosaccharide **34**.

[0022] FIGs. 8A and 8B depict a reaction scheme for conjugating the 6-mer thiol oligosaccharide **37** to BSA (A) or KLH (B) to form a 6-Mix-BSA conjugate **41** or a 6-Mix-KLH conjugate **42**, respectively. In the nomenclature used herein, e.g. "6-Mix," "12-NH_2" and "18-NHAc," the number refers to the

number of monosaccharide units, "Mix" refers to an oligosaccharide with a fixed pattern of both acetyl and amine groups at R^1 and R^2, "NH_2" refers to an oligosaccharide with amine groups at R^1 and R^2, and "NHAc" refers to an oligosaccharide with acetyl groups at R^1 and R^2.

5 **[0023]** FIGs. 9A and 9B depict a reaction scheme for conjugating the 12-mer thiol oligosaccharide **40** to BSA (A) or KLH (B) to form a 12-Mix-BSA conjugate **43** or 12-Mix-KLH conjugate **44**, respectively.

[0024] FIGs. 10A and 10B depict a reaction scheme for conjugating the 18-mer thiol oligosaccharide **34** to BSA (A) or KLH (B) to form an 18-Mix-BSA 10 conjugate **45** or 18-Mix-KLH conjugate **46**, respectively.

[0025] FIGs. 11A-11C depict IgG antibody titers as a function of antibody-antigen complex absorption (OD_{450}) at the indicated serum dilutions obtained from 3 succesive bleeds (pre-immune, 1st bleed, and final bleed) in rabbits immunized with KLH conjugates corresponding to: (A) 6-Mix-KLH **42**; 15 (B) 12-Mix-KLH **44**; and (C) 18-Mix-KLH **46**. In each case, the antisera were incubated on ELISA plates adsorbed with their corresponding BSA conjugate, specfically, (A) 6-Mix-BSA **41**; (B) 12-Mix-BSA **43**; and (C) 18-Mix-BSA **45**.

[0026] FIGs. 11D-11F depict IgG antibody titers from antigen-specific antigen-KLH conjugate-derived antibodies recovered at three successive 20 stages of affinity purification, including pre-affinity purification (3rd bleed), the flow-through fraction and the antibody-enriched (purified) fraction from rabbits immunized with KLH conjugates. Results are shown as a function of antibody-antigen complex absorption (OD_{450}) at the indicated serum dilutions obtained from the above-described antibody-enriched fractions generated 25 against antigen-KLH conjugates corresponding to: (A) 6-Mix-KLH **42**; (B) 12-Mix-KLH **44**; and (C) 18-Mix-KLH **46.** In each case, the antisera were incubated on ELISA plates adsorbed with their corresponding BSA conjugate, specifically, (A) 6-Mix-BSA **41**; (B) 12-Mix-BSA **43**; and (C) 18-Mix-BSA **45**.

[0027] FIGs. 12A-12G depict the results of a cross-ELISA assay examining 30 the specificity and cross-reactivity between fully non-acetylated (6-NH_2, 12-NH_2, 18-NH_2); mixed (6-Mix, 12-Mix, 18-Mix) and fully acetylated (6-NHAc,

12-NHAc, 18-NHAc) oligosaccharides **1a** and antibodies derived therefrom. In FIGs. 12A-12G, antisera from rabbits immunized with the indicated antigen-KLH conjugates corresponding to (left to right) 6-NH_2, 6-Mix, 6-NHAc, 12-NH_2, 12-Mix, 12-NHAc, 18-NH_2, 18-Mix, and 18-NHAc were incubated in each case
5 with an ELISA plate adsorbed with a different antigen-BSA conjugate, specifically: (A) 6-NH_2-BSA; (B) 6-Mix-BSA; (C) 6-NHAc-BSA; (D) 12-NH_2-BSA; (E) 12-Mix-BSA; (F) 12-NHAc-BSA; (G) 18-Mix-BSA. Results are shown as a function of antibody-antigen complex absorption (OD_{450}) representing the averages from two rabbit antiseras in each case at the
10 indicated serum dilutions, whereby total OD_{450} is measured by subtracting away the background OD_{450} from KLH antibodies alone.

[0028] FIGs. 13A-13D depict the results of a whole-cell ELISA assay examining the binding of pre-immune sera (A, C) or immune sera (B, D) generated from rabbits immunized against (left to right) KLH control, non-
15 acetylated (12-NH_2); mixed (12-Mix) and non-acetylated (12-NHAc) oligo-β-(1→6)-glucosamines and *S. epidermidis* coated onto ELISA fixed with methanol (A, B) or formalin (C, D). Results are shown as a function of antibody-antigen complex absorption (OD_{450}) at the indicated serum dilutions.

DETAILED DESCRIPTION

20 **[0029]** *Definitions*

[0030] In order to provide a clear and consistent understanding of the specification and claims, the following definitions are provided.

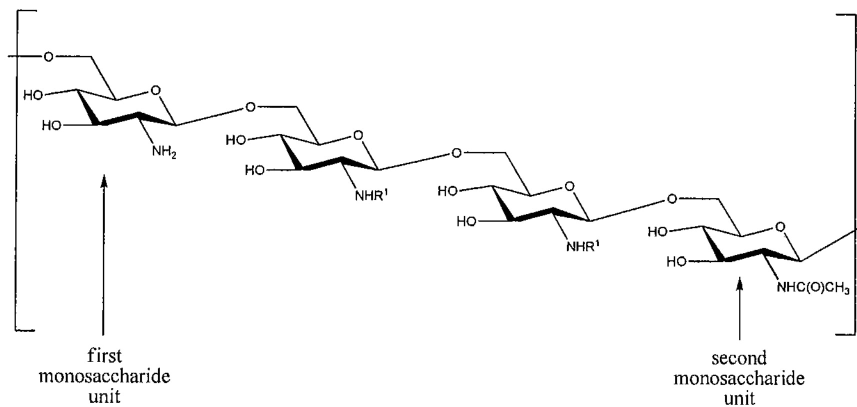
[0031] Units, prefixes, and symbols may be denoted in their SI accepted form. Numeric ranges recited herein are inclusive of the numbers defining the
25 range and include and are supportive of each integer within the defined range. Unless otherwise noted, the terms “a” or “an” are to be construed as meaning “at least one of.” The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. All documents, or portions of documents, cited in this application,
30 including but not limited to patents, patent applications, articles, books, and

treatises, are hereby expressly incorporated by reference in their entirety for any purpose.

[0032] As used herein, "oligosaccharide" refers to a compound containing two or more monosaccharides. Oligosaccharides are considered to have a reducing end and a non-reducing end, whether or not the monosaccharide at the reducing end is in fact a reducing sugar. In accordance with accepted nomenclature, oligosaccharides are depicted herein with the non-reducing end on the left and the reducing end on the right. All oligosaccharides described herein are described with the name or abbreviation for the non-reducing monosaccharide (e.g., Gal), preceded by the configuration of the glycosidic bond (α or β), the ring bond, the ring position of the reducing monosaccharide involved in the bond, and then the name or abbreviation of the reducing monosaccharide (e.g., GlcNAc). The linkage between two sugars may be expressed, for example, as 2,3, 2→3, or 2-3. Each monosaccharide is a pyranose or furanose.

[0033] As used herein, "monosaccharide" refers to a single sugar residue in an oligosaccharide, including derivatives therefrom. Within the context of an oligosaccharide, an individual monomer unit is a monosaccharide which is (or can be) bound through a hydroxyl group to another monosaccharide.

[0034] As used herein, the term "in a first monosaccharide unit R^1 is H, and in a second monosaccharide unit R^1 or R^2 is $C(O)CH_3$, said second monosaccharide unit is located three monosaccharide units from the first monosaccharide unit " refers to a substitution pattern as illustrated in the following scheme:



[0035] As used herein, “endotoxin-free” refers to an oligosaccharide that does not contain endotoxins or endotoxin components normally present in isolated bacterial carbohydrate structures.

[0036] As used herein, “synthetic” refers to material which is substantially or essentially free from components, such as endotoxins, glycolipids, oligosaccharides, etc., which normally accompany a compound when it is isolated. Typically, synthetic compounds are at least about 90% pure, usually at least about 95%, and preferably at least about 99% pure. Purity can be indicated by a number of means well known in the art. Preferably, purity is measured by HPLC. The identity of the synthetic material can be determined by mass spectroscopy and/or NMR spectroscopy.

[0037] As used herein, the term “carrier” refers to a protein, peptide, lipid, polymer, dendrimer, virosome, virus-like particle (VLP), or combination thereof, which is coupled to the oligosaccharide to enhance the immunogenicity of the resulting oligosaccharide-carrier conjugate to a greater degree than the oligosaccharide alone.

[0038] As used herein, “protein carrier” refers to a protein, peptide or fragment thereof, which is coupled or conjugated to an oligosaccharide to enhance the immunogenicity of the resulting oligosaccharide-protein carrier conjugate to a greater degree than the oligosaccharide alone. For example, when used as a carrier, the protein carrier may serve as a T-dependent antigen which can activate and recruit T-cells and thereby augment T-cell dependent antibody production.

[0039] As used herein, "conjugated" refers to a chemical linkage, either covalent or non-covalent, that proximally associates an oligosaccharide with a carrier so that the oligosaccharide conjugate has increased immunogenicity relative to an unconjugated oligosaccharide.

5 **[0040]** As used herein, "conjugate" refers to an oligosaccharide chemically coupled to a carrier through a linker and/or a cross-linking agent.

[0041] As used herein, "passive immunity" refers to the administration of antibodies to a subject, whereby the antibodies are produced in a different subject (including subjects of the same and different species) such that the

10 antibodies attach to the surface of the bacteria and cause the bacteria to be phagocytosed or killed.

[0042] As used herein, "protective immunity" means that a vaccine or immunization schedule that is administered to a animal induces an immune response that prevents, retards the development of, or reduces the severity of

15 a disease that is caused by a pathogen or diminishes or altogether eliminates the symptoms of the disease. Protective immunity may be predicted based on the ability of serum antibody to activate complement-mediated bactericidal activity or confer passive protection against a bacterial infection in a suitable animal challenge model.

20 **[0043]** As used herein, "immunoprotective composition" refers to a composition formulated to provide protective immunity in a host.

[0044] As used herein, "in a sufficient amount to elicit an immune response" (e.g., to epitopes present in a preparation) means that there is a detectable difference between an immune response indicator measured

25 before and after administration of a particular antigen preparation. Immune response indicators include but are not limited to: antibody titer or specificity, as detected by an assay such as enzyme-linked immunoassay (ELISA), bactericidal assay (e.g., to detect serum bactericidal antibodies), flow cytometry, immunoprecipitation, Ouchter-Lowry immunodiffusion; binding

30 detection assays of, for example, spot, Western blot or antigen arrays; cytotoxicity assays, and the like.

[0045] As used herein, “antibody” encompasses polyclonal and monoclonal antibody preparations, as well as preparations including hybrid antibodies, altered antibodies, $F(ab')^2$ fragments, F(ab) molecules, Fv fragments, single chain fragment variable displayed on phage (scFv), single
5 domain antibodies, chimeric antibodies, humanized antibodies, and functional fragments thereof which exhibit immunological binding properties of the parent antibody molecule.

[0046] As used herein, “monoclonal antibody” refers to an antibody composition having a homogeneous antibody population. The term is not
10 limited by the manner in which it is made. The term encompasses whole immunoglobulin molecules, as well as Fab molecules, $F(ab')_2$ fragments, Fv fragments, single chain fragment variable displayed on phage (scFv), and other molecules that exhibit immunological binding properties of the parent monoclonal antibody molecule.

15 **[0047]** As used herein, “specifically binds to an antibody” or “specifically immunoreactive with”, when referring to an oligosaccharide, protein or peptide, refers to a binding reaction which is based on and/or is probative of the presence of the antigen in a sample which may also include a heterogeneous population of other molecules. Thus, under designated
20 immunoassay conditions, the specified antibody or antibodies bind(s) to a particular antigen or antigens in a sample and does not bind in a significant amount to other molecules present in the sample. Specific binding to an antibody under such conditions may require an antibody or antiserum that is selected for its specificity for a particular antigen or antigens.

25 **[0048]** As used herein, “antigen” refers to include any substance that may be specifically bound by an antibody molecule.

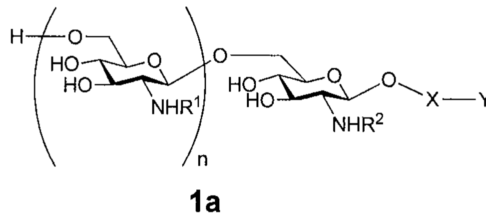
[0049] As used herein, “immunogen” and “immunogenic composition” refer to an antigenic composition capable of initiating lymphocyte activation resulting in an antigen-specific immune response.

30 **[0050]** As used herein, “epitope” refers to a site on an antigen to which specific B cells and/or T cells respond. The term is also used interchangeably

with "antigenic determinant" or "antigenic determinant site." B cell epitope sites on proteins, oligosaccharides, or other biopolymers may be composed of moieties from different parts of the macromolecule that have been brought together by folding. Epitopes of this kind are referred to as conformational or discontinuous epitopes, since the site is composed of segments the polymer that are discontinuous in the linear sequence but are continuous in the folded conformation(s). Epitopes that are composed of single segments of biopolymers or other molecules are termed continuous or linear epitopes. T cell epitopes are generally restricted to linear peptides. Antibodies that recognize the same epitope can be identified in a simple immunoassay showing the ability of one antibody to block the binding of another antibody to a target antigen.

[0051] *Synthetic oligosaccharides*

[0052] The present invention provides a subset of oligosaccharides **1a**:



1a

[0053] where R^1 and R^2 are each independently H or $COCH_3$, where at least one at least one R^1 or R^2 in the oligosaccharide is H and at least another is $COCH_3$; n is from 5-11, X is a bond or a linker, and Y is H or a carrier; and where each occurrence of R^1 can be the same or different. The subset is described below in Table A.

[0054] To design such dPNAG antigens many factors were considered, leading to specific antigen design criteria as summarized below:

1. approximately 50% or less of the GlcN residues should be N-acetylated (for odd-numbered sequences a ratio of (length +1)/2 N-acetyl groups were allowed)

2. de-N-acetylated groups spaced by two intervening residues were prohibited;

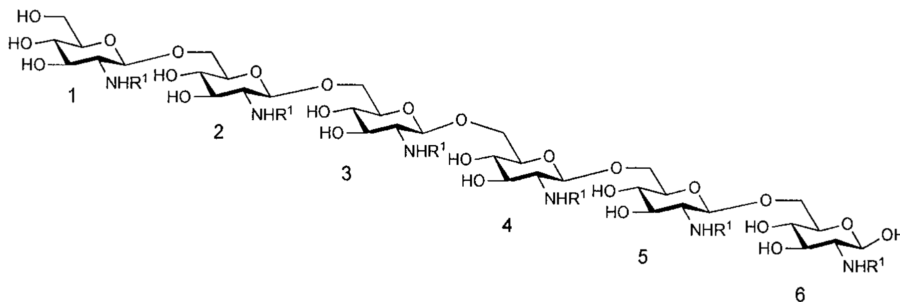
3. No more than two de-N-acetyl containing residues may be present in positions complementary to the CP ManAcA residues in an entire sequence;

4. conjugates containing PNAG/dPNAG antigens as small as five or six monosaccharide units in length are immunogenic, and can be immunoprotective. While the maximum allowable size has not been established, *in vivo* immunoprotection using conjugates containing dPNAG antigens of 12 units in length have been shown to be protective by the present inventors (see below).

5. sequences containing more than three GlcNAc (N-acetyl containing) units in a row were prohibited.

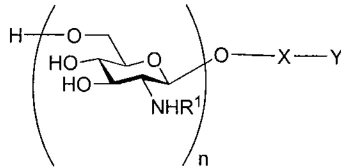
[0055] Specific embodiments of the present invention are shown below and include hexasaccharides (n=6), heptasaccharides (n=7), octasaccharides (n=8), nonasaccharides (n=9), decasaccharides (n=10), undecasaccharides (n=11), and dodecasaccharides (n=12).

[0056] In the following Table A, each monomeric unit in an oligosaccharide of n units is numbered, with position 1 being the non-reducing end. For example, the various positions in a hexasaccharide are shown below:



[0057] The 130 resulting sequences that meet the design criteria above are shown in the table below. Key: 1 = R^1 is acetyl (Ac), 0 = R^1 is H.

[0058] Thus, oligosaccharides are provided of the formula **1b**



1b

wherein X is a bond or a linker, Y is H or a carrier, n is the number of monomers (m) in an oligosaccharide and is from 6 to 12, and each R^1 in the oligosaccharide is either acetyl or H;

wherein the oligosaccharide has monomers with R^1 in position m selected from Table A, wherein when R^1 is 1 in the table R^1=acetyl and when R^1 is 0 in the table R^1=H, and wherein the monomer (m) in position 1 is the non-reducing end of the oligosaccharide. The oligosaccharides may be homogeneous and/or synthetic.

[0059] **Table A**

Position	1	2	3	4	5	6	7	8	9	10	11	12
6-MERS	0	0	0	1	1	1						
	0	0	1	1	1	0						
	0	1	0	1	0	1						
	0	1	1	1	0	0						
	1	0	0	0	1	1						
	1	0	1	0	1	0						
	1	1	0	0	0	1						
	1	1	1	0	0	0						
7-MERS	0	0	0	1	1	1	0					
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	1	1	1	0	0	0	1			
8-MERS	0	0	0	1	1	1	0	0		
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12-MERS	0	0	0	1	1	1	0	0	0	1	1	1
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	1	1	1	0	0	0	1	1	1	0	0	0

[0060] Suitable linkers comprise at one end a grouping able to enter into a covalent bonding with a reactive functional group of the carrier, e.g. an amino, thiol, or carboxyl group, and at the other end a grouping likewise able
5 to enter into a covalent bonding with a hydroxyl group of an oligosaccharide according to the present invention. Between the two functional groups of the linker molecule there is a biocompatible bridging molecule of suitable length, e.g. substituted or unsubstituted heteroalkylene, arylalkylene, alkylene, alkenylene, or (oligo)alkylene glycol groups. Linkers preferably include
10 substituted or unsubstituted alkylene or alkenylene groups containing 1-10 carbon atoms.

[0061] Linkers able to react with thiol groups on the carrier are, for example, maleimide and carboxyl groups; preferred groupings able to react with aldehyde or carboxyl groups are, for example, amino or thiol groups.
15 Preferred covalent attachments between linkers and carriers include thioethers from reaction of a thiol with an α-halo carbonyl or α-halo nitrile, including reactions of thiols with maleimide; hydrazides from reaction of a hydrazide or hydrazine with an activated carbonyl group (e.g. activated NHS-ester or acid halide); triazoles from reaction of an azide with an alkyne (e.g.
20 via "click chemistry"); and oximes from reaction of a hydroxylamine and an aldehyde or ketone as disclosed, for example, in Lees et al., *Vaccine*, 24:716, 2006. Although amine-based conjugation chemistries could be used in principle for coupling linkers and/or spacers to the oligosaccharides described herein, these approaches would typically sacrifice uniformity inasmuch as the

oligosaccharides of the present invention typically contain a plurality of amines bonded to second carbon of the respective monosaccharide units.

[0062] Further suitable linker molecules are known to skilled workers and commercially available or can be designed as required and depending on the functional groups present and can be prepared by known methods.

[0063] Suitable carriers are known in the art (See e.g., Remington's Pharmaceutical Sciences (18th ed., Mack Easton, PA (1990)) and may include, for example, proteins, peptides, lipids, polymers, dendrimers, virosomes, virus-like particles (VLPs), or combinations thereof, which by themselves may not display particular antigenic properties, but can support immunogenic reaction of a host to the oligosaccharides of the present invention (antigens) displayed at the surface of the carrier(s).

[0064] Preferably, the carrier is a protein carrier, including but are not limited to, bacterial toxoids, toxins, exotoxins, and nontoxic derivatives thereof, such as tetanus toxoid, tetanus toxin Fragment C, diphtheria toxoid, CRM (a nontoxic diphtheria toxin mutant) such as CRM 197, cholera toxoid, *Staphylococcus aureus* exotoxins or toxoids, *Escherichia coli* heat labile enterotoxin, *Pseudomonas aeruginosa* exotoxin A, including recombinantly produced, genetically detoxified variants thereof; bacterial outer membrane proteins, such as *Neisseria meningitidis* serotype B outer membrane protein complex (OMPC), outer membrane class 3 porin (rPorB) and other porins; keyhole limpet hemocyanin (KLH), hepatitis B virus core protein, thyroglobulin, albumins, such as bovine serum albumin (BSA), human serum albumin (HSA), and ovalbumin; pneumococcal surface protein A (PspA), pneumococcal adhesin protein (PsaA); purified protein derivative of tuberculin (PPD); transferrin binding proteins, polyamino acids, such as poly(lysine:glutamic acid); peptidyl agonists of TLR-5 (e.g. flagellin of motile bacteria like Listeria); and derivatives and/or combinations of the above carriers. Preferred carriers for use in humans include tetanus toxoid, CRM 197, and OMPC.

[0065] Depending on the type of bonding between the linker and the carrier, and the structural nature of the carrier and oligosaccharide, a carrier may display on average, for example, 1 to 500, 1 to 100, 1 to 20, or 3 to 9 oligosaccharide units on its surface.

5 **[0066]** Methods for attaching an oligosaccharide to a carrier, such as a carrier protein are conventional, and a skilled practitioner can create conjugates in accordance with the present invention using conventional methods. Guidance is also available in various disclosures, including, for example, U.S. Pat. Nos. 4,356,170; 4,619,828; 5,153,312; 5,422,427; and

10 5,445,817; and in various print and online Pierce protein cross-linking guides and catalogs (Thermo Fisher, Rockford, IL).

[0067] In one embodiment, the carbohydrate antigens of the present invention are conjugated to CRM 197, a commercially available protein carrier used in a number of FDA approved vaccines. CRM-conjugates have the

15 advantage of being easier to synthesize, purify and characterize than other FDA approved carriers such as OMPC. Carbohydrate antigens may be conjugated to CRM via thiol-bromoacetyl conjugation chemistry. CRM activation may be achieved by reacting the lysine side chains with the NHS ester of bromoacetic acid using standard conditions as previously described in

20 U.S. Pat. Appl. Publ. 2007-0134762, the disclosures of which are incorporated by reference herein. CRM may be functionalized with 10-20 bromoacetyl groups per protein (n=10-20) prior to conjugation. Conjugation may be performed at pH=9 to avoid aggregation of CRM. Careful monitoring of pH must be employed to ensure complete CRM reaction with NHS-bromoacetate

25 while minimizing background hydrolysis of CRM. Activated CRM may be purified by size exclusion chromatography prior to conjugation. Antigen-CRM conjugates may be synthesized by reacting thiol-terminated carbohydrate antigens with bromoacetamide-activated CRM.

[0068] CRM conjugates may be purified via size exclusion

30 chromatography to remove and recover any unreacted carbohydrate. MBTH (specific for GlcNAc residues) and Bradford assays may be used to determine

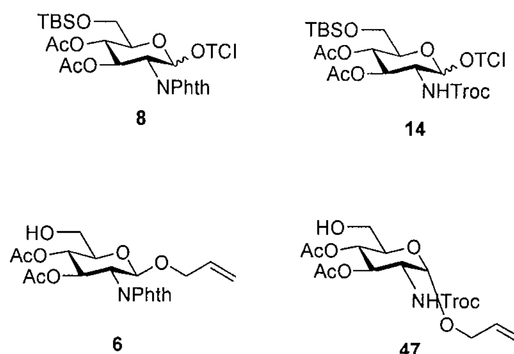
carbohydrate:protein ratio and protein content, respectively, as previously described (Manzi et al., Curr. Prot. Mol. Biol., section 17.9.1 (Suppl. 32), 1995. In preferred embodiments, a minimum carbohydrate content of about 15% by weight for each conjugate may be generated. Typically, a conjugate
5 may include about 3-20 antigens per protein carrier.

[0069] In another embodiment, carbohydrate antigens may be conjugated to one or more carriers suitable for development of diagnostic assays, including ELISAs and microarrays. Exemplary carriers for use in such assays include bovine serum albumin (BSA), keyhole limpet
10 hemocyanine (KLH), biotin, a label, a glass slide or a gold surface. By way of example, synthetic carbohydrate antigens may be conjugated to BSA by a thiol-maleimide coupling procedure (FIG. 5B). Maleimide-BSA contains 15-20 maleimide groups per protein (n=15-20). Accordingly, oligosaccharide antigens may be conjugated to maleimide functionalized BSA, whereby a 20-
15 fold molar excess of the antigen is reacted with commercially available Imject maleimide BSA (Pierce) in maleimide conjugation buffer (Pierce). Conjugation may be performed at pH=7.2 to avoid hydrolysis of the maleimide group during conjugation.

[0070] BSA conjugates may be purified via size exclusion
20 chromatography to remove and recover any unreacted carbohydrate. Characterization via MBTH and Bradford assays may be performed along with MALDI-MS to provide information on the carbohydrate content and valency of the conjugates. In preferred embodiments, conjugates will contain a minimum carbohydrate content of about 10% by weight per BSA conjugate and >8
25 antigen copies per conjugate.

[0071] *Methods for Synthesizing Oligo-β-(1-6)-Glucosamine Structures*

[0072] In another aspect, the invention provides a method for assembling mixed sequence oligo-β-(1→6)-glucosamine structures **1** from four building blocks **6**, **8**, **14**, and **47**. The four building blocks include donor building blocks
30 **8** and **14**, and acceptor building blocks **6** and **47** below.



[0073] Building blocks **6** and **8** further contain an –NPhth group for selective amine group protection of individual monosaccharide units. Building blocks **14** and **47** contain a protective -NHTroc group for selective protection and subsequent acetylation of individual monosaccharide units.

[0074] Acceptor building blocks **6** and **47** have a linker precursor incorporated at the reducing end, which may be reacted with thioacetic acid and deblocked to form a conjugation-ready thiol for conjugation to a carrier as further described above. For example, when incorporated into an oligosaccharide of the present invention, the -O-CH-CH_2=CH_2 group at the reducing end may be converted into a linker comprising the sequence, -O-$(CH_2)_3$-SH as further described in FIGs. 5B, 6B, and 7.

[0075] The four monosaccharide building blocks **6**, **8**, **14**, and **47** may be synthesized from a single monosaccharide **1** as shown in FIG. 3.

[0076] In a further aspect, the invention provides mixed disaccharide building blocks which can be used in combination with other monosaccharide- or disaccharide building blocks to form higher-order dPNAG/PNAG structures. In FIG. 4A, monosaccharide donor **14** is reacted with monosaccharide acceptor **6** to form a mixed disaccharide building block **15** in which the first monosaccharide unit is protected for selective acetylation and the amine in the second monosaccharide unit is selectively protected. In FIG. 4B, monosaccharide donor **8** is reacted with monosaccharide acceptor **47** to form a mixed disaccharide building block **49** in which the amine in the first monosaccharide unit is selectively protected and the second monosaccharide unit position is protected for selective acetylation.

[0077] In FIG. 4A, disaccharide building block **15** can be converted into a disaccharide donor **17** or a disaccharide acceptor **48** for further couplings to other acceptors or donors, respectively. Likewise, in FIG. 4B, disaccharide building block **49** can be converted into a disaccharide donor **51** or a disaccharide acceptor **52** for further couplings to other acceptors or donors, as well. For example, disaccharide donors **17** and **51** can be coupled with either of the monosaccharide acceptors **6** and **47** to form trisaccharides, or they can be coupled with disaccharide acceptors **48** and **52** to form tetrasaccharides.

[0078] In another aspect, the present invention provides disaccharide blocks for forming consecutive acetylated residues or consecutive non-acetylated resides. In FIG. 4C, monosaccharide donor **14** is reacted with monosaccharide acceptor **47** to form a disaccharide building block **53** in which each of the two monosaccharide units is protected for selective acetylation. In FIG. 4D, monosaccharide donor **8** is reacted with monosaccharide acceptor **6** to from a disaccharide building block **18** in which each of the 2-position amines in the two monosaccharide units is protected.

[0079] In FIG. 4C, disaccharide building block **53** can be converted into a disaccharide donor **55** or a disaccharide acceptor **56** for further couplings to other acceptors or donors, respectively. Likewise, in FIG. 4D, disaccharide building block **18** can be converted into a disaccharide donor **20** or a disaccharide acceptor **57** for further couplings to other acceptors or donors, as well. For example, disaccharide donors **20** and **55** can be coupled with either of the monosaccharide acceptors **6** and **47** to form trisaccharides or they can be coupled with any of the above-described disaccharide acceptors **48**, **52**, **56**, or **57** to form tetrasaccharides.

[0080] Any of the above-described donors can be coupled to any complementary acceptor. Accordingly, by coupling the monosaccharide-, disaccharide-, or other higher order donor modules of higher length with complementary monosaccharide-, disaccharide-, or other higher order acceptor modules of higher length, any mixed sequence oligosaccharide of

the present invention can be formed in which the individual acetylation positions and oligosaccharide length are engineered into a given synthesis process in a pre-determined fashion. Compositions and methods for synthesizing exemplary oligosaccharides are described in the Examples
5 below.

[0081] In FIGs. 3-10, syntheses of various oligosaccharides of the present invention proceed by a number of standard operating procedures (SOPs). In another aspect, the present invention provides a number of SOPs (or reaction steps) for synthesizing dPNAG/PNAG oligosaccharides, including
10 SOP 1, removal of 1° TBS group(s); SOP 2, removal of allyl group(s); SOP 3, trichloroacetimidate formation; SOP 4, glycosylation using trichloroacetimidate donors; SOP 5, removal of *N*-Troc group and *in situ N*-acetylation; SOP 6, thiol addition to olefin; and SOP 7, removal of *O*-acetate, *N*-phthaloyl and *S*-acetate groups. SOPs 1-7 are further detailed in the Examples below.
15 **[0082]** In some cases, the above-described protecting groups may be substituted with other protecting groups customarily considered in carbohydrate chemistry, including those mentioned in “Protective Groups in Organic Synthesis”, 3.sup.rd edition, T. W. Greene and P. G. M. Wuts (Ed.), John Wiley and Sons, New York, 1999. By way of example, O-acetate groups
20 may be replaced with O-benzoate groups for producing the antigens.

[0083] *Compositions*

[0084] In another aspect, the present invention provides compositions containing dPNAG/PNAG oligosaccharides **1a** and a pharmaceutically acceptable vehicle. The compositions are preferably immunogenic and
25 immunoprotective.

[0085] The compositions may be homogeneous and/or synthetic, and may contain one or more of the oligosaccharides of formula **1b**/Table A described above (e.g., a homogeneous composition consisting essentially of one of the oligosaccharides of formula **1b**/Table A; a homogeneous
30 composition consisting essentially of two of the oligosaccharides of formula **1b**/Table A; etc.).

[0086] The present invention contemplates the use of single- and multi-valent vaccines comprising any of the synthetic oligosaccharides described herein. The identification of a single oligosaccharide antigen eliciting a protective immune response can facilitate development of a single-antigen vaccine candidate against one or more bacterial target(s) expressing dPNAG/PNAG. Thus, in one embodiment, the compositions may contain a single oligosaccharide **1a**.

[0087] The present invention further contemplates multi-antigen vaccine candidates and vaccines thereof. In one embodiment, the invention provides a composition containing two, three, four or more different oligosaccharides **1a**.

[0088] Suitable vehicles and their formulation, inclusive of other human proteins, e.g., human serum albumin, are described, for example, in Remington's Pharmaceutical Sciences (18th ed., Mack Easton Pa. (1990)). Pharmaceutically acceptable vehicles may include any vehicle that does not itself induce the production of antibodies harmful to the individual receiving the composition. Suitable vehicles may include large, slowly metabolized macromolecules such as proteins, polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers; inactive virus particles, insoluble aluminum compounds, calcium phosphate, liposomes, virosomes, ISCOMS, microparticles, emulsions, and VLPs.

[0089] The compositions of the present invention may further include one or more adjuvants. An oligosaccharide-protein conjugate composition may further include one or more immunogenic adjuvant(s). An immunogenic adjuvant is a compound that, when combined with an antigen, increases the immune response to the antigen as compared to the response induced by the antigen alone so that less antigen can be used to achieve a similar response. For example, an adjuvant may augment humoral immune responses, cell-mediated immune responses, or both.

[0090] Those of skill in the art will appreciate that the terms "adjuvant," and "carrier," can overlap to a significant extent. For example, a substance

which acts as an "adjuvant" may also be a "carrier," and certain other substances normally thought of as "carriers," for example, may also function as an "adjuvant." Accordingly, a substance which may increase the immunogenicity of the synthetic oligosaccharide or carrier associated therewith is a potential adjuvant. As used herein, a carrier is generally used in the context of a more directed site-specific conjugation to an oligosaccharide of the present invention, whereby an adjuvant is generally used in a less specific or more generalized structural association therewith.

[0091] Exemplary adjuvants and/or adjuvant combinations may be selected from the group consisting of mineral salts, including aluminum salts, such as aluminum phosphate and aluminum hydroxide (alum) (e.g., Alhydrogel™, Superfos, Denmark) and calcium phosphate; RIBI, which contains three components extracted from bacteria, monophosphoryl lipid A, trehalose dimycolate, and cell wall skeleton (MPL+TDM+CWS) in a 2% squalene/Tween 80 emulsion, whereby any of the 3 components MPL, TDM or CWS may also be used alone or combined 2 by 2; toll-like receptor (TLR) agonists, including, for example, agonists of TLR-1 (e.g. tri-acyl lipopeptides); agonists of TLR-2 [e.g. peptidoglycan of gram-positive bacteria like streptococci and staphylococci; lipoteichoic acid]; agonists of TLR-3 (e.g. double-stranded RNA and their analogs such as poly 1:C); agonists of TLR-4 (e.g. lipopolysaccharide (endotoxin) of gram-negative bacteria like Salmonella and E. coli); agonists of TLR-5 (e.g. flagellin of motile bacteria like Listeria); agonists of TLR-6 (e.g. with TLR-2 peptidoglycan and certain lipids (diacyl lipopeptides)); agonists of TLR-7 (e.g. single-stranded RNA (ssRNA) genomes of such viruses as influenza, measles, and mumps; and small synthetic guanosine-base antiviral molecules like loxoribine and ssRNA and their analogs); agonists of TLR-8 (e.g. binds ssRNA); agonists of TLR-9 (e.g. unmethylated CpG of the DNA of the pathogen and their analogs; agonists of TLR-10 (function not defined) and TLR-11-(e.g. binds proteins expressed by several infectious protozoans (Apicomplexa), specific toll-like receptor agonists include monophosphoryl lipid A (MPL®), 3 De-O-acylated monophosphoryl lipid A (3 D-MPL), OM-174 (E. coli lipid A derivative); OM

triacyl lipid A derivative, and other MPL- or lipid A-based formulations and combinations thereof, including MPL®-SE, RC-529 (Dynavax Technologies), AS01 (liposomes+MPL+QS21), AS02 (oil-in-water PL + QS-21), and AS04 (Alum + MPL)(GlaxoSmith Kline, Pa.), CpG-oligodeoxynucleotides (ODNs)
5 containing immunostimulatory CpG motifs, double-stranded RNA, polyinosinic:polycytidylic acid (poly I:C), and other oligonucleotides or polynucleotides optionally encapsulated in liposomes; oil-in-water emulsions, including AS03 (GlaxoSmith Kline, Pa.), MF-59 (microfluidized detergent stabilized squalene oil-in-water emulsion; Novartis), and Montanide ISA-51
10 VG (stabilized water-in-oil emulsion) and Montanide ISA-720 (stabilized water/squalene; Seppic Pharmaceuticals, Fairfield, NJ); cholera toxin B subunit; saponins, such as Quil A or QS21, an HPLC purified non-toxic fraction derived from the bark of Quillaja Saponaria Molina (STIMULON™ (Antigenics, Inc., Lexington, Mass.) and saponin-based adjuvants, including
15 immunostimulating complexes (ISCOMs; structured complex of saponins and lipids) and other ISCOM-based adjuvants, such as ISCOMATRIX™ and AbISCO®-100 and -300 series adjuvants (Isconova AB, Uppsala, Sweden); QS21 and 3 D-MPL together with an oil in water emulsion as disclosed in U.S. Pat. Appl. No. 2006/0073171; stearyl tyrosine (ST) and amide analogs
20 thereof; virus-like particles (VLPs) and reconstituted influenza virosomes (IRIVs); complete Freund's adjuvant (CFA); incomplete Freund's adjuvant (IFA); E. coli heat-labile enterotoxin (LT); immune-adjuvants, including cytokines, such as IL-2, IL-12, GM-CSF, Flt3, accessory molecules, such as B7.1, and mast cell (MC) activators, such as mast cell activator compound
25 48/80 (C48/80); water-insoluble inorganic salts; liposomes, including those made from DNPC/Chol and DC Chol; micelles; squalene; squalane; muramyl dipeptides, such as N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP) as found in U.S. Pat. No. 4,606,918, N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), and N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-
30 alanine-2-(1'2'-dipalmitoyl-n-glycero-3-hydroxyphosphoryl; SAF-1 (Syntex); AS05 (GlaxoSmith Kline, Pa.); and combinations thereof.

[0092] In preferred embodiments, adjuvant potency may be enhanced by combining multiple adjuvants as described above, including combining various delivery systems with immunopotentiating substances to form multi-component adjuvants with the potential to act synergistically to enhance antigen-specific immune responses *in vivo*. Exemplary immunopotentiating substances include the above-described adjuvants, including, for example, MPL and synthetic derivatives, MDP and derivatives, oligonucleotides (CpG etc), double-stranded RNAs (ds RNAs), alternative pathogen-associated molecular patterns (PAMPs)(*E. coli* heat labile enterotoxin; flagellin, saponins (QS-21 etc), small molecule immune potentiators (SMIPs, e.g., resiquimod [R848]), cytokines, and chemokines.

[0093] *Methods of Treating or Preventing Staphyloccus infections*

[0094] *Oligosaccharide compositions*

[0095] In one embodiment, the present invention provides pharmaceutically acceptable immunogenic or immunoprotective oligosaccharide compositions and their use in methods for preventing Staphylococcus infection in a patient in need thereof. In one embodiment, comprising administering an effective amount of an oligosaccharide of the present invention. An immunogenic or immunoprotective composition will include a "sufficient amount" or "an immunologically effective amount" of a dPNAG/PNAG -protein conjugate according to the present invention, as well as any of the above mentioned components, for purposes of generating an immune response or providing protective immunity, as further defined herein. As mentioned above, the compositions may be homogeneous and the oligosaccharides may be synthetic.

[0096] Administration of the oligosaccharide- or oligosaccharide conjugate compositions or antibodies, as described herein may be carried out by any suitable means, including by parenteral administration (e.g., intravenously, subcutaneously, intradermally, or intramuscularly); by topical administration, of for example, antibodies to an airway surface; by oral

administration; by in ovo injection in birds, for example, and the like. Preferably, they are administered intramuscularly.

[0097] Typically, the compositions may be prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection. An aqueous composition for parenteral administration, for example, may include a solution of the immunogenic component(s) dissolved or suspended in a pharmaceutically acceptable vehicle or diluent, preferably a primarily aqueous vehicle. An aqueous composition may be formulated as a sterile, pyrogen-free buffered saline or phosphate-containing solution, which may include a preservative or may be preservative free. Suitable preservatives include benzyl alcohol, parabens, thimerosal, chlorobutanol, and benzalkonium chloride, for example. Aqueous solutions are preferably approximately isotonic, and its tonicity may be adjusted with agents such as sodium tartrate, sodium chloride, propylene glycol, and sodium phosphate. Additionally, auxiliary substances required to approximate physiological conditions, including pH adjusting and buffering agents, tonicity adjusting agents, wetting or emulsifying agents, pH buffering substances, and the like, including sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, sorbitan monolaurate, triethanolamine oleate, etc. may be included with the vehicles described herein.

[0098] Compositions may be formulated in a solid or liquid form for oral delivery. For solid compositions, nontoxic and/or pharmaceutically acceptable solid vehicles may include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like. For oral administration, a pharmaceutically acceptable nontoxic composition may be formed by incorporating any of the normally employed excipients, including those vehicles previously listed, and a unit dosage of an active ingredient, that is, one or more compounds of the invention, whether conjugated to a carrier or not. Topical application of antibodies to an airway surface can be carried out by intranasal administration (e.g., by use of dropper, swab, or inhaler

which deposits a pharmaceutical formulation intranasally). Topical application of the antibodies to an airway surface can also be carried out by inhalation administration, such as by creating respirable particles of a pharmaceutical formulation (including both solid particles and liquid particles) containing the antibodies as an aerosol suspension, and then causing the subject to inhale the respirable particles. Methods and apparatuses for administering respirable particles of pharmaceutical formulations are well known, and any conventional technique can be employed. Oral administration may be in the form of an ingestable liquid or solid formulation.

[0099] The preparation of such pharmaceutical compositions is within the ordinary skill in the art, and may be guided by standard reference books such as Remington the Science and Practice of Pharmacy, Lippincott Williams & Wilkins; 21 ed., May 1, 2005, which is incorporated herein by reference.

[00100] The concentration of the oligosaccharides of the invention in the pharmaceutical formulations can vary widely, i.e., from less than about 0.1%, usually at or at least about 0.1% to as much as 20% to 50% or more by weight, and may be selected on the basis of fluid volumes, viscosities, stability, etc., and/or in accordance with the particular mode of administration selected. A human unit dose form of the compounds and composition is typically included in a pharmaceutical composition that comprises a human unit dose of an acceptable vehicle, preferably an aqueous vehicle, and is administered in a volume of fluid that is known by those of skill in the art to be used for administration of such compositions to humans, and is adjusted according to commonly understood principles for a particular subject to be treated. Thus in one embodiment, the invention provides a unit dosage of the vaccine components of the invention in a suitable amount of an aqueous solution, such as 0.1-3 ml, preferably 0.2-2 mL.

[00101] The compositions of the present invention may be administered to any animal species at risk for developing an infection by a microbial species expressing a PNAG and/or PNAG antigen.

[00102] The present invention can also be used to treat or prevent other bacteria infections where the bacterium is known or suspected to express PNAG or dPNAG. Suitable bacteria that can be treated with the present invention include Staphylococcus species, such as *S. aureus* and *S. epidermidis*; *Escherichia coli*; *Yersinia* species (spp.), such as *Y. pestis*, *Y. pseudotuberculosis, and Y. entercolitica*; *Bordetella* spp., including *B. pertussis*, *B. bronchiseptica*, and *B. parapertussis; Aggregatibacter actinomycetemcomitans*, *Actinobacillus pleuropneumoniae*; *Acinetobacter* spp.; *Burkholderia* spp.; *Stenatrophomonas maltophilia* , *Klebsiella* spp., and *Shigella* spp. Accordingly, specific dPNAG/PNAG oligosaccharides may be modified, depending on the specific compositional makeup, including acetylation profiles of these antigens in their respective bacterial species.

[00103] The treatment may be given in a single dose schedule, or preferably a multiple dose schedule in which a primary course of treatment may be with 1-10 separate doses, followed by other doses given at subsequent time intervals required to maintain and or reinforce the response, for example, at 1-4 months for a second dose, and if needed, a subsequent dose(s) after several months. Examples of suitable treatment schedules include: (i) 0, 1 month and 6 months, (ii) 0, 7 days and 1 month, (iii) 0 and 1 month, (iv) 0 and 6 months, or other schedules sufficient to elicit the desired responses expected to reduce disease symptoms, or reduce severity of disease.

[00104] The amounts effective for inducing an immune response or providing protective immunity will depend on a variety of factors, including the oligosaccharide composition, conjugation to a carrier, inclusion and nature of adjuvant(s), the manner of administration, the weight and general state of health of the patient, and the judgment of the prescribing physician. By way of example, the amounts may generally range for the initial immunization (that is for a prophylactic administration) from about 1.0 μg to about 5,000 μg of oligosaccharide for a 70 kg patient, (e.g., 1.0 μg, 2.0 μg, 2.5 μg, 3.0 μg, 3.5 μg, 4.0 μg, 4.5 μg, 5.0 μg, 7.5 μg, 10 μg, 12.5 μg, 15 μg, 17.5 μg, 20 μg, 25 μg, 30 μg, 35 μg, 40 μg, 45 μg, 50 μg, 75 μg, 100 μg, 250 μg, 500 μg, 750 μg,

1,000 μg, 1,500 μg, 2,000 μg, 2,500 μg, 3,000 μg, 3,500 μg, 4,000 μg, 4,500 μg or 5,000 μg). The actual dose administered to a subject is often, but not necessarily, determined according to an appropriate amount per kg of the subject's body weight. For example, an effective amount may be about 0.1 μg to 5 μg/kg body weight.

[00105] A primary dose may optionally be followed by boosting dosages of from about 1.0 to about 1,000 of peptide (e.g., 1.0 μg, 2.0 μg, 2.5 μg, 3.0 μg, 3.5 μg, 4.0 μg, 4.5 μg, 5.0 μg, 7.5 μg, 10 μg, 12.5 μg, 15 μg, 17.5 μg, 20 μg, 25 μg, 30 μg, 35 μg, 40 μg, 45 μg, 50 μg, 75 μg, 100 μg, 250 μg, 500 μg, 750 μg, 1,000 μg, 1,500 μg, 2,000 μg, 2,500 μg, 3,000 μg, 3,500 μg, 4,000 μg, 4,500 μg or 5,000 μg) pursuant to a boosting regimen over weeks to months depending upon the patient's response and condition by measuring specific T cell activity in the patient's blood.

[00106] The immunogenic compositions comprising a compound of the invention may be suitable for use in adult humans or in children, including young children or others at risk for contracting an infection caused by a dPNAG/PNAG-expressing bacterial species. Optionally such a composition may be administered in combination with other pharmaceutically active substances, and frequently it will be administered in combination with other vaccines as part of a childhood vaccination program.

[00107] *Antibody Compositions*

[00108] In another embodiment, the invention provides an antibody preparation against one or more oligo-β-(1→6)-glucosamine **1a** in accordance with the present invention. The antibody preparation may include any member from the group consisting of polyclonal antibody, monoclonal antibody, mouse monoclonal IgG antibody, humanized antibody, chimeric antibody, fragment thereof, or combination thereof.

[00109] Pharmaceutical antibody compositions may be used in a method for providing passive immunity against a bacterial target species of interest, including *S. aureus* and other dPNAG/PNAG-expressing bacteria. A pharmaceutical antibody composition may be administered to an animal

subject, preferably a human, in an amount sufficient to prevent or attenuate the severity, extent of duration of the infection by the bacterial target species of interest.

[00110] The administration of the antibody may be either prophylactic (prior to anticipated exposure to a bacterial infection) or therapeutic (after the initiation of the infection, at or shortly after the onset of the symptoms). The dosage of the antibodies will vary depending upon factors as the subject's age, weight and species. In general, the dosage of the antibody may be in a range from about 1-10 mg/kg body weight. In a preferred embodiment, the antibody is a humanized antibody of the IgG or the IgA class. The route of administration of the antibody may be oral or systemic, for example, subcutaneous, intramuscular or intravenous.

[00111] _Antibodies in diagnostic assays_

[00112] In a further aspect, the present invention provides compositions and methods for inducing production of antibodies for diagnosing, treating, and/or preventing one or more infections caused by dPNAG/PNAG expressing bacteria.

[00113] Antisera to dPNAG/PNAG conjugates may be generated in New Zealand white rabbits by 3-4 subcutaneous injections over 13 weeks. A pre-immune bleed may generate about 5 mL of baseline serum from each rabbit. A prime injection (10 μg antigen equivalent) may be administered as an emulsion in complete Freund's adjuvant (CFA). Subsequent injections (5 μg antigen equivalent) may be given at three week intervals in incomplete Freund's adjuvant (IFA). Rabbits may be bled every two weeks commencing one week after the third immunization. Approximately 25 – 30 mL of serum per rabbit may be generated from each bleeding event and frozen at -80°C. Serum may be analyzed by ELISA against the corresponding dPNAG/PNAG conjugate as described below. In addition, antisera from later bleeds may be affinity purified as further described below.

[00114] The oligosaccharides and antibodies generated therefrom can be used as diagnostic reagents for detecting dPNAG-PNAG structures or

antibodies thereagainst, which are present in biological samples. The detection reagents may be used in a variety of immunodiagnostic techniques, known to those of skill in the art, including ELISA- and microarray-related technologies. In addition, these reagents may be used to evaluate antibody
5 responses, including serum antibody levels, to immunogenic oligosaccharide conjugates. The assay methodologies of the invention typically involve the use of labels such as fluorescent, chemiluminescent, radioactive, enzymatic labels or dye molecules, and/or secondary immunologic reagents for direct or indirect detection of a complex between an antigen or antibody in a biological
10 sample and a corresponding antibody or antigen bound to a solid support.

[00115] Such assays typically involve separation of unbound antibody in a liquid phase from a solid phase support to which antibody-antigen complexes are bound. Solid supports which can be used in the practice of the invention include substrates such as nitrocellulose (e.g., in membrane or microtiter well
15 form); polyvinylchloride (e.g., sheets or microtiter wells); polystyrene latex (e.g., beads or microtiter plates); polyvinylidine fluoride; diazotized paper; nylon membranes; activated beads, magnetically responsive beads, and the like.

[00116] Typically, a solid support is first reacted with a first binding
20 component (e.g., an anti- dPNAG-PNAG antibody or dPNAG-PNAG oligosaccharide) under suitable binding conditions such that the first binding component is sufficiently immobilized to the support. In some cases, mobilization to the support can be enhanced by first coupling the antibody or oligosaccharide to a protein with better binding properties, or that provides for
25 immobilization of the antibody or antigen on the support without significant loss of antibody binding activity or specificity. Suitable coupling proteins include, but are not limited to, macromolecules such as serum albumins including bovine serum albumin (BSA), keyhole limpet hemocyanin (KLH), immunoglobulin molecules, thyroglobulin, ovalbumin, and other proteins well
30 known to those skilled in the art. Other molecules that can be used to bind antibodies the support include polysaccharides, polylactic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, and the like. Such

molecules and methods of coupling these molecules are well known to those of ordinary skill in the art and are described in, for example, U.S. Pat. No. 7,595,307, U.S. Pat. Appl. No. US 2009/0155299, the disclosures and cited references therein of which are incorporated by reference herein.

5 **[00117]** The following examples are included for purposes of illustration and are not intended to limit the scope of the invention.

EXAMPLES

[00118] *Example 1 - Generation of dPNAG/PNAG test libraries*

[00119] The above-described monosaccharide- and disaccharide building
10 blocks were used in higher antigen assembly to initially provide a route for forming three large, defined poly-glucosamine structures. Thus, an initial library was generated from three basic hexamer units or "analog cores" (FIG. 2B) corresponding to: (1) a fully acetylated N-acetyl PNAG (ANC-PNAG; exemplified by thiol oligosaccharide **58**), (2) a partially acetylated (16.7%)
15 PNAG (ANC-dPNAG exemplified by thiol oligosaccharide **37**) and (3) a fully non-acetylated PNAG (ANC-PNG; exemplified by thiol oligosaccharide **61**).

[00120] Molecules generated with the first analog core in the initial PNAG library represented by **58**, **59**, **60** can serve as a control group and to mimic functionally the major secreted component of staphylococcal biofilm.

20 **[00121]** The second analog core containing a partially acetylated PNAG molecule exemplified by oligosaccharides **37**, **40**, **34**, and conjugates thereof (see FIG. 2B) represents one possible product of chemical or *ica* B-mediated deacetylation (16.7% *N*-acetyl), which was shown by the semi-synthetically prepared version to confer opsonic and immunoprotective capability. The
25 synthetic antigens herein are structurally defined molecules wherein the *N*-acetyl groups are regularly spaced every 6 glucosamine units. A significant portion of the multiple free amine groups in these antigens are likely to be protonated at physiologic pH and will have a major impact on the physical properties, such as tertiary structure and solubility.

30 **[00122]** The third analog core exemplifying a fully deacetylated PNAG molecule as represented by **61**, **62**, **63** in FIG. 2B. The third analog core

presents a three-dimensional structure or tertiary structure distinguished from naturally derived deacetylated PNG structures and is similar to synthetic PNAGs recently described (Gening et al., *Infect. Immun.*, 78:764, 2010, epub 11/30/2009).

5 **[00123]** Key building blocks were divided into two batches; in each case, one was converted into an acceptor, the other into a donor. Coupling of the donor and acceptor units provided a key monosaccharide or disaccharide units for use in higher antigen assembly so as to provide an efficient route to large, defined poly-glucosamine structures. To produce the mixed-*N*-acetyl
10 sequences (Ag 4-6), an *N*-Troc protective group was employed to facilitate selective replacement with an *N*-acetyl. This chemistry was highly selective and produced a set of three mixed *N*-acetyl sequences (6, 12, and 18-mer) with one, two and three *N*-acetyl groups respectively. This corresponds to 16.7% incorporation of *N*-acetyl, similar to the average degree of *N*-
15 acetylation found in the most active naturally-derived heterogeneous materials (Maira-Litran et al., *Infect. Immun.*, 73:6752, 2005).

[00124] Each protected antigen was reacted with thioacetic acid to install a thioacetate at the reducing end as a conjugation site (*See* Scheme 2 of Buskas et al., J. Org. Chem., 65:958, 2000). Removal of the protecting
20 groups provided two sets of compounds, the 100% poly-NH_2 sequences (by **61**, **62**, **63**) and the 16.7% *N*-acetyl substituted structures (thiol oligosaccharides **37**, **40**, **34**). Reaction of a portion of the 100% poly-NH_2 sequences with acetic anhydride under aqueous conditions provided the 100% poly-*N*-acetyl sequences (**58**, **59**, **60**). All synthetic antigens were
25 purified via size exclusion chromatography (BioGel P2 or P4) and fully characterized by ^{1}H-NMR, ^{13}C-NMR and mass spectroscopy.

[00125] The PNAG-based library set contains 9 structures comprised of molecules varying by the 3 core unit analogs used and by 3 different molecule lengths (FIG. 2B). Complete analytical characterization (NMR, MS, HPLC,
30 elemental analysis) demonstrated that each molecule was over 98% pure compound.

[00126] *Example 2 - Standard Operating Procedure (SOP) 1: Removal of 1° TBS group*

[00127] To a solution of the starting tert-butyldimethylsilyl (TBS) containing material (35 mmol) in CH_3CN (500 mL) were added H_2O (50 mL) over 10 minutes. Scandium trifluoromethanesulfonate trihydrate ($Sc(OTf)_3$, 400mg, 0.8 mmol) were added and the reaction mixture was stirred at room temperature for 24 hours. The reaction mixture was then diluted with EtOAc (500 mL) and washed with saturated aqueous $NaHCO_3$ and brine. The organic solution was dried over Na_2SO_4, filtered and concentrated. Purification via silica gel chromatography (EtOAc/Heptanes; 50-100% EtOAc gradient) afforded the desired deprotected product. Typical isolated yields for the product formation varied between 60-92%.

[00128] *Example 3 - SOP 2: Removal of allyl group*

[00129] A solution of 1,5-cyclooctadienebis(methyldiphenylphosphine)iridium(I) hexafluorophosphate (Ir cat.; 0.3 mmol) in THF (50 mL) was purged with hydrogen bubbling until a clear yellow solution remained (~15 minutes). The activated Ir catalyst solution was then purged with nitrogen bubbling for 15 minutes. A solution of the allyl glycoside (10 mmol) in THF (20 mL) was added in one portion to the Ir catalyst solution and the resulting reaction mixture was stirred for 30 minutes. The inert atmosphere was removed and a solution of *N*-methylmorpholine *N*-oxide (NMO, 50% aqueous, 20 mL) was added followed by osmium tetroxide (0.03 mmol). The resulting biphasic reaction mixture was stirred in the dark for 2h, then quenched with 20 mL 1M aqueous $Na_2S_2O_4$. After vigorous stirring for 1h, the organic phase was partitioned, diluted with EtOAc (400 mL) and washed with 1M HCl (aq.), H_2O and brine. The organics were dried over Na_2SO_4, filtered and concentrated. Purification via silica gel chromatography (EtOAc/Heptanes; 50-100% EtOAc gradient) afforded the desired hydroxyl product. Typical isolated yields for the product formation varied between 80-95%.

[00130] *Example 4 - SOP 3: Tricholoracetimidate formation*

[00131] *Formation with DBU.* A solution of the starting sugar (4 mmol) in CH_2Cl_2 (20 mL) was treated with trichloroacetonitrile (5 mL). To the reaction mixture were added 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 0.1 mL, 0.6 mmol) dropwise. The reaction mixture was stirred at room temperature for 1h, then concentrated to a viscous oil. Purification via filtration through a silica gel plug pre-treated with EtOAc containing 0.1% TEA afforded the desired trichloroacetimidate product. Typical isolated yields for the product formation varied between 80-95%.

[00132] *Formation with K_2CO_3.* A solution of the starting sugar (4 mmol) in CH_2Cl_2 (20 mL) was treated with trichloroacetonitrile (5 mL). K_2CO_3 (5g) was added to the reaction mixture and the heterogeneous solution was stirred for 12h. The reaction mixture was filtered through celite, rinsed with CH_2Cl_2 and concentrated *in vacuo*. Purification via filtration through a silica gel plug pre-treated with EtOAc containing 0.1% TEA afforded the desired trichloroacetimidate product. Typical isolated yields for the product formation varied between 80-95%.

[00133] *Example 5 - SOP 4: Glycoslyation using trichloroacetimidate donors*

[00134] Glycosyl trichloroacetimidate (12.0 mmol) and glycosyl acceptor (10.0 mmol) were combined, co-evaporated with toluene (3 x 20 mL) and dried *in vacuo* for 1h. The resulting mixture was dissolved in dry CH_2Cl_2 (50 mL) under nitrogen and the reaction mixture was cooled to -20°C. A solution of trimethylsilyl trifluoromethanesulfonate (TMSOTf, 0.10 M in CH_2Cl_2, 0.12 mL, 1.2 mmol) was added dropwise over 10 minutes and the reaction stirred for an additional 30 minutes. The reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with saturated aqueous $NaHCO_3$ and brine. The organic solution was dried over Na_2SO_4, filtered and concentrated. Purification via silica gel chromatography (EtOAc/Heptanes; 50-100% EtOAc gradient) afforded the desired coupling product. Typical isolated yields for the product formation varied between 60-97%.

[00135] *Example 6 - SOP 5: Removal of N-Troc group and in situ N-acetylation*

[00136] Starting *N*-Troc oligosaccharide (0.22 mmol) was dissolved in THF:Ac_2O:AcOH (8:3:1, v:v:v; 20mL). The reaction mixture was treated with activated Zn (15 mmol) and stirred at room temperature for 1h. The reaction mixture was diluted with EtOAc, filtered through celite and washed with saturated aqueous $NaHCO_3$ and brine. Purification via silica gel chromatography (EtOAc/Heptanes; 50-100% EtOAc gradient) afforded the desired *N*-Acetate product. Typical isolated yields for the product formation varied between 60-90%.

[00137] Zinc activation: Zinc (50g, powdered) was washed with 200 mL each: 2M HCl(aqueous), H_2O, EtOH and THF. The solids were dried *in vacuo* overnight to a constant weight.

[00138] *Example 7 - SOP 6: Thiol addition to olefin*

[00139] Starting allyl glycoside (0.22 mmol) was dissolved in 1,4-dioxane (5 mL). The solution was degassed with nitrogen. Thiol acetic acid (2.2 mmol) and 2,2'-azobis(isobutyronitrile) (0.088 mmol) were added and the reaction mixture was degassed a second time. The reaction was heated to 75°C for 3h, then cooled to room temperature and quenched with cyclohexene (0.2 mL). After concentration, the crude reaction mixture was purified via silica gel chromatography (EtOAc/Heptanes; 50-100% EtOAc gradient) to provide the thiolated reaction product. Typical isolated yields for the product formation varied between 70-95%.

[00140] *Example 8 - SOP7: Removal of O-acetate, N-phthaloyl, and S-acetate groups*

[00141] Starting protected oligosaccharide (0.06 mmol) was dissolved in MeOH (10 mL). Hydrazine hydrate (1 mL) was added and the reaction mixture was heated to 65°C for 3h. White precipitates formed upon heating. After 3h at 65°C, H_2O (5 mL) was added and the reaction mixture was stirred at 65°C for an additional 12h. The reaction mixture was concentrated *in vacuo* and co-evaporated with H_2O (3 x 5 mL). Purification via size exclusion

chromatography (Biogel P-2 Media, 1"x24" column, gravity pressure, H_2O eluent) afforded the desired fully deprotected oligosaccharides as a mixture of thiol-disulfide products. Typical isolated yields for the product formation varied between 60-85%.

5 **[00142]** _Example 9 - Synthesis of monosaccharide building blocks_

[00143] FIG. 3 outlines the reaction scheme for synthesizing the monosaccharide building blocks, which proceeds according to the following steps.

[00144] *Synthesis of building blocks **6** and **8** for selective amine group*
10 *protection of individual monosaccharide units*

[00145] Glucosamine **1** (90 g, 0.23 mol), (prepared as described in Tetrahedron **1997**, *53*, 12, 4159) was dissolved in pyridine (405 mL) and triethylamine (36.5 mL). The solution was stirred for 15 minutes followed by the addition of phthalic anhydride (22 g, 0.15 mmol). The reaction mixture
15 was maintained at room temperature in a water bath for 30 minutes. Triethylamine (40.5 mL) and phthalic anhydride (22 g, 0.15 mmol) were added and stirred at room temperature for an additional 45 minutes. The reaction mixture was heated to 90°C and acetic anhydride (121.5 mL) was added. The reaction mixture was maintained at 90-95°C for 10 minutes followed by
20 concentration *in vacuo* to a thick, yellow syrup. The crude product was dissolved in CH_2Cl_2 (1.0 L) and washed with H_2O (3 x 500 mL). The organic solution was dried over Na_2SO_4, filtered and concentrated to a syrup. The product was recovered via recrystallization from ethanol (300 mL, 190 proof, 0.1% MeOH). Recovered 103 g product **2**, 93% yield. To a solution of **2** (103
25 g, 0.21 mol) in CH_2Cl_2 (700 mL) were added allyl alcohol (62 mL). The reaction mixture was purged with N_2 and cooled to 0°C. $SnCl_4$ (62 mL) was added dropwise over 1h. The reaction mixture was kept at 0°C for 6h then warmed to room temperature and stirred for 48h. The solution was poured into ice water (1L), separated and the organics were washed with 3 x 500 mL
30 H_2O, dried over Na_2SO_4, filtered and concentrated to a syrup. Recovered **3** (100 g) as a yellow oil. A solution of **3** (1.0 g, 2.1 mmol) was dissolved in

MeOH (10mL) and cooled to 0°C. Acetyl chloride (0.75 mL) was added dropwise over 5 minutes. The solution was allowed to warm to room temperature over 2h and stirred for an additional 48h. The reaction mixture was concentrated *in vacuo* to afford **4** (0.7 g) as a white solid. Starting
5 monomer **4** (49.2 g, 0.141 mol) was dissolved in pyridine (160 mL) and cooled to 0°C under N_2. A solution of tert-butyldimethylsilyl chloride (TBSCl, 21.3 g, 0.141 mol) in CH_2Cl_2 (70 mL) was added over 1h and the temperature was maintained at <1°C. The reaction mixture was stirred for an additional 1h at 0°C, then a second portion of TBSCl (2.0g, 0.014 mol) in CH_2Cl_2 (7 mL) was
10 added and stirred at 0°C for 1h. The reaction mixture was warmed to room temperature for 2h and then re-cooled to 0°C. Acetic anhydride (80 mL) was added over 1h at 0°C and the solution was warmed to room temperature overnight. After 12h at rt, the reaction mixture was poured onto ice water (1L) and stirred for 1h. The biphasic solution was extracted with EtOAc in 3
15 portions (1L, then 2 x 250 mL). The combined organics were concentrated and the product recrystallized from hot ethanol (300 mL) to give **5** (66 g, 86% yield) as a white solid. TBS removal was performed as described in **SOP 1** using **5** (32 g, 59 mmol) and $Sc(OTf)_3$ (400mg, 0.8 mmol). Product **6** was formed in 88% yield (22.5 g). Allyl removal was performed as described in
20 **SOP 2** using Ir catalyst (1.0 g, 1.2 mmol), **5** (70 g, 127 mmol), 50% aqueous NMO (100 mL) and OsO_4 (20 mg, 0.08 mmol). Product **7** was formed in 94% yield (61 g). Glycosyl trichloroacetimidate **8** was formed as described in **SOP 3a** using **7** (61 g, 120 mmol), trichloroacetonitrile (30 mL) and DBU (1 mL). Product **8** was formed in 96% yield (75.9 g).

25 **[00146]** *Synthesis of monosaccharide building blocks **14** and **47** for selective protection and subsequent acetylation of individual monosaccharide units*

[00147] Starting sugar **1** (50g, 130 mmol; see FIG. 3) was dissolved in THF (350 mL). With constant stirring, a solution of $NaHCO_3$ (22 g in 170 mL
30 H_2O) was added slowly over 20 minutes. The reaction mixture was stirred for an additional 20 minutes. Troc-Cl (18.5 mL, 60 mmol) was added over 10 minutes followed by a second portion (18.5 mL, 60 mmol). After 1 hour, the

reaction mixture was diluted with H_2O (500 mL) and EtOAc (1L). The organics were partitioned and washed with 2 x 250 mL brine, dried over Na_2SO_4, filtered and concentrated. Product **9** was recovered in quantitative yield (69 g). To a solution of **9** (78 g, 0.15 mol) in CH_2Cl_2 (350 mL) were
5 added allyl alcohol (43 mL). The reaction mixture was purged with N_2 and cooled to 0°C. $SnCl_4$ (43 mL) was added dropwise over 1h. The reaction mixture was kept at 0°C for 6h then warmed to room temperature and stirred for 24h. The solution was poured into ice water (1L), separated and the organics were washed with 3 x 500 mL H_2O, dried over Na_2SO_4, filtered and
10 concentrated to a syrup. Recovered **10** (72 g) as a yellow oil in 92% yield. A solution of **10** (72 g, 138 mmol) was dissolved in MeOH (300 mL) and cooled to 0°C. Acetyl chloride (30 mL) was added dropwise over 5 minutes. The solution was allowed to warm to room temperature over 2h and stirred for an additional 48h. The reaction mixture was concentrated *in vacuo* to afford **11**
15 (54 g) as a yellow syrup that was used in the next step without further purification. Starting monomer **11** (54 g, 0.138 mol) was dissolved in pyridine (180 mL) and cooled to 0°C under N_2. A solution of *tert*-butyldimethylsilyl chloride (TBSCl, 23.7 g, 0.157 mol) in CH_2Cl_2 (75 mL) was added over 1h and the temperature was maintained at <1°C. The reaction mixture was stirred for
20 an additional 1h at 0°C, then a second portion of TBSCl (4.0g, 0.028 mol) in CH_2Cl_2 (10 mL) was added and stirred at 0°C for 1h. The reaction mixture was warmed to room temperature for 2h and then re-cooled to 0°C. Acetic anhydride (80 mL) was added over 1h at 0°C and the solution was warmed to room temperature overnight. After 12h at rt, the reaction mixture was poured
25 onto ice water (1L) and stirred for 1h. The biphasic solution was extracted with EtOAc in 3 portions (1L, then 2 x 250 mL). The combined organics were concentrated and the product recrystallized from hot ethanol (300 mL) to give **12** (65 g, 80% yield) as a white solid. Allyl removal was performed as described in **SOP 2** using Ir catalyst (0.6 g, 0.7 mmol), **12** (32 g, 54 mmol),
30 50% aqueous NMO (50 mL) and OsO_4 (10 mg, 0.04 mmol). Product **13** was formed in 70% yield (21 g, 38 mmol). Glycosyl trichloroacetimidate **14** was

formed as described in **SOP 3** using **13** (21 g, 38 mmol), trichloroacetonitrile (30 mL) and K_2CO_3 (20 g). Product **14** was formed in 97% yield (25.5 g).

[00148] *Example 10 - Synthesis of disaccharide building blocks*

[00149] FIG. 4A-4D outline reaction schemes for synthesizing various disaccharide building blocks, including those depicted in FIG. 4A which proceeds according to the following steps. In FIG. 4A, coupling was performed as described in **SOP 4** using trichloroacetimidate **14** (13.2g, 19.1 mmol), acceptor **6** (6.34 g, 14.7 mmol) and TMSOTf (2.94 mL, 0.5 M in CH_2Cl_2, 1.47 mmol). Product **15** was formed in 80% yield (11.04 g). Allyl removal was performed as described in **SOP 2** using Ir catalyst (0.25 g, 0.3 mmol), **15** (10 g, 10 mmol), 50% aqueous NMO (25 mL) and OsO_4 (5 mg, 0.02 mmol). Product **16** was formed in 90% yield (9 g, 9 mmol). Glycosyl trichloroacetimidate **17** was formed as described in **SOP 3** using **16** (9 g, 9 mmol), trichloroacetonitrile (20 mL) and K_2CO_3 (10 g). Product **17** was formed in quantitative yield (10 g).

[00150] The reaction schemes depicted in FIGs. 4B-4D employ monosaccharide building block starting materials (as shown), but essentially the same steps and SOPs (-1, -2, -3, and -4) as described above. For example, in FIG. 4C, coupling was performed as described in **SOP 4** using: trichloroacetimidate **14** (12 g, 17.2 mmol), acceptor **47** (6.86 g, 14.3 mmol) and TMSOTf (1.43 mL, 1.0 M in CH_2Cl_2, 1.43 mmol). Product **48** was formed in 98% yield (14.2 g, 14 mmol).

[00151] *Example 11 - Synthesis of mixed-N-acetyl oligo-β-(1→6)-glucosamine structures*

[00152] FIG. 5A-5B depict a reaction scheme for synthesizing a mixed-*N*-acetyl oligo-β-(1→6)-glucosamine 6-mer (**37** in FIG. 2B), which proceeds according to the following steps. Utilization of monosaccharide and disaccharide building blocks described in FIGs. 3 and 4A-4D in conjunction with the indicated SOPs additionally described in Examples 2-8 exemplify the methodologies and reagents for synthesizing mixed-*N*-acetyl oligo-β-(1→6)-glucosamine structures, including oligosaccharides **37**, **40**, and **34** in FIG. 2B,

the syntheses of which are outlined in FIGs. 5A, 5B (6-mer, **37**), FIGs. 6A, 6B (12-mer, **40**), and FIGs. 7A, 7B (18-mer, **34**) and further described below.

[00153] *Synthesis of mixed-N-acetyl oligo-β-(1→6)-glucosamine 6-mer thiol* ***37***

5 **[00154]** Referring to FIG. 5A, coupling was performed as described in **SOP 4** using trichloroacetimidate **8** (65 g, 101 mmol), acceptor **6** (36.3 g, 84 mmol) and TMSOTf (8.4 mL, 1.0 M in CH_2Cl_2, 8.4 mmol). Product **18** was formed in 96% yield (75 g). TBS removal was performed as described in **SOP 1** using **18** (35 g, 38 mmol) and $Sc(OTf)_3$ (400mg, 0.8 mmol). Product **21** was 10 formed in 84% yield (26 g, 32 mmol). Allyl removal was performed as described in **SOP 2** using Ir catalyst (0.50 g, 0.6 mmol), **18** (50 g, 55 mmol), 50% aqueous NMO (50 mL) and OsO_4 (10 mg, 0.04 mmol). Product **19** was formed in 77% yield (37.5 g, 42.5 mmol). Glycosyl trichloroacetimidate **20** was formed as described in **SOP 3** using **19** (37 g, 42 mmol), 15 trichloroacetonitrile (20 mL) and DBU (0.6 mL). Product **20** was formed in quantitative yield (44 g). Coupling was performed as described in **SOP 4** using trichloroacetimidate **20** (35 g, 34 mmol), acceptor **21** (23 g, 28 mmol) and TMSOTf (2.8 mL, 1.0 M in CH_2Cl_2, 2.8 mmol). Product **22** was formed in 82% yield (38 g). TBS removal was performed as described in **SOP 1** using 20 **22** (37 g, 22 mmol) and $Sc(OTf)_3$ (400mg, 0.8 mmol). Product **23** was formed in 68% yield (24 g, 15 mmol).

[00155] Referring now to FIG. 5B, coupling was performed as described in **SOP 4** using trichloroacetimidate **17** (9.9 g, 9.2 mmol), acceptor **23** (12 g, 7.7 mmol) and TMSOTf (0.77 mL, 1.0 M in CH_2Cl_2, 0.77 mmol). Product **24** was 25 formed in 83% yield (15.7 g, 6.4 mmol). TBS removal was performed as described in **SOP 1** using **24** (4.55 g, 1.8 mmol) and $Sc(OTf)_3$ (60mg, 0.12 mmol). Product **27** was formed in 83% yield (3.6 g, 1.5 mmol). Exchange of N-Troc for N-Acetate groups was performed as described in **SOP 5** using **27** (0.50g, 0.23 mmol) and Zn (1g) in THF:Ac_2O:AcOH (20 mL). Product **35** was 30 formed in 51% yield (0.25 g, 0.12 mmol). Thiol addition was performed as described in **SOP 6** using **35** (0.20 g, 0.09 mmol), HSAc (0.5 mL) and AIBN (50 mg). Product **36** was formed in 78% yield (0.16 g, 0.07 mmol).

Deprotection was performed as described in **SOP 7** using **36** (0.15 g, 0.06 mmol) and hydrazine (1 mL). Product **37** was formed in 90% yield (0.07 g, 0.054 mmol).

[00156] *Synthesis of mixed-N-acetyl oligo-β-(1→6)-glucosamine 12-mer*
5 *thiol* ***40***

[00157] As depicted in FIGs. 6A and 6B, synthesis of the mixed-N-acetyl oligo-β-(1→6)-glucosamine 12-mer thiol proceeded according to the following steps. With reference to FIG. 6A, allyl removal in **24** was performed as described in **SOP 2** using Ir catalyst (0.25 g, 0.3 mmol), **24** (7.5 g, 3.0 mmol),
10 50% aqueous NMO (25 mL) and OsO_4 (5 mg, 0.04 mmol). Product **25** was formed in 93% yield (7.0 g, 2.8 mmol). Glycosyl trichloroacetimidate **26** was formed from **25** as described in **SOP 3b** using **25** (7.0 g, 2.8 mmol), trichloroacetonitrile (10 mL) and K_2CO_3 (7 g). Product **26** was formed i_n 86% yield (6.3 g, 2.4 mmol). TBS removal of was performed as described in **SOP**
15 **1** using **24** (4.55 g, 1.8 mmol) and $Sc(OTf)_3$ (60mg, 0.12 mmol). Product **27** was formed in 83% yield (3.6 g, 1.5 mmol). Coupling of **26** and **27** was performed as described in **SOP 4** using trichloroacetimidate **26** (4.2 g, 1.6 mmol), acceptor **27** (3.2 g, 1.36 mmol) and TMSOTf (1.36 mL, 0.1 M in CH_2Cl_2, 0.136 mmol). Product **28** was formed in 65% yield (4.2 g, 0.88
20 mmol). TBS removal in **28** was performed as described in **SOP 1** using **28** (4.0 g, 0.84 mmol) and $Sc(OTf)_3$ (60mg, 0.12 mmol). Product **29** was formed in 63% yield (2.6 g, 0.55 mmol);

[00158] Referring now to FIG. 6B, (6) exchange of N-Troc for N-Acetate groups in **29** was performed as described in **SOP 5** using **29** (0.47 g, 0.10
25 mmol) and Zn (1g) in THF:Ac_2O:AcOH (20 mL). Product **38** was formed in 90% yield (0.4 g, 0.09 mmol). Thiol addition to **38** was performed as described in **SOP 6** using **38** (0.40 g, 0.09 mmol), HSAc (0.5 mL) and AIBN (50 mg). Product **39** was formed in 57% yield (0.23 g, 0.51 mmol). Deprotection of **39** and formation of **40** was performed as described in **SOP 7**
30 using **39** (0.22 g, 0.065 mmol) and hydrazine (3 mL). Product **40** was formed in 73% yield (0.10 g, 0.047 mmol).

[00159] *Synthesis of mixed-N-acetyl oligo-β-(1→6)-glucosamine 18-mer thiol* ***34***

[00160] As depicted in FIG. 7, synthesis of the mixed-N-acetyl oligo-β-(1→6)-glucosamine 18-mer thiol proceeded according to the following steps.
5 Coupling of **26** and **29** was performed as described in **SOP 4** using trichloroacetimidate **26** (1.17 g, 0.455 mmol), acceptor **29** (1.41 g, 0.303 mmol) and TMSOTf (0.3 mL, 0.1 M in CH_2Cl_2, 0.03 mmol). Product **30** was formed in 67% yield (1.4 g, 0.20 mmol). TBS removal in **30** was performed as described in **SOP 1** using **30** (2 g, 0.29 mmol) and $Sc(OTf)_3$ (40mg, 0.08
10 mmol). Product **31** was formed in 90% yield (0.18 g, 0.26 mmol). Exchange of N-Troc for N-Acetate groups in **31** was performed as described in **SOP 5** using **31** (0.73g, 0.10 mmol) and Zn (1g) in THF:Ac_2O:AcOH (20 mL). Product **32** was formed in 92% yield (0.6 g, 0.092 mmol). Thiol addition to **32** was performed as described in **SOP 6** using **32** (0.6 g, 0.092 mmol), HSAc
15 (0.5 mL) and AIBN (50 mg). Product **33** was formed in 83% yield (0.5 g, 0.076 mmol). Deprotection of **33** and formation of **34** was performed as described in **SOP 7** using **33** (0.5 g, 0.076 mmol) and hydrazine (3 mL). Product **34** was formed in 79% yield (0.19 g, 0.06 mmol).

[00161] Table 1 provides supporting characterization data for selected
20 antigens and intermediates described in Example 11.

[00162] Table 1

			NMR		Mass Spec		
Series	Description	Molecule #	^{1}H	^{13}C	Theo.	ESI (Pos. Mode)	MALDI
6-mer	Troc/Phth	27	√		2355	2376 (M+Na$^+$)	
	NHAc/Phth	35	√		2221	2277 (M$^+$+HOAc)	
	NHAc/Phth/SAc	36	√		2340	2353 (M$^+$)	
	NHAc/NH$_2$	37	√	√	1101	1101 (M$^+$)	
12-mer	Troc/Phth	29	√		4652		4674 (M+Na$^+$)
	NHAc/Phth	38	√		4386		4409 (M+Na$^+$)
	NHAc/Phth/SAc	39	√		4459	4482 (M+Na$^+$)	
	NHAc/NH$_2$	40	√	√	2109		2110 (M+H$^+$)
18-mer	Troc/Phth	31	√		6949		6973 (M+Na$^+$)
	NHAc/Phth	32	√		6549		6572 (M+Na$^+$)
	NHAc/Phth/SAc	33	√		6622		6668 (M$^+$+HOAc)
	NHAc/NH$_2$	34	√	√	3117	1039 (M^{+3})	3140 (M+Na$^+$)

[00163] *Example 12 - Conjugation of mixed-N-acetyl oligo-β-(1→6)-*
5 *glucosamine thiols to BSA and KLH*

[00164] *Conjugation of mixed-N-acetyl oligo-β-(1→6)-glucosamine 6-mer thiol* ***34*** *to BSA and KLH*

[00165] FIGs. 8A and 8B depict reaction schemes for conjugating a mixed-*N*-acetyl oligo-β-(1→6)-glucosamine 6-mer thiol **37** to BSA and KLH as
10 follows. First, a conjugation stock solution of hexamer thiol **37** was prepared by dissolving the hexamer thiol **37** (4.2 mg, 3.81 μmol) in water (0.300 mL). A solution of tris(2-carboxyethyl)phosphine (TCEP) in water (40 μL, 0.05 M, 1.95 μmol) was added and stirred for 1 hour. Imject® Conjugation Buffer (Pierce, 300 μL) was added to provide a stock solution for conjugation to BSA (FIG.
15 8A) and KLH (FIG. 8B).

[00166] With reference to FIG. 8A, the conjugation stock solution of hexamer thiol **37** (500 μL, 3.0 μmol) was added to a solution of maleimide-activated bovine serum albumin (Imject® BSA, Pierce, Rockford, IL) (5 mg, ~ 1.5 μmol maleimide) in Imject® Conjugation Buffer (Pierce, 250 μL diluted
20 with 250 μL water) and the resulting solution stirred for 18 hours at room temperature. The reaction mixture was purified by de-salting on D-Salt P-6000 10 mL column (Pierce, Rockford, IL). The column was pre-equilibrated

with 30 mL of purification buffer (Pierce, Prod. No. 77159), the crude material loaded onto the column and eluted with purification buffer. 1-mL fractions were collected and analyzed for protein content by absorbance at 280 nm (A_{280}). Fractions containing protein were combined and lyophilized to give the
5 desired hexamer-BSA conjugate **41**.

[00167] Turning now to FIG. 8B, the conjugation stock solution of hexamer thiol **37** (140 μL, 0.86 μmol) was added to a solution of maleimide-activated keyhole limpet hemocyanin (Imject® KLH, Pierce, Rockford, IL) (5 mg, ~ 0.43 μmol maleimide) in water (0.5 mL) was added and the resulting solution
10 stirred overnight at room temperature. The reaction mixture was purified by de-salting on D-Salt P-6000 10 mL column (Pierce, Rockford, IL). The column was pre-equilibrated with 30 mL of purification buffer (Pierce, Prod. No. 77159), the crude material loaded onto the column and eluted with purification buffer. 1-mL fractions were collected and analyzed for protein
15 content by absorbance at 280 nm (A_{280}). Fractions containing protein were combined and lyophilized to give the desired hexamer-KLH conjugate **42**.

[00168] *Conjugation of mixed-N-acetyl oligo-β-(1→6)-glucosamine 12-mer thiol **40** to BSA and KLH*

[00169] FIGs. 9A and 9B depict reaction schemes for conjugating mixed-
20 *N*-acetyl oligo-β-(1→6)-glucosamine 6-mers (Ag 5 in FIG. 2B) to BSA and KLH as follows. First, a conjugation stock solution of 12-mer thiol **40** was prepared by dissolving the 12-mer **40** (8.1 mg, 3.84 μmol) in water (300 μL). A solution of tris(2-carboxyethyl)phosphine (TCEP) in water (30 μL, 0.05 M, 1. 5 μmol) was added and stirred for 1 hour. Imject® Conjugation Buffer (Pierce,
25 300 μL) was added to provide a stock solution for conjugation to BSA (FIG. 9A) and KLH (FIG. 9B).

[00170] With reference to FIG. 9A, the conjugation stock solution of 12-mer thiol **40** (500 μL, 3.0 μmol) was added to a solution of maleimide-activated bovine serum albumin (Imject® BSA, Pierce, Rockford, IL) (5 mg, ~
30 1.5 μmol maleimide) in Imject® Conjugation Buffer (Pierce, 250 μL diluted with water, 250 μL) and the resulting solution stirred for 18 hours at room

temperature. The reaction mixture was purified by de-salting on D-Salt P-6000 10 mL column (Pierce, Rockford, IL). The column was pre-equilibrated with 30 mL of purification buffer (Pierce, Prod. No. 77159), the crude material loaded onto the column and eluted with purification buffer. 1-mL fractions were collected and analyzed for protein content by absorbance at 280 nm (A_{280}). Fractions containing protein were combined and lyophilized to give the desired 12-mer-BSA conjugate **43**.

[00171] Turning now to FIG. 9B, the conjugation stock solution of 12-mer thiol **40** (140 μL, 0.86 μmol) was added to a solution of maleimide-activated keyhole limpet hemocyanin (Imject® KLH, Pierce, Rockford, IL) (5 mg, ~ 0.43 μmol maleimide) in water (0.5 mL) was added and the resulting solution stirred overnight at room temperature. The reaction mixture was purified by de-salting on D-Salt P-6000 10 mL column (Pierce, Rockford, IL). The column was pre-equilibrated with 30 mL of purification buffer (Pierce, Prod. No. 77159), the crude material loaded onto the column and eluted with purification buffer. 1-mL fractions were collected and analyzed for protein content by absorbance at 280 nm (A_{280}). Fractions containing protein were combined and lyophilized to give the desired 12-mer-KLH conjugate **44**.

[00172] *Conjugation of mixed-N-acetyl oligo-β-(1→6)-glucosamine 18-mer thiol **34** to BSA and KLH*

[00173] FIGs. 10A and 10B depict reaction schemes for conjugating mixed-*N*-acetyl oligo-β-(1→6)-glucosamine 18-mers (Ag 6 in FIG. 2B) to BSA and KLH as follows. First, a conjugation stock solution of 18-mer thiol **34** was prepared by dissolving the 18-mer **34** (12 mg, 3.8 μmol) in water (0.3 mL). A suspension of tris(2-carboxyethyl)phosphine (TCEP)-bound agarose resin in water (200 μL, ~1 μmol) was added and stirred for 1 hour. The TCEP-resin was filtered and to the filtrate was added Imject® Conjugation Buffer (Pierce, 300 μL) to provide a stock solution for conjugation to KLH (FIG. 10A) and BSA (FIG. 10B).

[00174] With reference to FIG. 10A, the conjugation stock solution of 18-mer thiol **34** (0.5 mL, 3.0 μmol) was added to a solution of maleimide-

activated bovine serum albumin (Imject® BSA, Pierce, Rockford, IL) (5 mg, ~ 1.5 μmol maleimide) in Imject® Conjugation Buffer (Pierce, 250 μL diluted with water, 2500 μL) and the resulting solution stirred for 18 hours at room temperature. The reaction mixture was purified by de-salting on D-Salt P-6000 10 mL column (Pierce, Rockford, IL). The column was pre-equilibrated with 30 mL of purification buffer (Pierce, Prod. No. 77159), the crude material loaded onto the column and eluted with purification buffer. 1-mL fractions were collected and analyzed for protein content by absorbance at 280 nm (A_{280}). Fractions containing protein were combined and lyophilized to give the desired 18-mer-BSA conjugate **45**.

[00175] Turning now to FIG. 10B, the conjugation stock solution of 18-mer thiol **34** (0.14 mL, 0.86 μmol) was added to a solution of keyhole limpet hemocyanin (Imject® KLH, Pierce, Rockford, IL) (5 mg, ~ 0.43 μmol maleimide) in water (500 μL) and the resulting solution stirred for 18 hours at room temperature. The reaction mixture was purified by de-salting on D-Salt P-6000 10 mL column (Pierce, Rockford, IL). The column was pre-equilibrated with 30 mL of purification buffer (Pierce, Prod. No. 77159), the crude material loaded onto the column and eluted with purification buffer. 1-mL fractions were collected and analyzed for protein content by absorbance at 280 nm (A_{280}). Fractions containing protein were combined and lyophilized to give the desired 18-mer-KLH conjugate **46**.

[00176] Table 2 provides supporting characterization data for the antigen conjugates described in Example 12.

[00177] Table 2

		Protein Assay				% Carbohydrate		
Conjugate #	Description	BSA (mg/mL)	KLH (mg/mL)	MALDI	Antigen MW	Ave Copy Number	MALDI	Sample
41	S-6Mix-BSA	1.62		100,900	1101	23	33%	
42	S-6Mix-KLH		0.85		1101			12%
43	S-12Mix-BSA	0.98		112,100	2109	17	48%	
44	S-12Mix-KLH		1.32		2109			18%
45	S-18Mix-BSA	1.33		126,251	3117	16	66%	
46	S-18Mix-KLH		1.21		3117			25%

[00178] In Table 2, protein assays were performed according to the method of Bradford, M. *Anal. Biochem.* **1976**, *72*, 248. Maldi analysis was performed using 2,5-dihydroxybenzoid acid as a matrix. Copy numbers were determined by the formula: copy number = [Maldi $_{(observed)}$-76,000 $_{(Maldi\ of\ BSA\ alone)}$]/antigen MW. Carbohydrate content in KLH sample was extrapolated from BSA using the formula: KLH carbohydrate content = BSA carbohydrate content/2.65.

[00179] *Example 13 - Serum Antibody Production and Purification*

[00180] Antisera to antigen-KLH conjugates were raised in New Zealand white rabbits by four subcutaneous injections of antigen-KLH conjugate over 13 weeks. A pre-immune bleed generated 5 mL of baseline serum from each rabbit. The prime injection (10 μg antigen equivalent) was given as an emulsion in complete Freund's adjuvant (CFA). Subsequent injections (5 μg antigen equivalent) were given at three week intervals in incomplete Freund's adjuvant (IFA). Rabbits were bled every two weeks commencing one week after the third immunization. Approximately 25 – 30 mL of serum per rabbit was generated for each bleeding event, and was aliquoted into 1-mL aliquots and frozen at -80°C. Serum was analyzed by ELISA against the corresponding antigen-BSA conjugate as described in Example 3 below.

[00181] Affinity purification of antisera was conducted with serum from the third bleed from each rabbit. Affinity purification was carried out by coupling of antigen-BSA conjugates to CNBr-activated Sepharose 4B. Briefly, CNBr-activated Sepharose 4B (0.8 g, 2.5ml of final gel volume) was washed and re-swelled on a sintered glass filter with 1mM HCl, then coupling buffer (0.1M $NaHCO_3$, 0.25M NaCl, pH 8.5). Antigen-BSA conjugate (1 mg) was dissolved in coupling buffer, mixed with the gel suspension and incubated overnight at 40°C. Unreacted active groups were capped with glycine buffer (0.2M, pH 8.1) and excess adsorbed conjugated was washed away with coupling buffer, then acetate buffer (0.1 M containing 0.5M NaCl, pH 4.3). The column was equilibrated with phosphate buffered saline (PBS), pH 7.7.

[00182] Antisera were affinity purified by diluting clear antiserum (5 mL) 1:1 with PBS pH 7.7 and applying the diluted antisera to the affinity column at the rate of 0.3ml/min and absorbance of eluate was monitored at 280 nm. Unbound material (flow through) was collected and analyzed by ELISA using the general ELISA procedure. The column was washed with PBS until A280 reached baseline. Bound antibodies were eluted with 0.2M glycine (pH 1.85) into one fraction until the A280 returned to baseline. Fractions were neutralized with 1M Tris-HCl, pH 8.5 immediately after collection and the OD at 280 nm was determined. ELISA analysis was conducted using the corresponding antigen-BSA conjugate according to the general ELISA protocol to confirm the recovered antibody and the removal of all the antibodies from the original serum. Antibody quantification was determined by A280 reading of the antibody (a small amount was diluted to give an OD value of about 1.0) and this value was divided by the extinction coefficient of IgG, 1.4, to give mg/mL. The solutions were concentrated to ~1-2mg/mL, dialyzed against PBS with 0.02% sodium azide, aliquoted and frozen at -80°C.

[00183] *Example 14 - ELISA Plate Preparation and Use*

[00184] An oligosaccharide-BSA conjugate solution was prepared by dissolving the conjugate in carbonate buffer (1.59 g Na_2CO_3, 2.93 g $NaHCO_3$, 0.20 g NaN_3, dissolved and diluted to 1 L in H_2O, final pH 9.5) at a concentration of 5 – 10 μg/mL. COSTAR flat bottom EIA 8-well strips were incubated with oligosaccharide-BSA conjugate solution (100 μL per well) for 24 hours in a humidity chamber to coat the well surfaces. Coating solution was removed, each well was rinsed twice with water, and dried on a paper towel. Blocking solution (0.1% BSA in PBS with 0.02% thimerosal, 200 μL) was added to each well and incubated for 2 hours in a humidity chamber. Blocking solution was removed; each well was rinsed with water and dried on a paper towel.

[00185] Serum samples were prepared by 1:5 serial dilutions starting from a 1:1,000 dilution of serum in 0.1% BSA in PBS with 0.02% thimerosal.

Diluted serum (100 μL) was added to each well and incubated for 2 hours at room temperature in a humidity chamber. The serum solution was removed, the wells were rinsed twice with water and dried on a paper towel. Goat anti-rabbit-HRP conjugate solution (100 μL/well) was added and incubated for 2 hours at room temperature in a humidity chamber. The HRP-conjugate solution was removed, and wells were washed three times with PBS/0.02% thimerosal/0.05% tween-20, twice with water, and dried on a paper towel. TMB solution (100 μL/well) was added and developed at room temperature. The reaction was stopped by the addition of 1N HCl (100 μL/well) and the wells were read immediately at A450 (absorbance at 450 nm). The titer of the test serum was designated as the dilution which gave an optical density (OD_{450}) reading of 0.1 above background.

[00186] _Example 15 - Rabbit Immunogenicity and ELISA Results_

[00187] *Immunogenicity of synthetic antigen-KLH conjugates in rabbits (ELISA).*

[00188] FIGs. 11A-11C depict IgG antibody titers as a function of antibody-antigen complex absorption (OD_{450}) at 3 serum dilutions of immune sera obtained from 3 succesive bleeds (pre-imune, 1st bleed, and final bleed) in rabbits (n=2) immunized with antigen-KLH conjugates corresponding to (A) 6-Mix-KLH **42**; (B) 12-Mix-KLH **44**; and (C) 18-Mix-KLH **46**. In each case, the antisera were incubated on ELISA plates adsorbed with their corresponding BSA conjugate, specfically, (A) 6-Mix-BSA **41**; (B) 12-Mix-BSA **43**; and (C) 18-Mix-BSA **45** as described the ELISA protocol above (Example 14).

[00189] FIGs. 11D-11F depict antigen-specific IgG antibody titers from antigen-KLH conjugate-derived antibodies recovered at three successive stages of purification, including the pre-affinity purification fraction (3rd bleed), the flow-through fraction, and the antibody-enriched (purified) fraction. Results are shown as a function of antibody-antigen complex absorption (OD_{450}) at the indicated serum dilutions obtained from the above-described antibody-enriched fractions generated against antigen-KLH conjugates corresponding to (A) 6-Mix-KLH **42**; (B) 12-Mix-KLH **44**; and (C) 18-Mix-KLH

46. In each case, the antisera were incubated on ELISA plates adsorbed with their corresponding BSA conjugate, specifically, (A) 6-Mix-BSA **41**; (B) 12-Mix-BSA **43**; and (C) 18-Mix-BSA **45**.

[00190] Affinity purification of 10 mL of 3rd bleed sera (in each case) yielded: 5.5 mL of a purified 6-Mix Ab solution at 2.3 mg/mL (12.7 mg purified Ab total); 5.4 mL of a purified 12-Mix Ab solution at 5.5 mg/mL (29.7 mg purified Ab total); and 5.4 mL of purified 18-Mix Ab solution at 1.7 mg/mL (9.2 mg purified Ab total).

[00191] _Example 16 - Specificity and Cross-Reactivity of Antisera to Different Antigens_

[00192] FIGs. 12A-12G depict the results of a cross-ELISA assay examining the specificity and cross-reactivity between fully non-acetylated (6-NH_2 **61**, 12-NH_2 **62**, 18-NH_2 **63**;); mixed (6-Mix **37**, 12-Mix **40**, 18-Mix **34**) and fully acetylated (6-NHAc **58**, 12-NHAc **59**, 18-NHAc **60**;) oligo-β-(1→6)-glucosamines and antibodies derived therefrom. In FIGs. 12A-12G, antisera from rabbits immunized with the indicated antigen-KLH conjugates corresponding to (left to right) 6-NH_2, 6-Mix **42**, 6-NHAc, 12-NH_2 **44**, 12-Mix, 12-NHAc, 18-NH_2, 18-Mix **46**, and 18-NHAc were incubated in each case with an ELISA plate adsorbed with a different antigen-BSA conjugate, specifically (A) 6-NH_2-BSA **64**; (B) 6-Mix-BSA **41**; (C) 6-NHAc-BSA **65**; (D) 12-NH_2-BSA **66**; (E) 12-Mix-BSA **43**; (F) 12-NHAc-BSA;. (G) 18-Mix-BSA **45**. Results are shown as a function of antibody-antigen complex absorption (OD_{450}) representing the averages from of antisera from two rabbits in each case at the indicated serum dilutions, whereby total OD_{450} is measured by subtracting away the background OD_{450} from KLH antibodies alone. The results surprisingly showed that antibodies against the 12-Mix-KLH conjugate **44** antigen (16.7% acetylation) cross-reacted strongly with all of the antigens screened, including fully non-acetylated, mixed, and fully acetylated antigens, and typically to a greater extent than antibodies against the fully acetylated or fully aminylated oligosaccharides.

[00193] *Example 17 - Whole-cell ELISA experiment*

[00194] FIGs. 13A-13D depict the results of a whole-cell ELISA assay examining the binding of pre-immune sera (A, C) or immune sera (B, D) generated from rabbits immunized against (left to right) KLH control, fully non-
5 acetylated antigen (12-NH_2) **62**; mixed antigen (12-Mix) **44** and fully acetylated antigen (12-NHAc) **59** and *Staphylococcus epidermidis* coated onto ELISA fixed with methanol (A, B) or formalin (C, D). Results are shown as a function of antibody-antigen complex absorption (OD_{450}) at the indicated serum dilutions. The results surprisingly showed that at the higher dilutions,
10 antibodies against the poorly acetylated 12-Mix-KLH conjugate **44** antigen (16.7% acetylation) reacted more strongly to *S. epidermidis* than antibodies against the fully non-acetylated (12-NH_2) or fully acetylated (12-NHAc) antigens even though the PNAG/dPNAG from natural *S. epidermidis* isolates are highly acetylated.

15 [00195] *Example 18 - Opsonophagocytic Assay*: The opsonophagocytic (bacterial killing) activity of serum samples will be determined in an assay using *S. aureus* ATCC strain 25904 in the presence of phagocytic cells and complement.

[00196] HL-60 cells (human promyelocytic cells; ATCC Cat #CCL240) will
20 be used as the effector cells in this assay. The cells will be in differentiation medium for 5-7 days prior to use (RPMI 1640 with 15% heat-inactivated fetal bovine serum and 1.25% dimethylsulfoxide). Approximately 50 ul of a stock solution of target bacteria will be grown on tryptic soy agar plates with 5% sheep red blood cells (blood agar plates) and incubated overnight at 36-37°C.
25 The bacterial lawn will be transferred to a sterile 50 ml conical containing 30 mls of tryptic soy broth with 1% (w/v) glucose. The bacteria will be grown in a shaking water batch set for 80 strokes per minute at 36-37°C. The bacterial suspension will be adjusted to a %T of 72-75% (1 cm light path) and 2.7 – 3.0 ul of this suspension was mixed with 1.4 mls of TSB for a final concentration
30 of approximately 5-6 X 104 cfu/ml.

[00197] Ten ul of the bacterial suspension will be mixed with 40 ul of heat-inactivated serum samples or reference antibody in a 96-well, round-bottom assay plate and incubated at 36-37°C in a shaking incubator at ~ 100 rpm for 30-40 minutes. Antibody dilutions, if required, will be made in DMEM/F12 medium buffered with 10 mM HEPES to maintain a pH of 7.2-7.6.

[00198] Differentiated HL-60 cells will be pelleted by centrifugation at ~1000 X g at room temperature for 10 minutes and the supernatant removed. The cells will be resuspended in DMEM/F12 medium buffered with 10 mM HEPES to maintain a pH of 7.2 – 7.6 and pelleted twice more to remove residual DMSO. After the final centrifugation the supernatant will be removed to near dryness and the cells suspended to a final concentration of 5 X 107viable cells per ml.

[00199] Forty ul of the cell suspension will be added to each well of the assay plate and the reactions were initiated by addition of 10 ul of complement per well. The complement will be derived from human serum treated with protein A and protein L to extract inherent antibodies reactive with the target bacteria.

[00200] Following addition of the complement to the assay plate, the reagents will be mixed by rapid pipetting up and down 20-25 times using a multichannel pipettor set at 10 ul. After mixing a sample will be removed from each well, diluted 20-fold in water containing 0.1% BSA and 0.01% Tween20. These samples will be designated the T_0 samples and 100 ul of each T_0 sample will be transferred to a blood agar plate, allowed to dry, inverted and incubated overnight at 36-37°C.

[00201] After transfer of the T_0 samples the assay plate will be incubated at 36-37°C in an orbital shaking incubator at 250-300 rpm for an additional 90 minutes. At the end of this incubation period samples will be taken from each well, diluted and plated as described above (T_{90} samples).

[00202] Assay controls included HL-60's alone, HL-60's with complement and reference antibody. The percentage of bacterial killing will be calculated using the formula:

$$\frac{(\text{Number of colonies } T_0 - \text{Number of colonies } T_{90})}{(\text{Number of Colonies } T_0)} * 100$$

[00203] *Example 19 - In vivo challenge protocol:* Five week old female Crl:CD-1®Swiss outbred mice will be acclimated for 7 days prior to study start. Mice will be randomized into study groups (n= 10 per group) the day before initial immunization. Test articles will be reconstituted using sterile saline to the appropriate dosing concentration (10µg glycan equivalent of test article/100µL solution). Each dose will be mixed with an equal volume (100µL) of CFA or IFA to form a stable emulsion. On Day 0, mice will be administered a single subcutaneous (SC) treatment of the appropriate test or control article at a volume of 100µL. Prime immunizations will be followed by two boost immunizations on Days 11 and 22. A separate group of untreated mice (untreated control) will be not be vaccinated.

[00204] On Day 29, each mouse will be challenged via intravenous tail injection (IV) route with *Staphylococcus aureus* Newman strain at a concentration of approximately 4 x 109 CFU/mL in a dose volume of 0.2 mL. Bacteria inoculation suspensions will be prepared by harvesting isolated colonies seeding 50-mL of fresh Trypticase Soy Broth (TSB). The culture will be incubated (37°C) with shaking for approximately 3-5 hours, washed and resuspended. The concentration in the final culture was adjusted to 4.0 x 109 CFU/mL dose using a spectrophotometer (Target OD_{620} = 3.0). The concentration was verified using the dilution plate count method.

[00205] On Days -3, 10, 21 and 28 mice will be bled via retro-orbital sinus (approx. 0.2mL) into serum separator tubes for processing of sera (stored frozen at -20°C). On Days 30, 31 and 32, mice will be bled in the sub-mandibular region (approx. 0.1mL) onto solid media for bacteremia analysis. All surviving animals will be euthanized via CO_2 asphyxiation on Day 36. The percentage of survival and mortality for each group will be determined and microbiological analyses of blood were expressed as ± bacteremia.

[00206] *Example 20* – Each of the synthetic, mixed-N-acetyl oligo-β-(1→6)-glucosamine hexamers and decamers listed in Tables 3 and 4 below were synthesized from building blocks 8, 14, 6 and 47 using methods exemplified in Example 11 as described below. Characterization data (NMR 5 and mass spec) for each individual compound synthesized are listed below in Tables 3 and 4.

[00207] *Synthesis of hexamer set:*

[00208] Briefly, the hexamers were assembled in a 2 + 2 + 2 approach from the reducing end. Building blocks 8, 14, 6 and 47 were combined, in the 10 appropriate combination, and coupled according to SOP 4 to afford the reducing end disaccharides. Removal of the primary TBS group on the reducing end disaccharides following SOP 1 provided free hydroxyl compounds that were subsequently coupled with disaccharide building blocks (prepared as in Example 10) according to SOP 4 to afford tetramers. 15 Removal of the primary TBS group on the reducing end tetramers following SOP 1 provided free hydroxyl compounds that were subsequently coupled with disaccharide building blocks (prepared as in Example 10) according to SOP 4 to afford the hexamer set. Removal of the primary TBS group on the hexamers following SOP 1 provided free hydroxyl compounds that were 20 subsequently per-acetylated with pyridine/acetic anhydride (as in Example 9) to afford hexamers 67-74 (Table 3).

[00209] Installation of the N-Acetate functionality was accomplished according to SOP 5 to afford hexamers 85-92 (Table 4).

[00210] *Synthesis of decamer set:*

25 **[00211]** Briefly, the decamer set was assembled via a 4 + 4 + 2 approach from the reducing end. The appropriately designed tetramer acceptors were assembled using the strategy outlined above for the hexamer set. Separately, a set of tetramers (assembled as above) was treated according to SOP 2 to remove each allyl group. Subsequent treatment of the de-allylated tetramers, 30 according to SOP 3 (formation with K2CO3), gave a set of tetramers as trichloroacetimidate donors. Coupling of the tetramer donors with the

tetramer acceptors (SOP 4) afforded an octamer set. Removal of the primary TBS group on the octamers following SOP 1 provided free hydroxyl compounds that were subsequently coupled with disaccharide building blocks (prepared as in Example 10) according to SOP 4 to afford the decamer set. Removal of the primary TBS group on the decamers following SOP 1 provided free hydroxyl compounds that were subsequently per-acetylated with pyridine/acetic anhydride (as in Example 9) to afford decamers 75-84 (Table 3).

[00212] Installation of the N-Acetate functionality was accomplished according to SOP 5 to afford decamers 93-102 (Table 4).

[00213] Table 3. Characterization data for defined, synthetic mixed sequences (R1 = Troc)

1 = Troc; 0 = Phth

												Mass Spec	
Position	1	2	3	4	5	6	7	8	9	10	Molecule	Theo. MW	MALDI (Obs.)
6-MERS	0	0	0	1	1	1					67	2488.0	2510 (M+Na$^+$)
	0	0	1	1	1	0					68	2488.0	2510 (M+Na$^+$)
	0	1	0	1	0	1					69	2488.0	2510 (M+Na$^+$)
	0	1	1	1	0	0					70	2488.0	2510 (M+Na$^+$)
	1	0	0	0	1	1					71	2488.0	2510 (M+Na$^+$)
	1	0	1	0	1	0					72	2488.0	2510 (M+Na$^+$)
	1	1	0	0	0	1					73	2488.0	2510 (M+Na$^+$)
	1	1	1	0	0	0					74	2488.0	2510 (M+Na$^+$)
10-MERS	0	0	0	1	1	1	0	0	0	1	75	4034.6	4056 (M+Na$^+$)
	0	0	0	1	1	1	0	0	1	1	76	4079.9	4102 (M+Na$^+$)
	0	0	0	1	1	1	0	1	0	1	77	4079.9	4101 (M+Na$^+$)
	0	0	1	1	1	0	0	0	1	1	78	4079.9	4102 (M+Na$^+$)
	0	0	1	1	1	0	1	0	1	0	79	4079.9	4101 (M+Na$^+$)
	0	1	0	1	0	1	0	1	0	1	80	4079.9	4100 (M+Na$^+$)
	1	0	0	0	1	1	1	0	0	0	81	4034.6	4057 (M+Na$^+$)
	1	0	0	0	1	1	1	0	0	1	82	4079.9	4101 (M+Na$^+$)
	1	0	0	1	1	1	0	0	0	0	83	4034.6	4056 (M+Na$^+$)
	1	0	1	0	1	0	1	0	0	1	84	4079.9	4101 (M+Na$^+$)

[00214] Table 4. Characterization data for defined, synthetic mixed sequences (R1 = Acetate)

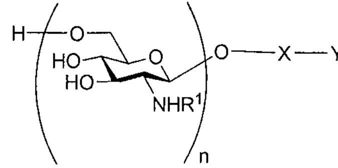
1 = Acetyl; 0 = Phth

Position	1	2	3	4	5	6	7	8	9	10	Molecule	Mass Spec Theo. MW	Mass Spec MALDI (Obs.)
6-MERS	0	0	0	1	1	1					85	2087.9	2110 (M+Na+)
	0	0	1	1	1	0					86	2087.9	2110 (M+Na+)
	0	1	0	1	0	1					87	2087.9	2110 (M+Na+)
	0	1	1	1	0	0					88	2087.9	2110 (M+Na+)
	1	0	0	0	1	1					89	2087.9	2110 (M+Na+)
	1	0	1	0	1	0					90	2087.9	2110 (M+Na+)
	1	1	0	0	0	1					91	2087.9	2110 (M+Na+)
	1	1	1	0	0	0					92	2087.9	2110 (M+Na+)
10-MERS	0	0	0	1	1	1	0	0	0	1	93	3501.2	3524 (M+Na+)
	0	0	0	1	1	1	0	0	1	1	94	3413.1	3435 (M+Na+)
	0	0	0	1	1	1	0	1	0	1	95	3413.1	3435 (M+Na+)
	0	0	1	1	1	0	0	0	1	1	96	3413.1	3435 (M+Na+)
	0	0	1	1	1	0	1	0	1	0	97	3413.1	3435 (M+Na+)
	0	1	0	1	0	1	0	1	0	1	98	3413.1	3435 (M+Na+)
	1	0	0	0	1	1	1	0	0	0	99	3501.2	3524 (M+Na+)
	1	0	0	0	1	1	1	0	0	1	100	3413.1	3435 (M+Na+)
	1	0	0	1	1	1	0	0	0	0	101	3501.2	3524 (M+Na+)
	1	0	1	0	1	0	1	0	0	1	102	3413.1	3435 (M+Na+)

[00215] It is intended that the foregoing detailed description be regarded as illustrative rather than limiting, and that it be understood that it is the following claims, including all equivalents, that are intended to define the spirit and scope of this invention.

CLAIMS

1. An oligosaccharide of the formula:



wherein X is a bond or a linker, Y is H or a carrier, n is the number of monomers (m) in an oligosaccharide and is from 6 to 12, and each R^1 in the oligosaccharide is either acetyl or H;

wherein the oligosaccharide has monomers with R^1 in position m selected from the following table, wherein when R^1 is 1 in the table R^1=acetyl and when R^1 is 0 in the table R^1=H, and wherein the monomer (m) in position 1 is the non-reducing end of the oligosaccharide:

	R^1 in position m											
	1	2	3	4	5	6	7	8	9	10	11	12
n=6	0	0	0	1	1	1						
	0	0	1	1	1	0						
	0	1	0	1	0	1						
	0	1	1	1	0	0						
	1	0	0	0	1	1						
	1	0	1	0	1	0						
	1	1	0	0	0	1						
	1	1	1	0	0	0						
n=7	0	0	0	1	1	1	0					
	0	0	1	1	1	0	0					
	0	1	0	1	0	1	0					
	0	1	1	1	0	0	0					
	0	0	1	1	1	0	1					
	0	1	0	1	0	1	1					
	0	1	0	1	1	1	0					
	0	1	1	1	0	0	1					
	0	1	1	1	0	1	0					
	1	0	0	0	1	1	1					
	1	0	0	1	1	1	0					
	1	0	1	0	1	0	1					
	1	0	1	1	1	0	0					
	1	1	0	0	0	1	1					
	1	1	0	1	0	1	0					
	1	1	1	0	0	0	1					
n=8	0	0	0	1	1	1	0	0				
	0	0	0	1	1	1	0	1				

	0	0	1	1	1	0	0	0			
	0	0	1	1	1	0	0	1			
	0	0	1	1	1	0	1	0			
	0	1	0	1	0	1	0	0			
	0	1	0	1	1	1	0	0			
	0	1	1	1	0	0	0	1			
	1	0	0	0	1	1	1	0			
	1	0	0	1	1	1	0	0			
	1	0	1	0	1	0	1	0			
	1	0	1	1	1	0	0	0			
n=9	0	0	0	1	1	1	0	0	0		
	0	0	0	1	1	1	0	0	1		
	0	0	0	1	1	1	0	1	0		
	0	0	1	1	1	0	0	0	1		
	0	1	0	1	0	1	0	1	0		
	0	1	0	1	1	1	0	0	0		
	1	0	0	0	1	1	1	0	0		
	1	0	0	1	1	1	0	0	0		
	0	0	0	1	1	1	0	1	1		
	0	0	1	1	1	0	0	1	1		
	0	0	1	1	1	0	1	0	1		
	0	1	0	1	0	1	0	1	1		
	0	1	0	1	0	1	1	1	0		
	0	1	0	1	1	1	0	0	1		
	0	1	0	1	1	1	0	1	0		
	0	1	1	1	0	0	0	1	1		
	0	1	1	1	0	1	0	1	0		
	1	0	0	0	1	1	1	0	1		
	1	0	0	1	1	1	0	0	1		
	1	0	0	1	1	1	0	1	0		
	1	0	1	0	1	0	1	0	1		
	1	0	1	0	1	1	1	0	0		
	1	0	1	1	1	0	0	0	1		
	1	1	0	0	0	1	1	1	0		
	1	1	0	0	1	1	1	0	0		
	1	1	0	1	0	1	0	1	0		
	1	1	0	1	1	1	0	0	0		
n=10	0	0	0	1	1	1	0	0	0	1	
	0	0	0	1	1	1	0	0	1	1	
	0	0	0	1	1	1	0	1	0	1	
	0	0	1	1	1	0	0	0	1	1	
	0	0	1	1	1	0	1	0	1	0	
	0	1	0	1	0	1	0	1	0	1	
	1	0	0	0	1	1	1	0	0	0	
	1	0	0	0	1	1	1	0	0	1	
	1	0	0	1	1	1	0	0	0	0	
	1	0	1	0	1	0	1	0	0	1	
n=11	0	0	0	1	1	1	0	0	0	1	1

	0	0	0	1	1	1	0	1	0	1	0	
	0	1	0	1	0	1	0	1	0	1	0	
	0	1	0	1	0	1	1	1	0	0	0	
	0	1	1	1	0	1	1	1	0	0	0	
	1	0	0	0	1	1	1	0	0	0	1	
	1	1	0	0	0	1	1	1	0	0	0	
	0	0	0	1	1	1	0	0	1	1	1	
	0	0	0	1	1	1	0	1	0	1	1	
	0	0	0	1	1	1	0	1	1	1	0	
	0	0	1	1	1	0	0	0	1	1	1	
	0	0	1	1	1	0	0	1	1	1	0	
	0	0	1	1	1	0	1	0	1	0	1	
	0	0	1	1	1	0	1	1	1	0	0	
	0	1	0	1	0	1	0	1	0	1	1	
	0	1	0	1	0	1	0	1	1	1	0	
	0	1	0	1	0	1	1	0	1	0	1	
	0	1	0	1	0	1	1	1	0	0	1	
	0	1	0	1	0	1	1	1	0	1	0	
	0	1	0	1	1	1	0	0	0	1	1	
	0	1	0	1	1	1	0	1	0	1	0	
	0	1	1	1	0	0	0	1	1	1	0	
	0	1	1	1	0	0	1	1	1	0	0	
	0	1	1	1	0	1	0	1	0	1	0	
	1	0	0	0	1	1	1	0	0	1	1	
	1	0	0	0	1	1	1	0	1	0	1	
	1	0	0	1	1	1	0	0	0	1	1	
	1	0	0	1	1	1	0	1	0	1	0	
	1	0	1	0	1	0	1	0	1	0	1	
	1	0	1	0	1	0	1	1	1	0	0	
	1	0	1	0	1	1	1	0	0	0	1	
	1	1	0	0	0	1	1	1	0	0	1	
	1	1	0	0	0	1	1	1	0	1	0	
	1	1	0	0	1	1	1	0	0	0	1	
	1	1	0	1	0	1	0	1	0	1	0	
	1	1	0	1	0	1	1	1	0	0	0	
	1	1	1	0	0	0	1	1	1	0	0	
	1	1	1	0	0	1	1	1	0	0	0	
n=12	0	0	0	1	1	1	0	0	0	1	1	1
	0	0	0	1	1	1	0	0	1	1	1	0
	0	0	0	1	1	1	0	1	0	1	0	1
	0	0	0	1	1	1	0	1	1	1	0	0
	0	0	1	1	1	0	0	0	1	1	1	0
	0	0	1	1	1	0	0	1	1	1	0	0
	0	0	1	1	1	0	1	0	1	0	1	0
	0	0	1	1	1	0	1	1	1	0	0	0
	0	1	0	1	0	1	0	1	0	1	0	1
	0	1	0	1	0	1	0	1	1	1	0	0

	0	1	0	1	0	1	1	1	0	0	0	1
	0	1	1	1	0	0	0	1	1	1	0	0
	0	1	1	1	0	0	1	1	1	0	0	0
	1	0	0	0	1	1	1	0	0	0	1	1
	1	0	0	0	1	1	1	0	1	0	1	0
	1	0	1	0	1	0	1	0	1	0	1	0
	1	0	1	0	1	0	1	1	1	0	0	0
	1	1	0	0	0	1	1	1	0	0	0	1
	1	1	1	0	0	0	1	1	1	0	0	0

2. The oligosaccharide of claim 1, where n is 6.

3. The oligosaccharide of claim 1, where n is 7.

4. The oligosaccharide of claim 1, where n is 8.

5. The oligosaccharide of claim 1, where n is 9.

6. The oligosaccharide of claim 1, where n is 10.

7. The oligosaccharide of claim 1, where n is 11.

8. The oligosaccharide of claim 1, where n is 12.

9. The oligosaccharide of claim 1, where X is a substituted or unsubstituted alkylene or alkenylene moiety.

10. The oligosaccharide of claim 1, where Y is a carrier selected from the group consisting of protein, peptide, lipid, polymer, dendrimer, virosome, and virus-like particle or combination thereof.

11. The oligosaccharide of claim 10, where the carrier is a carrier protein.

12. The oligosaccharide of claim 11, where the carrier protein is selected from the group consisting of diphtheria CRM, diphtheria toxoid, tetanus toxoid, Neisseria meningitidis serotype B outer membrane protein complex, and Pseudomonas aeruginosa exotoxoid A.

13. A composition comprising an oligosaccharide of claim 1 and a pharmaceutically acceptable vehicle.

14. The composition of claim 13, further comprising a second different oligosaccharide of claim 1.

15. The composition of claim 13, further comprising a second different oligosaccharide that is a poly-dPNAG.

16. The composition of claim 14 or 15, wherein the ratio of the first and second oligosaccharides is 0.01:1.0 to 1.0:0.01.

17. The composition of claim 16, wherein the ratio is 0.1:1 to 1:0.1.

18. The composition of claim 16, wherein the ratio is 0.5:1 to 1:0.5.

5 19. The composition of claim 16, wherein the ratio is 0.75:1 to 1:0.75.

20. The composition of claim 16, wherein the ratio is greater than 0.1:1.

21. The composition of claim 16, wherein the ratio is greater than 10 0.25:1.

22. The composition of claim 16, wherein the ratio is greater than 0.5:1.

23. The composition of claim 16, wherein the ratio is greater than 0.75:1.

15 24. The composition of claim 16, wherein the ratio is less than 1:0.1.

25. The composition of claim 16, wherein the ratio is less than 1:0.25.

26. The composition of claim 16, wherein the ratio is less than 1:0.5.

27. The composition of claim 16, wherein the ratio is less than 20 1:0.75.

28. The composition of claim 16, wherein the activity of the composition against Staphylococcus aureus in an opsonophagocytic assay is not substantially lower than a composition containing just the first oligosaccharide.

25 29. The composition of one of claims 13-28, further comprising an adjuvant.

30. The composition of one of claims 13-29, where the adjuvant is selected from the group consisting of aluminum salts, RIBI, toll-like receptor agonists, AS01 AS02 AS03, AS04, AS05, CpG-oligodeoxynucleotide, MF-59, 30 Montanide ISA-51 VG , Montanide ISA-720, Quil A, QS21, immunostimulating complexes, stearyl tyrosine, virus-like particles, reconstituted influenza

virosomes, cytokines, mast cell activator compound 48/80, liposomes, muramyl dipeptides, SAF-1, and combinations thereof.

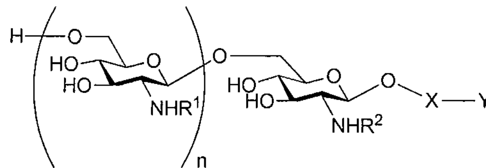
31. The composition of one of claims 13-30, which comprises an amount of oligosaccharide of claim 1 sufficient to confer immunity against
5 Staphylococcus.

32. An antibody preparation against an oligosaccharide of claim 1.

33. The antibody preparation of claim 32, where the antibody preparation comprises at least one member from the group consisting of polyclonal antibody, monoclonal antibody, mouse monoclonal IgG antibody,
10 humanized antibody, chimeric antibody, fragment thereof, or combination thereof.

34. A method of treating or preventing a Staphylococcus infection in a patient in need thereof comprising administering an effective amount of a synthetic oligosaccharide of any of claims 1-12 or an antibody thereto.

15 35. A synthetic oligosaccharide of the formula:



wherein R^1 and R^2 are independently selected from H or $C(O)CH_3$; n is an integer of at least 3, X is a bond or a linker, and Y is H or a carrier;

20 wherein each occurrence of R^1 can be the same or different and wherein in a first monosaccharide unit wherein R^1 is H is present, and for a second monosaccharide-unit which is located three monosaccharide units from the first monosaccharide unit, R^1 or R^2 is $C(O)CH_3$.

36. The synthetic oligomer of claim 35 wherein no more than 3
25 subsequent monosaccharide units are substituted by R^1 and R^2 being $C(O)CH_3$.

37. A homogeneous composition consisting essentially of an oligosaccharide of one of claims 1-12 and 35.

38. The composition of claim 37, further comprising a second different oligosaccharide of one of claims 1-12 and 35.

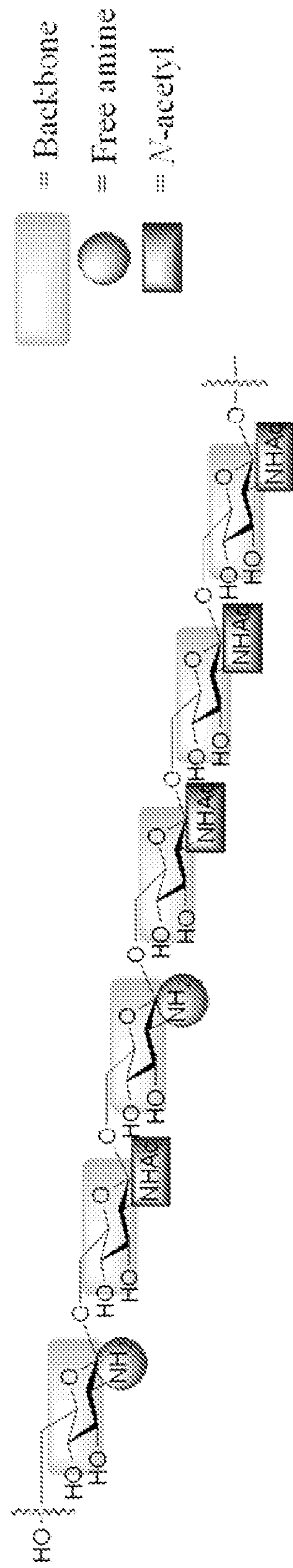


FIG. 1

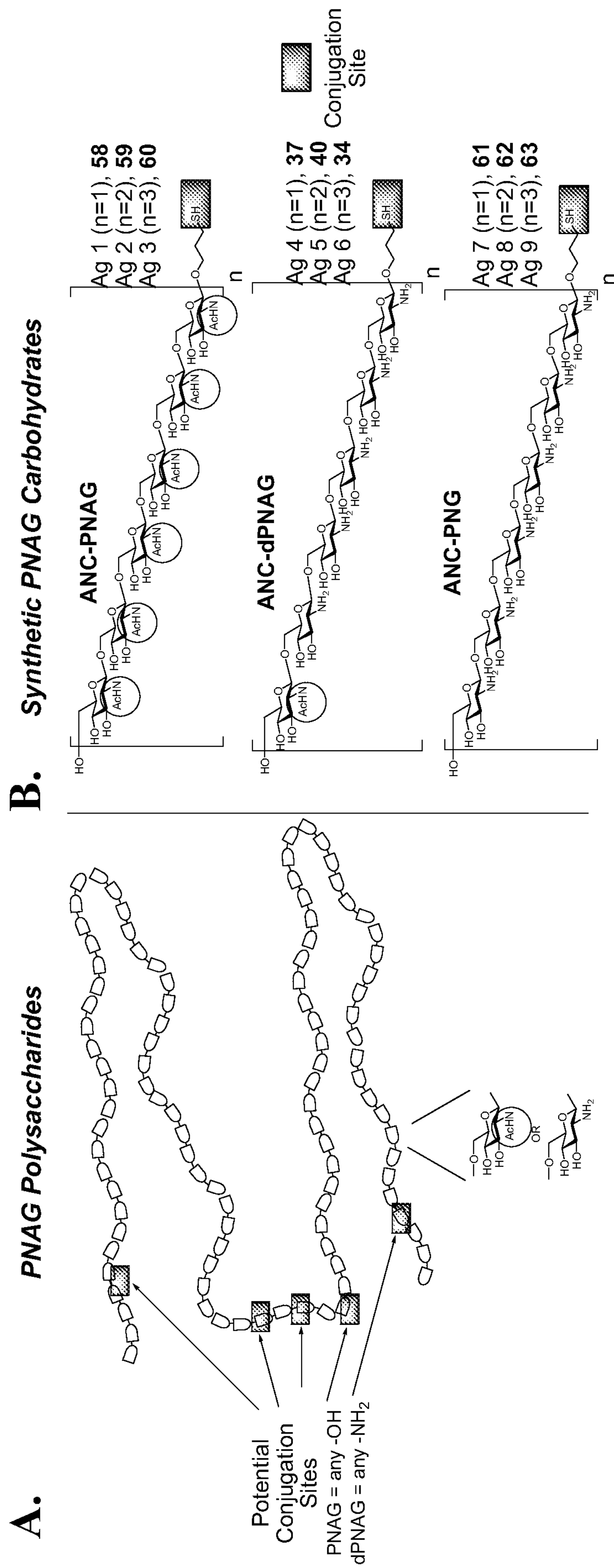


FIG. 2

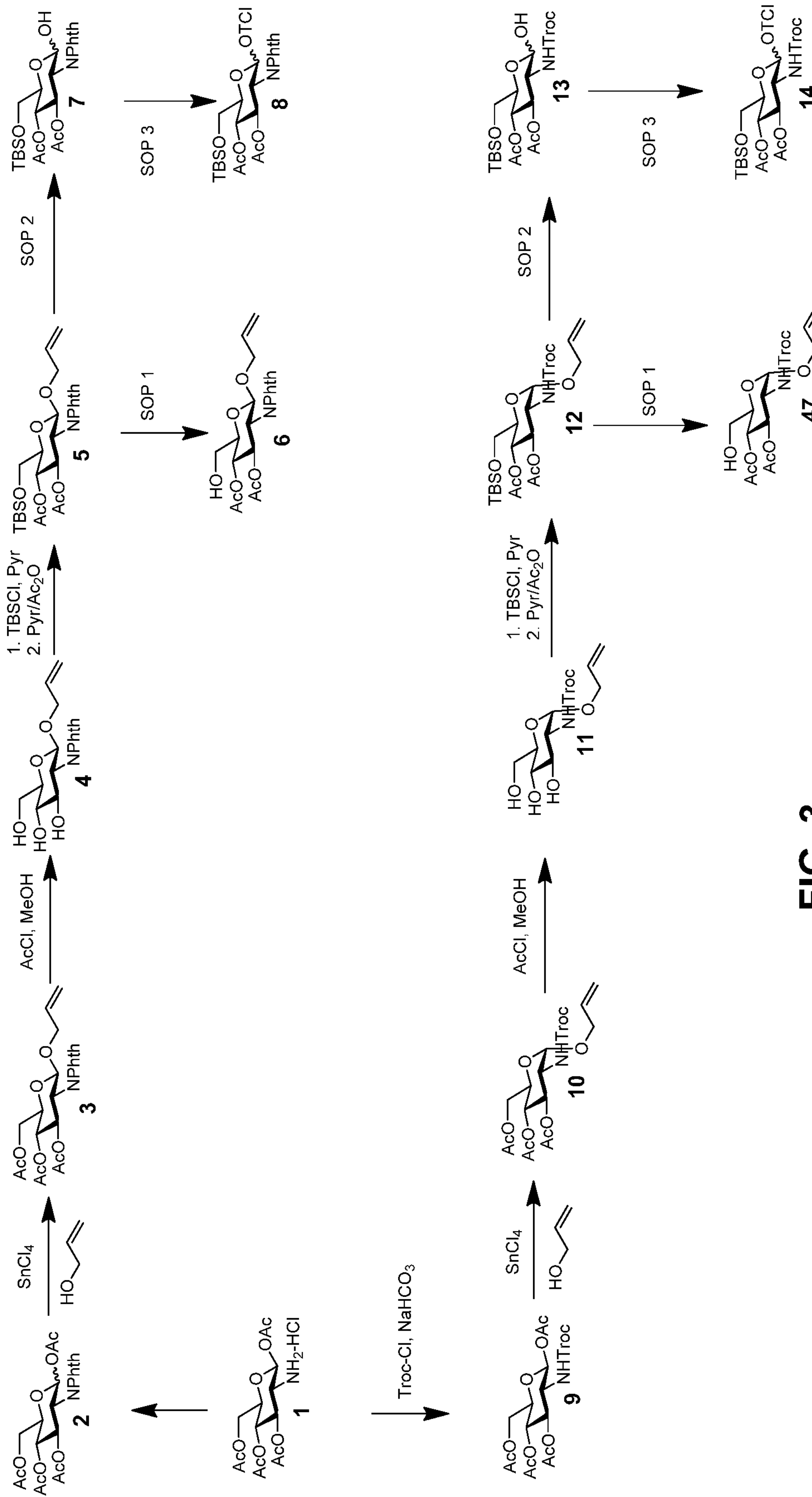


FIG. 3

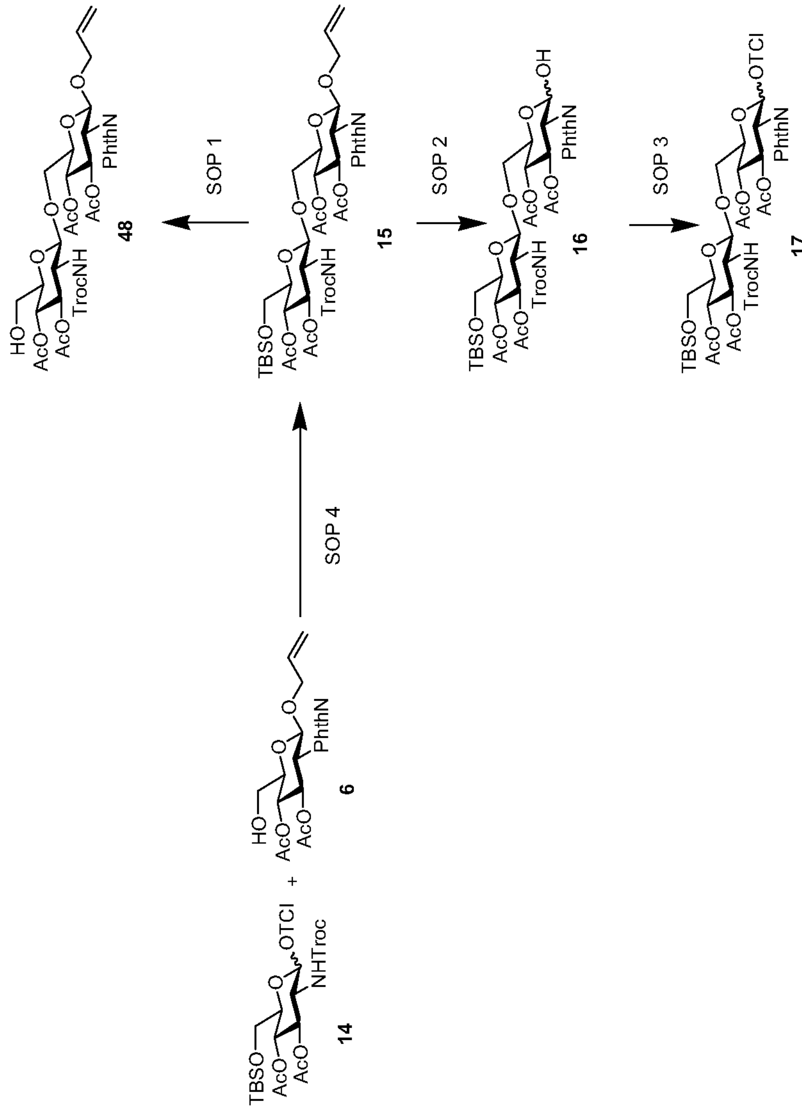


FIG. 4A

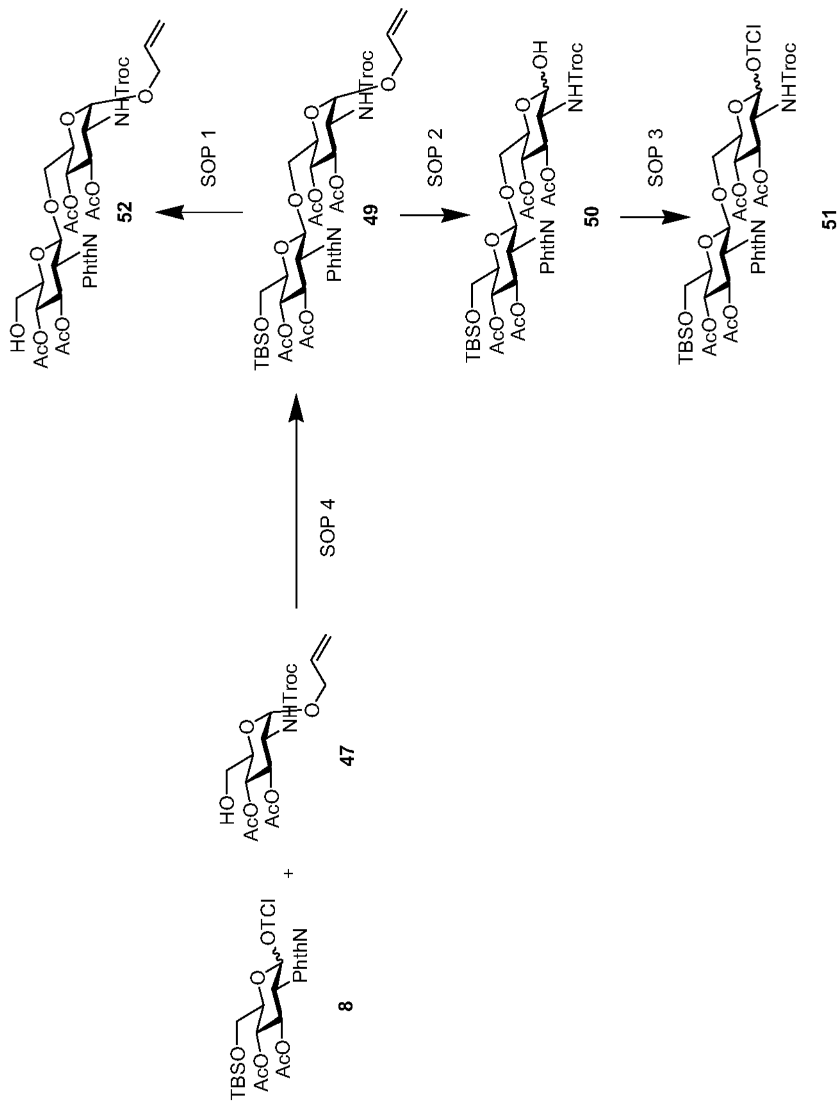


FIG. 4B

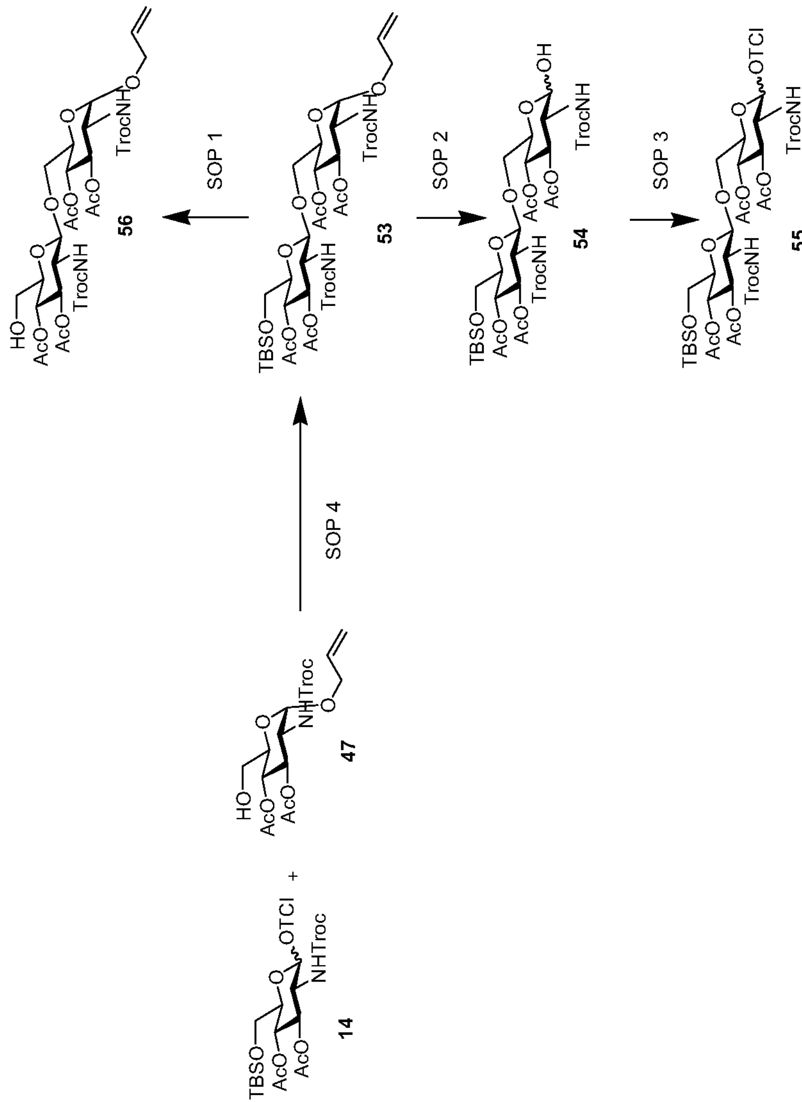


FIG. 4C

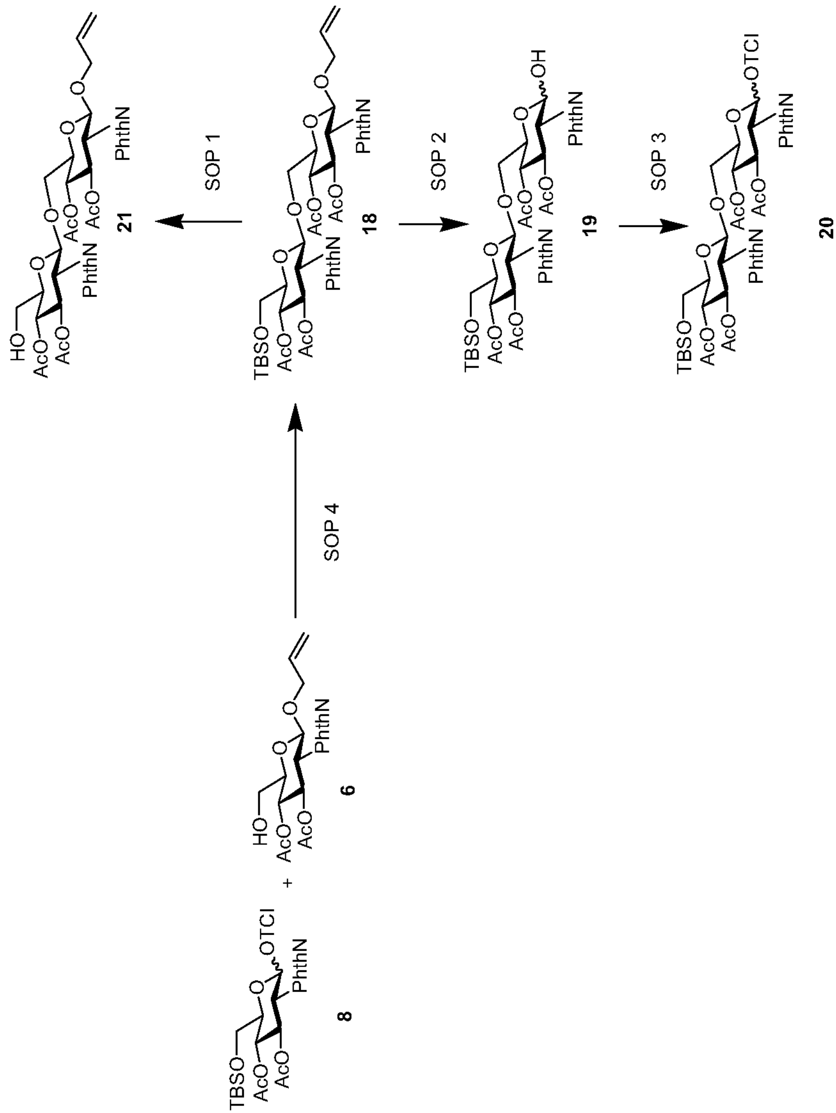


FIG. 4D

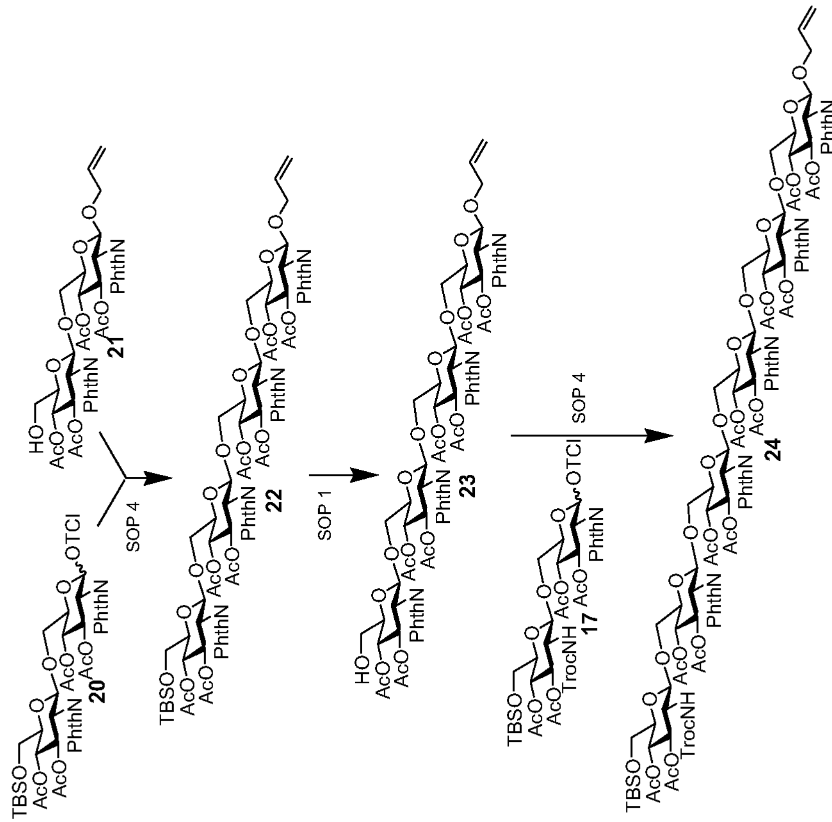


FIG. 5A

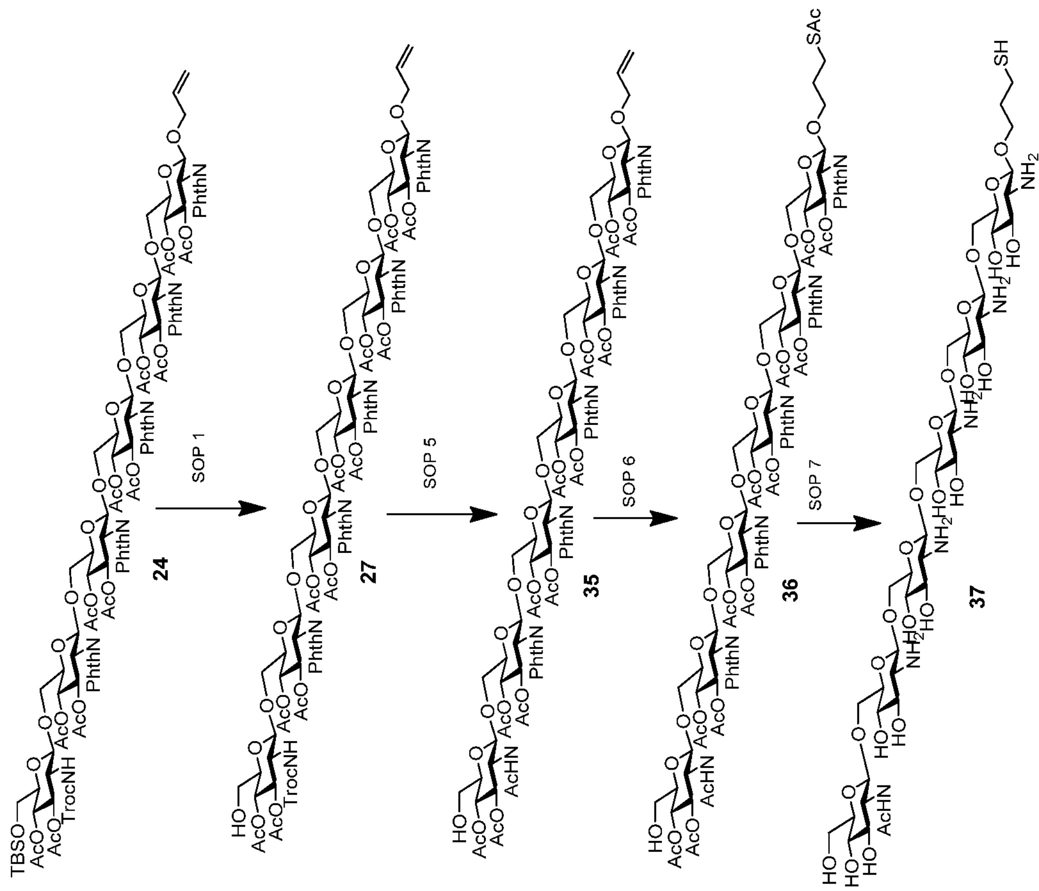


FIG. 5B

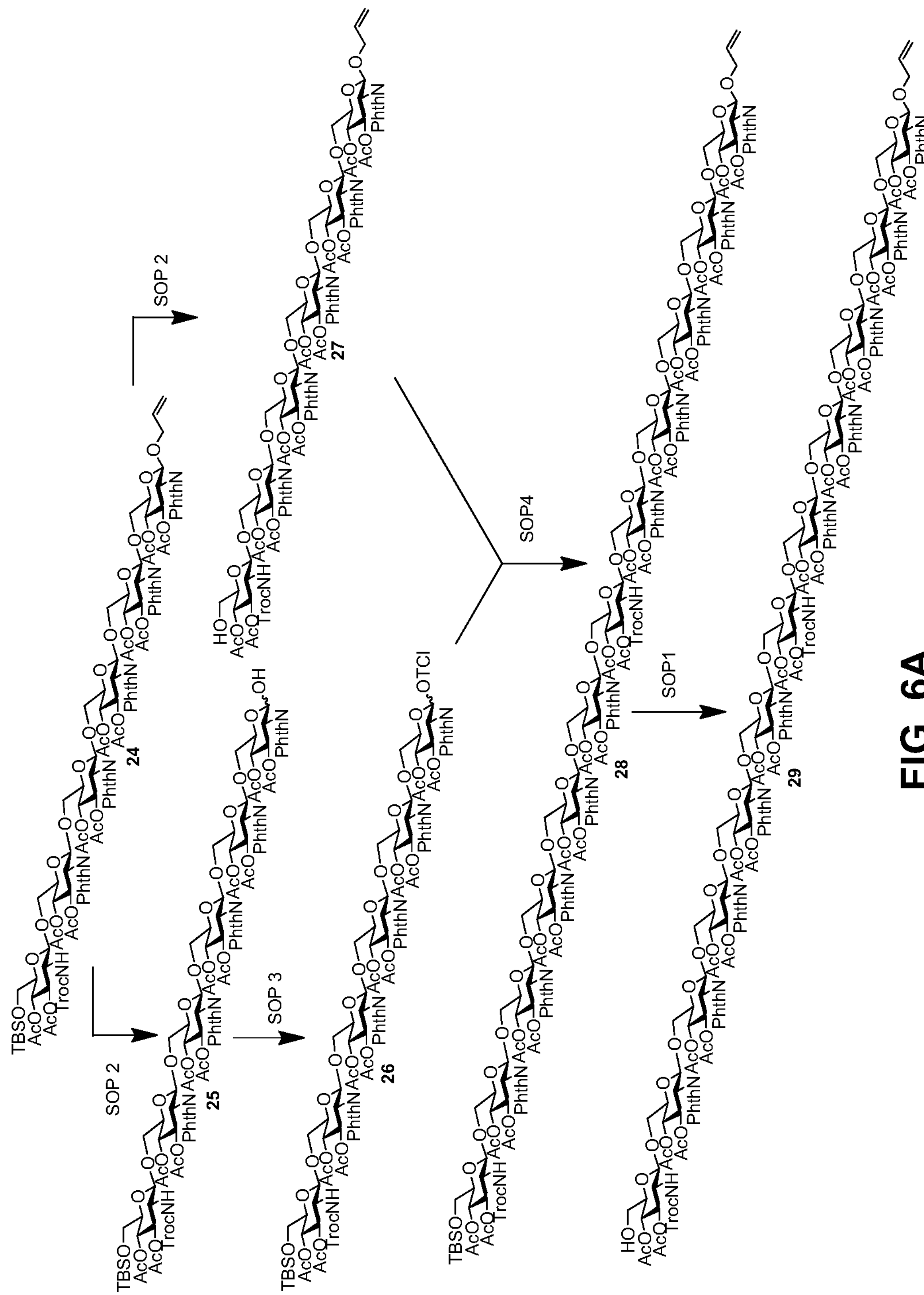


FIG. 6A

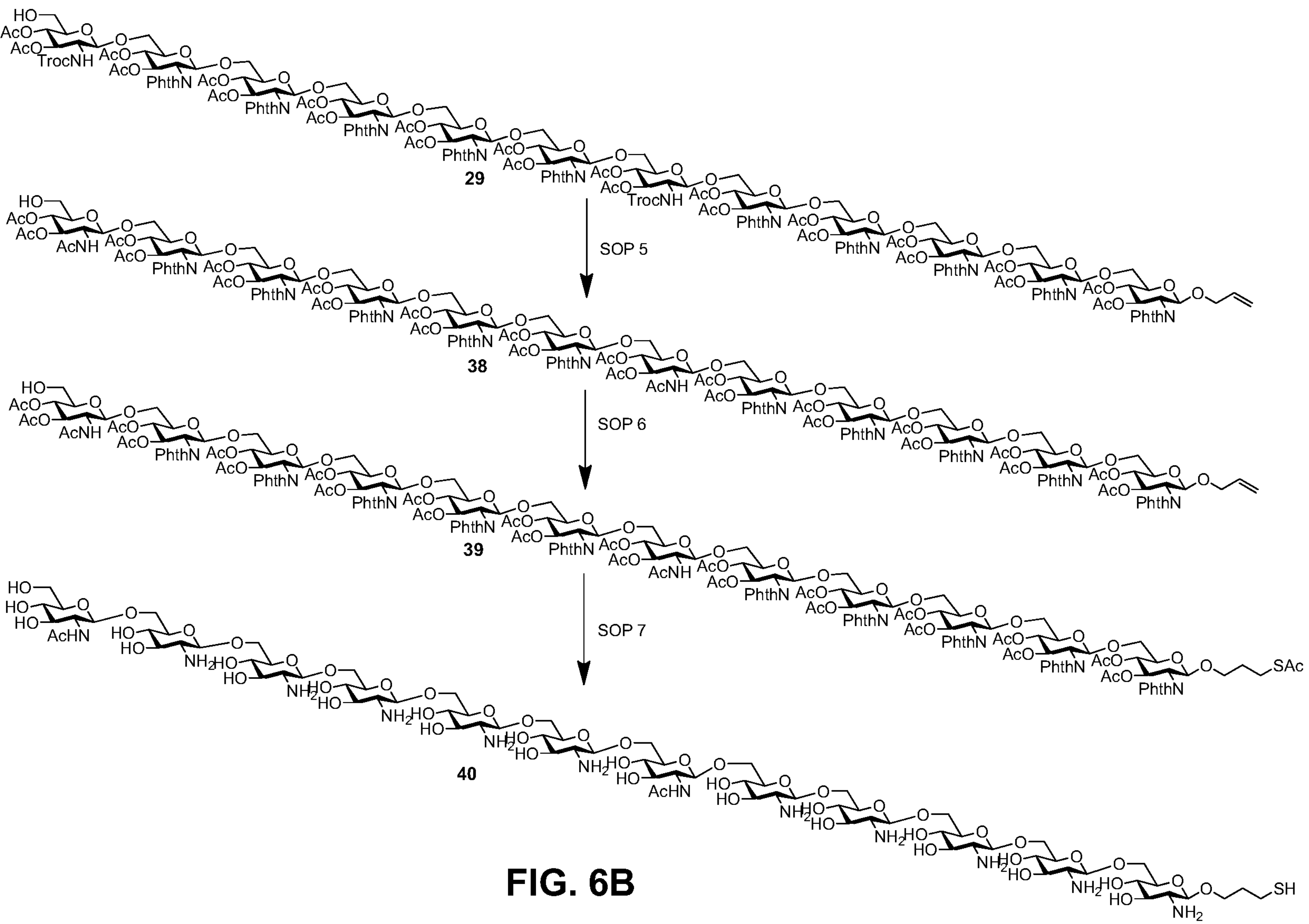


FIG. 6B

26 + 29

SOP 4

30

SOP 1

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SOP 5

32

SOP 6

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SOP 7

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FIG. 7

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FIG. 8A

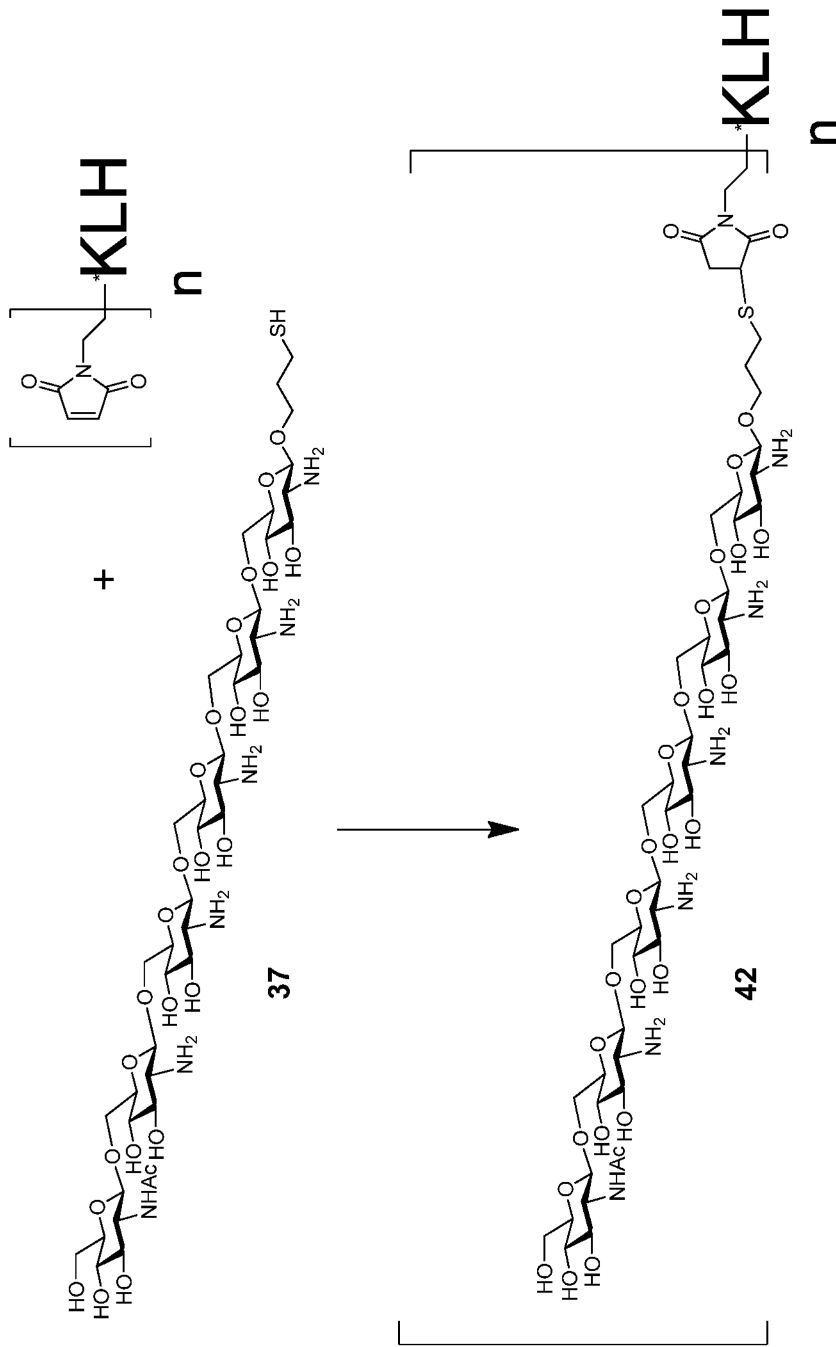

FIG. 8B

FIG. 9A

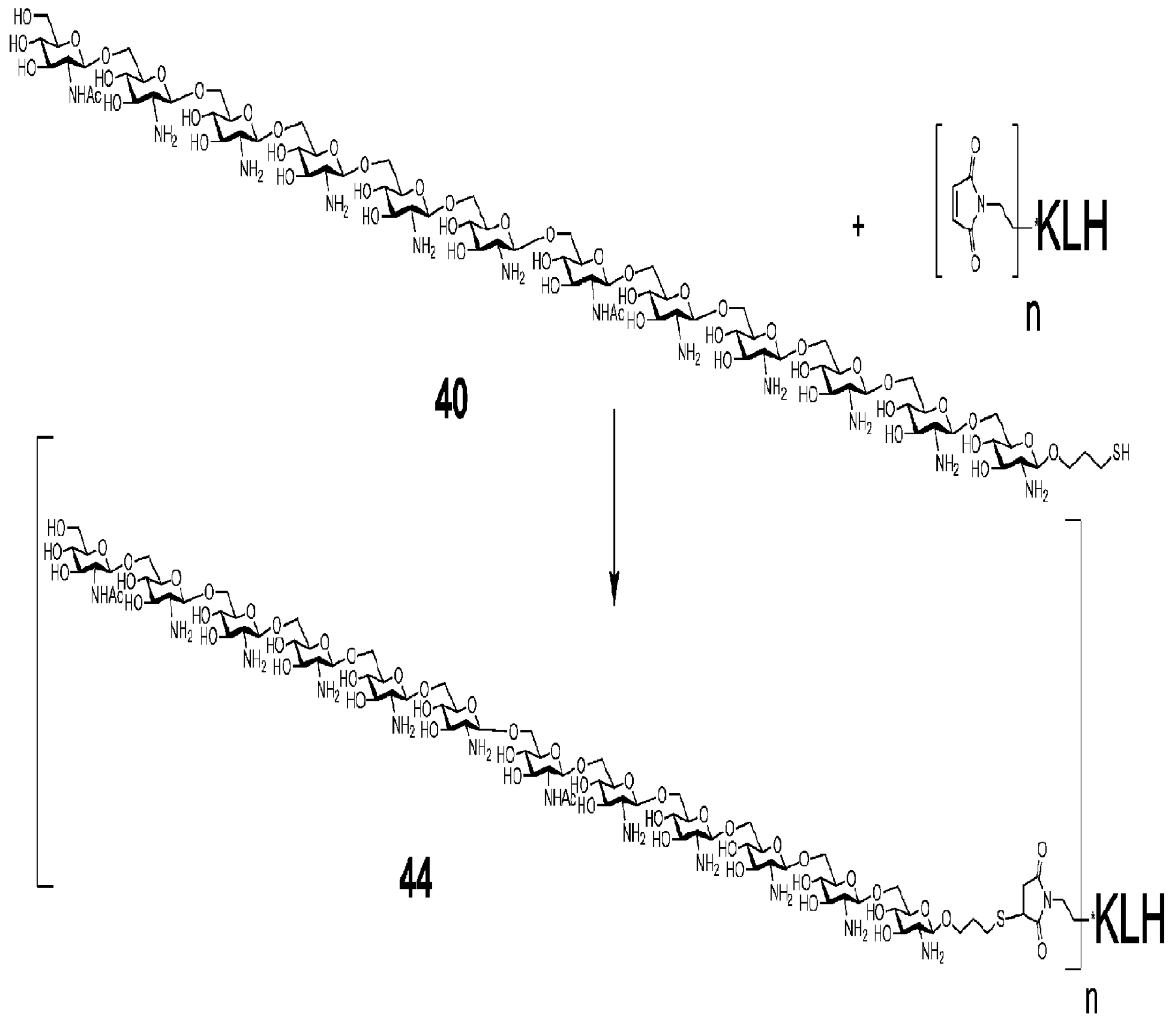

FIG. 9B

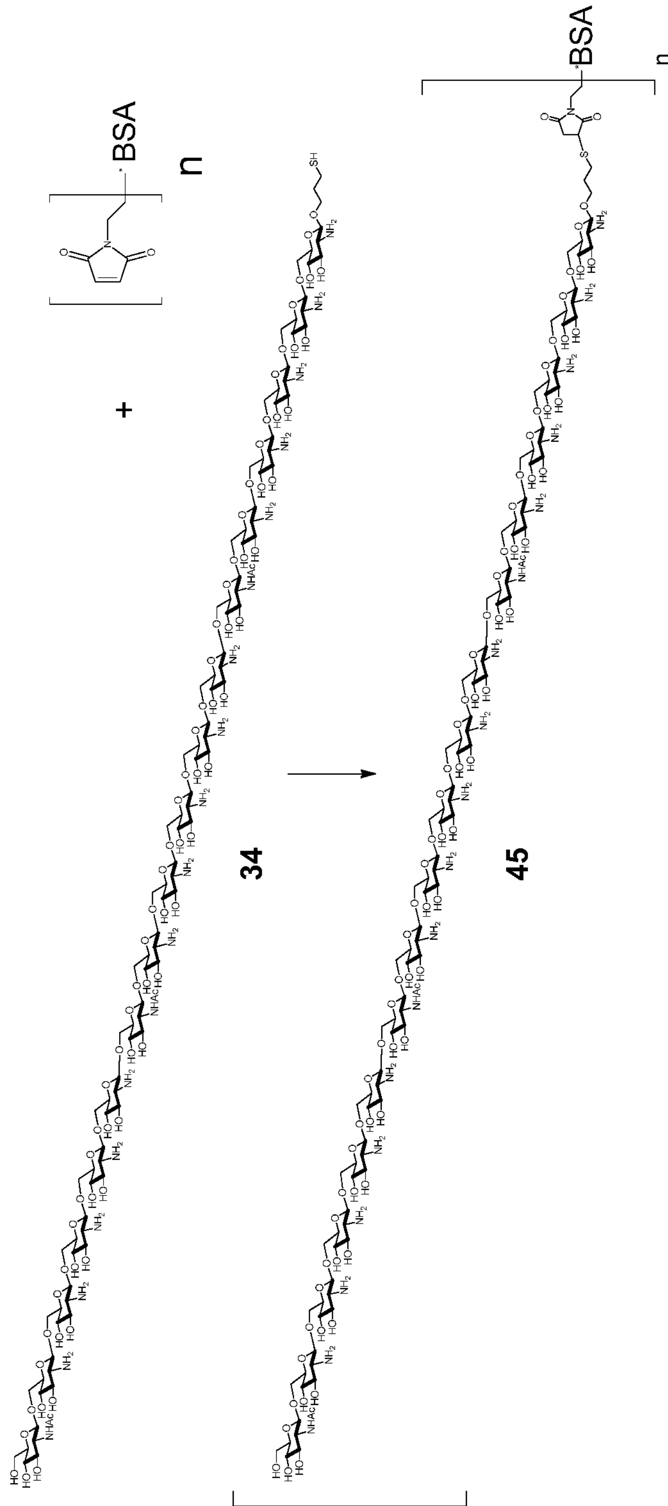

FIG. 10A

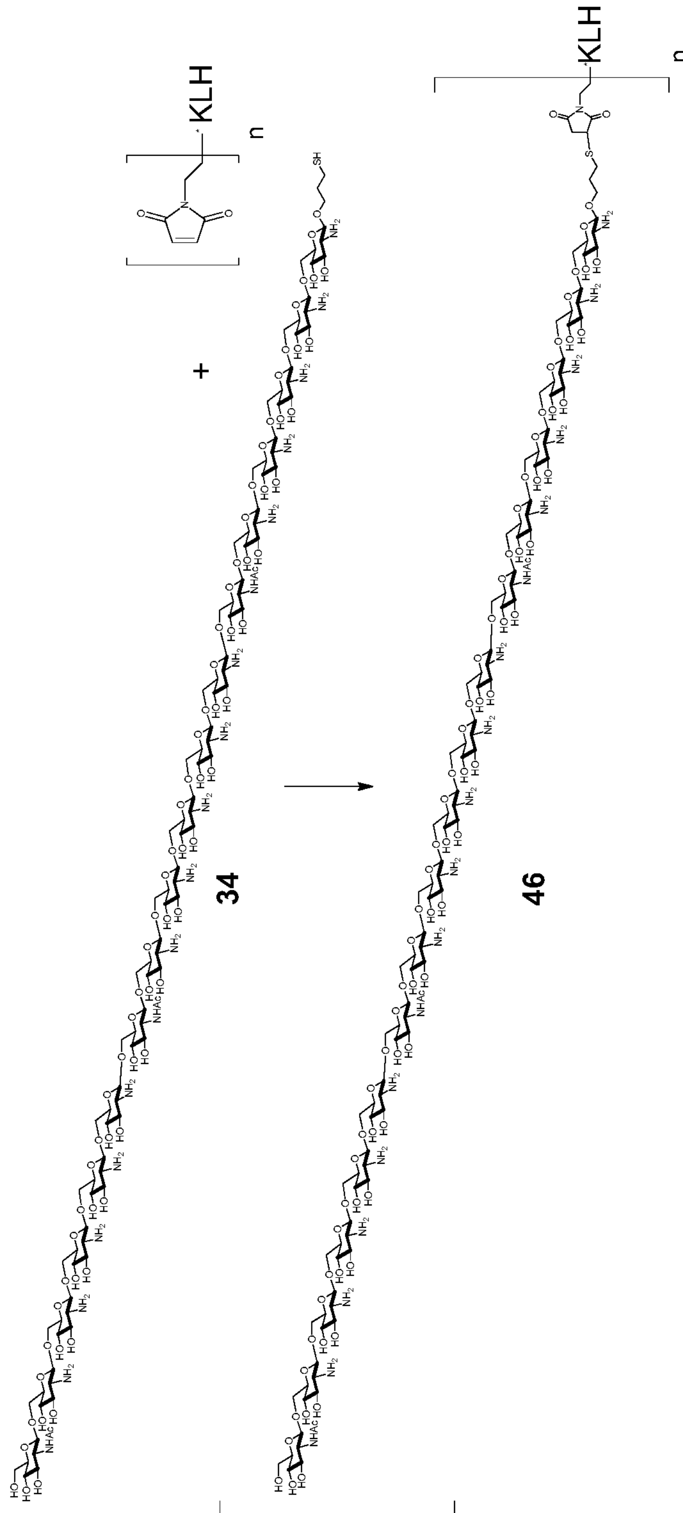

FIG. 10B

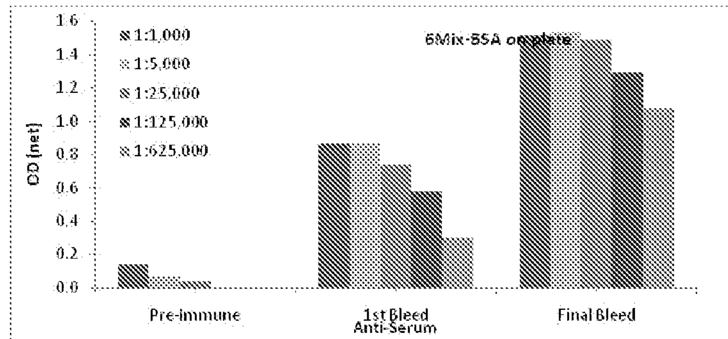

FIG. 11A

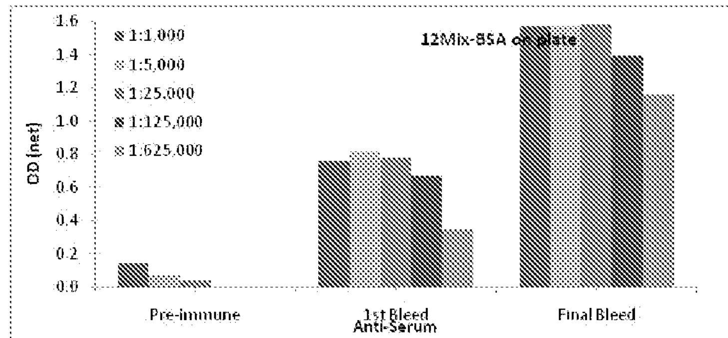

FIG. 11B

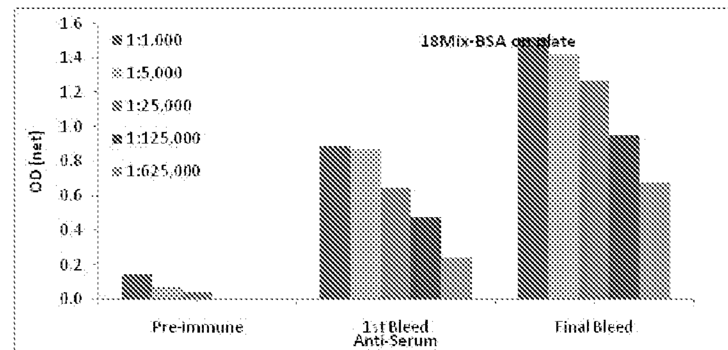

FIG. 11C

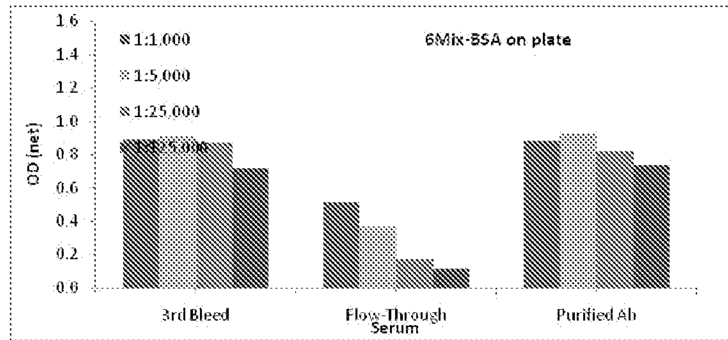

FIG. 11D

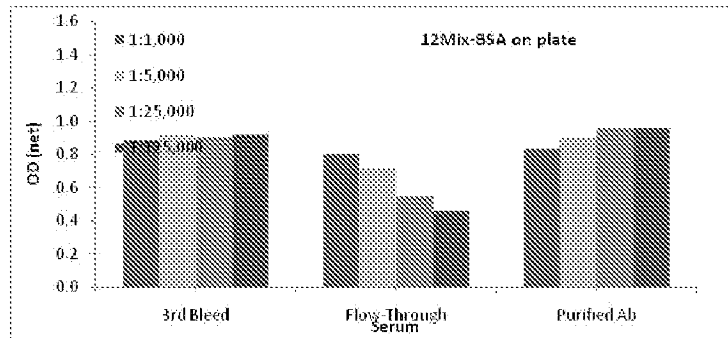

FIG. 11E

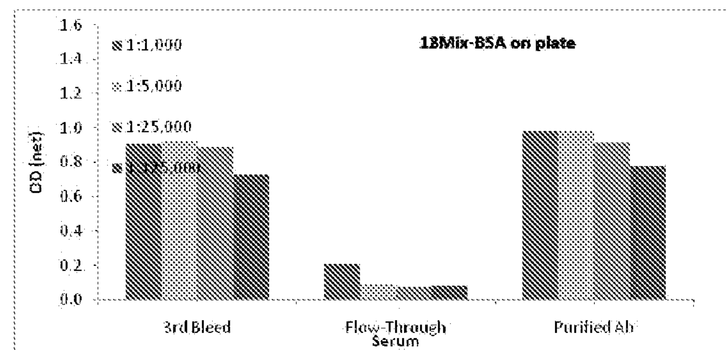

FIG. 11F

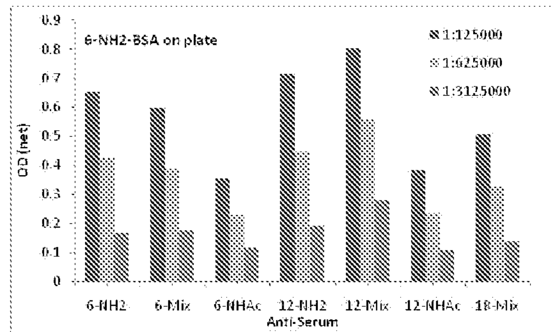

FIG. 12A

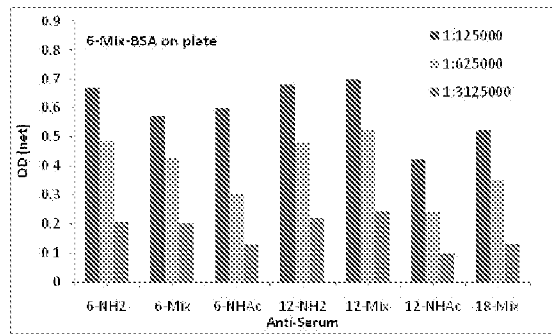

FIG. 12B

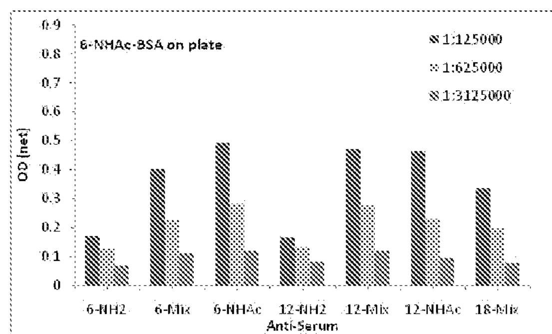

FIG. 12C

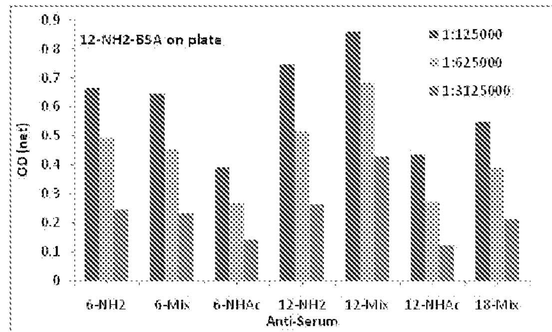

FIG. 12D

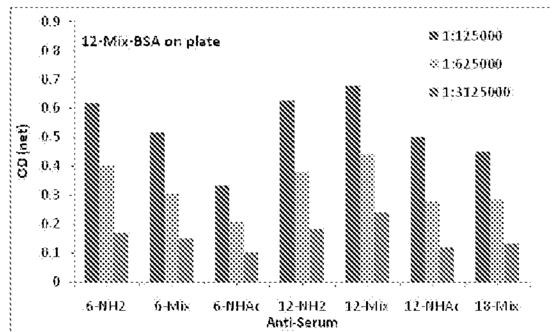

FIG. 12E

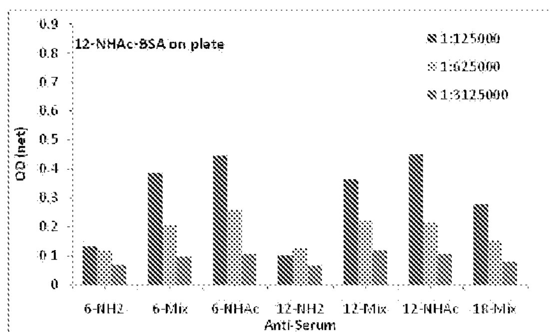

FIG. 12F

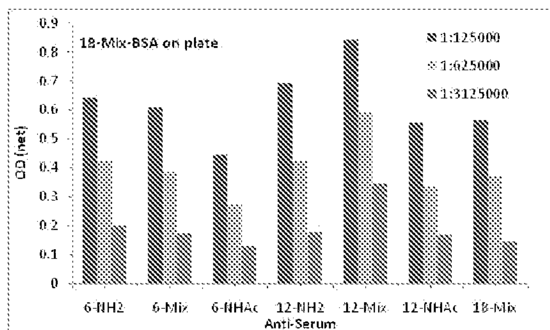

FIG. 12G

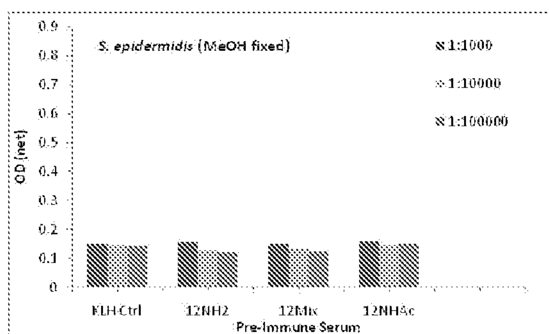

FIG. 13A

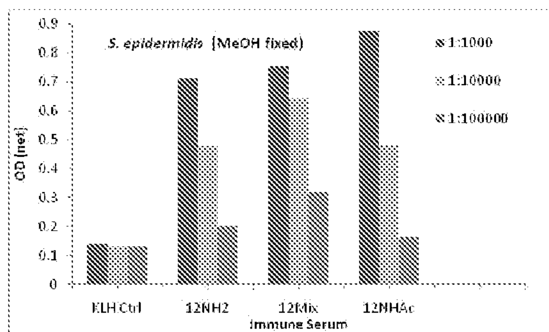

FIG. 13B

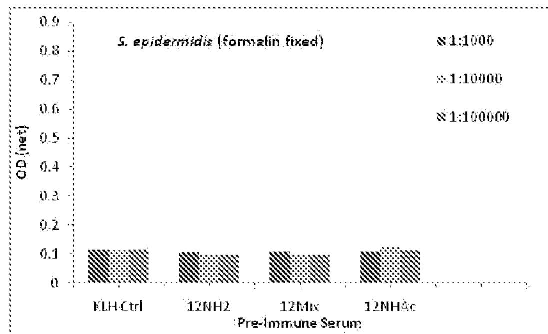

FIG. 13C

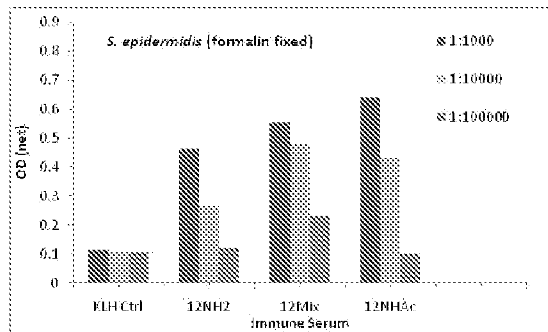

FIG. 13D

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/34449

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A01N 43/04; A61K 31/70 (2012.01)
USPC - 514/23
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC: 514/23

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 514/24-25 (see search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST (USPT, PGPB, EPAB, JPAB), Google Patents/Scholar
Search Terms Used: N-acetyl glucosamine, monomer, acetylated, deacetylated, alternating, antibody, vaccine

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2010/011284 A2 (Pier et al.) 28 January 2010 (28.01.2010) pg 2, ln 24-32; pg 4 ln 23 to pg 5, ln 5; pg 5, ln 10-13; pg 17, ln 22-33; pg 21, ln 13-23; pg 22, ln 4-20; pg 24, ln 5-14; pg 30, ln 1 -19; pg 31, compound 11, Fig 5	1-13, 15, 32-37
Y	US 2009/0281058 A1 (Gislason et al.) 12 November 2009 (12.11.2009) para [0011]-[0012], [0032], [0134]; Table 5	1-13, 15, 32-37
Y	US 2004/0191834 A1 (Laferriere et al.) 30 September 2004 (30.09.2004) para [00445]	15

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
07 July 2012 (07.07.2012)	03 AUG 2012

Name and mailing address of the ISA/US
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Facsimile No. 571-273-3201

Authorized officer:
Lee W. Young
PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/34449

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 14, 16-31 and 38
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.